# Systematic evaluation of clinical and experimental evidence for the application of Chinese herbal medicines in the management of colorectal cancer

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy


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# DECLARATION

I certify that except where due acknowledgement has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any other academic award; the content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program; and, any editorial work, paid or unpaid, carried out by a third party is acknowledged.

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Signed:

Meng Hua Chen
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# ABBREVIATIONS

| Acronym | Description |
|---|---|
| 5-FU | 5- Fluorouracil |
| ACRC | Advanced Colorectal Cancer |
| ADCC | Antibody-Dependent Cell-Mediated Cytotoxicity |
| ADM | Adriamycin |
| AEs | Adverse Events |
| AJCC | American Joint Committee on Cancer |
| ANC | Absolute Neutrophil Count |
| APC | Adenomatous Polyposis Coli Gene |
| ASR | Age-Standardized Rate |
| AUC | Area Under the Concentration Time Curve |
| BIM | Bcl-2-like Protein 11 |
| BMI | Body Mass Index |
| BRAF | Serine/Threonine-Protein Kinase B-raf |
| BW | Body Weight |
| CAM | Complementary and Alternative Medicine |
| CCK-8 | Cell Counting Kit-8 |
| CD | Cluster of Differentiation (T-Lymphocytes Co-receptor) |
| CEA | Carcinoembryonic Antigen |
| CF | Calcium Folinate/Leucovorin |
| c-FLIP | FLICE Inhibitory Protein |
| CHM | Chinese Herbal Medicine |
| CI | Confidence Interval |
| CIMP | CpG Island Methylation |
| CIN | Chemotherapy induced neutropenia |
| CINV | Chemotherapy-induced nausea and vomiting |
| CMI | Cell Mediated Immunity |
| CMT | Chemotherapy |
| CNKI | China Academic Journals |
| COX-2 | Cyclooxygenase-2 |
| CpG | Cytosine-phosphate-Guanine |
| CQVIP | Chinese Science and Technology Journals |
| CR | Complete Remission |
| CRC | Colorectal Cancer |
| CRP | C-Reactive Protein |
| CSI | Chromosomal Instable |

| | |
|---|---|
| DALY | Disability-Adjusted Life Year |
| DC | Dendritic Cells |
| DFS | Disease Free Survival |
| DMEM | Dulbecco’s Modified Eagle's Medium |
| DMSO | Dimethyl Sulfoxide (polar aprotic solvent) |
| EGFR | Epidermal Growth Factor Receptors |
| EORTC | European Organisation for Research Treatment of Cancer |
| ERK | Extracellular Signal-Related Kinases |
| FAP | Familial Adenomatous Polyposis |
| FasL | Fas Ligand |
| FBS | Fetal Bovine Serum |
| FDA | US Food and Drug Administration |
| FE | Fixed-Effect |
| FN | Febrile Neutropenia |
| FOBTs | Fecal Occult Blood Tests |
| FOLFIRI | 5-Fluorouracil (5-FU) plus Leucovorin (LV) plus Irinotecan |
| FOLFOX | 5-Fluorouracil (5-FU) plus Leucovorin (LV) plus Oxaliplatin |
| G-CSF | Granulocyte Colony-Stimulating Factor |
| HCPT | Hydroxycamptothecine |
| HLA | Human Leukocyte Antigen |
| HM | Herbal Medicine |
| HNPCC | Hereditary Nonpolyposis Colorectal Cancer |
| HRT | Hormone Replacement Therapy |
| IAP | Immunosuppressive Acidic Protein |
| IARC | International Agency for Researchon Cancer |
| IFN-γ | Interferon Gamma |
| Ig | Immune Globulins |
| IHPC | Intraperitoneal Hyperthermic Perfusion Chemotherapy |
| IL | Interleukin |
| IM | Integrative Medicine |
| ITT | Intent to Treat |
| Jak | Janus kinase |
| KPS | Karnofsky Performance Status |
| KRAS | GTPase KRas also known as V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog |
| LV | Leucovorin |
| MD | Mean Difference |
| MDSCs | Myeloid-Derived Suppressor Cells |
| MeCCNu | Semustine |

| | |
|---|---|
| MEKK3 | Mitogen-activated protein kinase kinase kinase 3 |
| MHC | Major Histocompatibility Complex |
| MKK3 | Dual Specificity Mitogen-Activated Protein Kinase Kinase 3 |
| MMC | Mitomycin |
| MMP | Matrix Metalloproteinases |
| MOMP | Mitochondrial Outer Membrane Permeabilization |
| mOS | Median OS |
| mPFS | Median Progression Free Survival |
| MSI | Microsatellite Instability |
| MTX | Methotrexate |
| NCI-CTC | National Cancer Institute Common Toxicity Criteria |
| NK | Natural Killer (a type of cytotoxic lymphocytes) |
| NSAIDs | Nonsteroidal Anti-Inflammatory Drugs |
| OS | Overall Survival |
| OXA | Oxaliplatin |
| PARP | Poly (ADP-ribose) Polymerase |
| PBS | Phosphate-Buffered Saline |
| PCD | Programmed Cell Death |
| PD | Progressive Disease |
| PDD | Cisplatin |
| PFS | Pregression Free Survival |
| PGE2 | Prostaglandin E2 |
| PHT | Postmenopausal Hormone Therapy |
| PI3K | Phosphoinositide 3-Kinase |
| PIK3CA | Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha |
| PPARγ | Peroxisome Proliferator-Activated Receptor γ |
| PR | Partial Remission |
| PRRs | Pattern Recognition Receptors |
| PSK | Polysaccharide K |
| QoL | Quality of Life |
| Rac | Rac GTPase |
| Raf | RAF proto-oncogene serine/threonine-protein kinase |
| Ras | Ras GTPases |
| RCT | Randomised Control trial |
| RD | Risk Difference |
| RE | Random-Effect |
| RECIST | Response Evaluation Criteria in Solid Tumours |
| ROCK | Rho-associated Protein Kinase |

| | |
|---|---|
| ROS | Reactive Oxygen Species |
| RR | Risk Ratio |
| SD | Stable Disease |
| SMAD4 | SMAD Family Member 4 (Mothers against decapentaplegic homolog 4) |
| TAA | Tumour Associated Antigen |
| TACE | Transcatheter Arterial Chemoembolization |
| TCM | Traditional Chinese Medicine |
| TCR | T-cell Receptor |
| TGF-β | Transforming Growth Factor Beta |
| TIL | Tumour infiltrating lymphocytes |
| TNF-α | Tumour Necrosis Factor-alpha |
| TNM | Cancer staging system, T: tumour, N: lymph nodes, M: metastasis |
| TP53 | Cellular Tumour Antigen p53 |
| TRAIL | Tumour Necrosis Factor–related Apoptosis-inducing Ligand |
| tRR | Tumour Response Rate |
| TTP | Time to Progression |
| UICC | The International Union Against Cancer (renamed Union for International Cancer Control) |
| VCR | Vincristine |
| VEGF | Vascular Endothelial Growth Factor |
| WHO | World Health Organisation |
| XELOX | Xeloda (capecitabine) plus Oxaliplatin |

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# LIST OF PUBLICATIONS ARISING FROM THIS PROJECT

## List of articles in peer-reviewed journals

1. **Chen MH**, Chen, M., Cui, J., Zhang, A.L., Sze, D.M., Xue, C.C., May, B.H. (2018). Adherence to CONSORT Items in Randomized Controlled Trials of Integrative Medicine for Colorectal Cancer Published in Chinese Journals. *J Altern Complement Med*, 24(2), 115-24. doi: 10.1089/acm.2017.0065.

2. **Chen MH**, May BH, Zhou IW, Sze DM, Xue CC, Zhang AL (2016c). Oxaliplatin-based chemotherapy combined with traditional medicines for neutropenia in colorectal cancer: A meta-analysis of the contributions of specific plants. *Crit Rev Oncol Hematol*. 2016 Sep;105:18-34. doi: 10.1016/j.

3. **Chen MH**, May BH, Zhou IW, Zhang AL, Xue CCL (2016b). Integrative Medicine for Relief of Nausea and Vomiting in the Treatment of Colorectal Cancer Using Oxaliplatin-Based Chemotherapy: A Systematic Review and Meta-Analysis. *Phytother Res*, 30(5):741-53 doi: 10.1002/ptr.5586.

4. **Chen MH**, May BH, Zhou IW, Xue CCL, Zhang AL (2016a). Meta-analysis of oxaliplatin-based chemotherapy combined with traditional medicines for colorectal cancer: contributions of specific plants to tumor response. *Integr Cancer Ther*, Mar;15(1):40-59. doi: 10.1177/1534735415596424

5. **Chen MH**, May BH, Zhou IW, Xue CC, Zhang AL. (2014). FOLFOX 4 Combined with herbal medicine for advanced colorectal cancer: A systematic review. *Phytother Res*. Jul,28(7): 976-91. doi: 10.1002/ptr.5092.

## List of conference presentations

1. **Menghua Chen**, Brian H. May, Iris W. Zhou, Daniel Man-yuen Sze, Charlie C. Xue, Anthony L. Zhang. Oxaliplatin-based chemotherapy combined with traditional medicines for neutropenia in colorectal cancer: A meta-analysis of the contributions of specific plants, by poster presentation on the 16th Meeting of Consortium for Globalization of Chinese Medicine (CGCM) held in Guangzhou on August 18-20, 2017

2. **Menghua Chen**, Brian H. May, Iris W. Zhou, Charlie C. L. Xue, and Anthony L. Zhang. Meta-Analysis of Oxaliplatin-Based Chemotherapy Combined With Traditional Medicines for Colorectal Cancer: Contributions of Specific Plants to Tumor Response, by poster & oral presentation on the 15th Meeting of Consortium for Globalization of Chinese Medicine (CGCM) held in Academia Sinica, Taipei on August 23-25, 2016

3. **Menghua Chen**, Brian H. May, Iris W. Zhou, Charlie C. L. Xue and Anthony L Zhang. FOLFOX 4 Combined with Herbal Medicine for Advanced Colorectal Cancer: A SystematicReview, by poster & oral presentation on the 13th Meeting of Consortium for Globalization of Chinese Medicine (CGCM) held in Beijing on August 27-29, 2014

# SUMMARY

Worldwide, colorectal cancer (CRC) is the third most common cancer in men and the second in women. CRC is curable by surgical resection if it is diagnosed in early stage but requires more complex therapeutic approaches including chemotherapy at more advanced stages. Although overall patient survival is improving, survival rates for advanced CRC are poor and adverse events (AEs) associated with multi-drug combination chemotherapy can severely compromise quality of life in CRC sufferers.

In China, integrative treatments which combine herbal medicines (HM) and chemotherapy are applied in hospital settings with the aim of enhancing the benefits of conventional treatments and alleviating the side effects of chemotherapy. Outside China, there is widespread use of HMs by cancer patients. However, the number of different HMs in use is large, they are often used in combination, and the evidence for their effects (if any) is limited.

Hence, the primary objectives of this study were to:

- Evaluate the efficacy and safety of HMs in the clinical management of CRC;
- Identify potentially effective HMs and combinations of HMs that warrant further research;
- Investigate the actions and mechanisms of action of promising HMs in experimental models of CRC; and
- Determine directions for future research.

The first stage of the project involved a comprehensive systematic review of randomised controlled trials (RCTs) that evaluated HMs in patients with CRC. Eighty-eight (88) RCTs were included (Chapter 4). The majority of studies were of integrative treatments for CRC. Meta-analyses found the addition of HM interventions to conventional chemotherapy provided benefits for tumour response, survival, alleviation of chemotherapy-related AEs and improved quality of life. This suggested that at least some of the included HMs improved clinical outcomes and warranted further study. However, the variety of HMs and chemotherapy regimens tested in the studies was considerable, participants were at different stages of the disease and there was potential for bias in the published studies (publication 1 Chen et al 2018).

To further explore the effects of HMs, more focussed meta-analyses were conducted of RCTs that only enrolled people with advanced CRC and all employed FOLFOX4, which is the most commonly used regimen (Chapter 5, publication 5 Chen et al 2014). The result showed that even in advanced CRC patients, the addition of HMs to FOLFOX4 enhanced the tumour response rate by 9% based on data from 12 RCTs (880 participants) without statistical heterogeneity, and improved Quality of Life based on Karnofsky Performance Status. Importantly, there were significant reductions in severe

(grade 3/4) chemotherapy-induced AEs for nausea & vomiting (9.5% reduction, 9 RCTs) and neutropenia (8.7% reduction, 10 RCTs) without heterogeneity. Both AEs are clinically important since they can lead to cessation of treatment which shortens overall survival.

The HMs were composed of multiple ingredients, so the question was which of these ingredients made greater contributions to the overall effects detected in the pooled data in the meta-analyses? To approach this question, a larger meta-analysis pool was identified and a novel approach to sensitivity analyses was developed. The inclusion criteria for studies were broadened to encompass other oxaliplatin-based chemotherapies besides FOLFOX4, since these are known to have similar effects on tumour response and similar AE profiles. The resultant studies were all of Chinese HMs, most of which used orally administered formulae. Meta-analyses of the oral HM studies were conducted for tumour response rate (31 studies, 2,145 participants), nausea and vomiting (21 studies, 1,322 participants) and neutropenia (24 studies, 1,319 participants) (Chapter 6). In each case, there were significant improvements in the oral HM plus chemotherapy groups compared to the chemotherapy alone groups for all grades of the AE without important heterogeneity, based on large sample sizes. This lack of statistical heterogeneity combined with large sample sizes provided the opportunity for a series of sensitivity analyses aimed at determining which (if any) specific herbs, or combinations of herbs, improved the meta-analysis results of the pool of studies in which the herb was an ingredient. If a herb consistently improved the outcome, whenever it was included in a formula along with a variety of other herbs, it was considered a candidate for further research.

The above meta-analyses and sensitivity analyses identified promising herbs for reducing chemotherapy-induced nausea and vomiting (publication 3 Chen et al 2016b) and chemotherapy-induced neutropenia (publication 2 Chen et al 2016c); and improving tumour response rate (publication 4 Chen et al 2016a). Of these outcomes, tumour response was selected as particularly relevant for further research. Of the three herbs identified as most likely to have contributed to improved tumour response (*ku shen*, *chi shao* and *e zhu*), one was selected for further research. This herb (*ku shen*) is always derived from the root of the plant *Sophora flavescens*, is well characterised, and some of its constituent compounds have been identified. Of these, the alkaloid matrine has been reported to have antitumour effects, so this was selected for a series of experiments.

Matrine was tested in four human CRC cell lines: LS 174T, Caco-2, SW1116 and RKO (Chapter 7). Cell viability, measured using CCK-8 assays, showed that matrine inhibited proliferation of these cell-lines, time- and dose-dependently. Optical microscopy of cell morphology indicated cells underwent apoptosis rather than necrosis. Matrine was much less cytotoxic than oxaliplatin. Flow cytometry was used to measure DNA content for cell cycle analysis, and Annexin V-FITC/PI double staining was used to measure cellular apoptosis. The results showed that matrine induced cell cycle arrest at the G1 phase, and induced apoptosis in each cell-line in a time- and dose-dependent manner.

To explore the likely molecular mechanisms of action of *S. flavescens* and its various constituent compounds, including matrine, a detailed review was conducted of published *in vitro* and *in vivo* studies in models of CRC (Chapter 8). This identified a number of intracellular signalling pathways, including WNT signalling, MAPK signalling, TGF-β signalling, and p53 signalling, as likely to be central to the anti-proliferative actions of this HM. In conclusion, matrine and other *S. flavescens* compounds show important bioactivities in CRC. Future studies in CRC cell-lines and *in vivo* models of CRC could investigate the effects of *Sophora* alkaloids and flavonoids on the protein components of the above pathways (Chapter 9).

# Chapter 1. Introduction

## 1.1 Background to the disease

Colorectal cancer (CRC) refers to malignant tumours that develop in the colon or rectum. The majority of CRCs are carcinomas that mutate from the epithelial cells (Stewart et al., 2006). The *International Statistical Classification of Diseases* (ICD 10) specifies colorectal cancer under: malignant neoplasms of the colon (code C18), rectosigmoid junction (code C19) and rectum (code C20) (World Health Organisation, 2016).

According to the World Health Organization (WHO) Cancer Report 2012, CRC is the third most common cancer in men and the second in women. Worldwide, it is estimated that there were over 1.4 million new CRC cases and more than 694,000 deaths from CRC in 2012 (Ferlay et al., 2014). In Australia, CRC was estimated to be the second most commonly diagnosed cancer in both sexes in 2017 and was the cancer with the third highest mortality (Australian Institute of Health and Welfare, 2017). In China in 2011, CRC was ranked fifth in men and third in women and was the fifth leading cause of cancer death in both men and women (Chen et al., 2015).

Epidemiological studies have identified factors that may increase the risk of CRC including hereditary factors (in a small proportion of cases), increased age, chronic inflammatory bowel diseases, and lifestyle-related factors such as excessive consumption of red meat, preserved meat and animal fat, lack of exercise, obesity, alcohol consumption and smoking. Conversely, the risk of CRC is reduced by higher consumption of vegetables and fruit, exercise, vitamin D, calcium, folic acid, non-steroidal anti-inflammatory drugs (NSAIDs) and hormone replacement therapy (HRT) for postmenopausal women (American Cancer Society, 2008).

CRC can be curable when diagnosed at a sufficiently early stage to enable complete surgical resection of the pre-carcinoma polyp or early stage carcinoma. Bowel cancer screening programs can detect possible CRC and an endoscope can be used to diagnose the disease and remove early stage polyps (Hayat, 2009; Winawer et al., 1993; Faivre et al., 2004). However, CRC is asymptomatic at the early stage and may have progressed to invade the intestinal wall and have spread to lymph nodes by the time clinical symptoms are evident. Although survival rates are high (90%) at the early stages of the disease, in advanced colorectal cancer (ACRC) the median survival time, even with the best available treatment, may only be 12 months (Sargent et al., 2005; Stewart et al., 2006; Ries et al., 2008). Therefore, it is imperative that new methods for the prevention and management of CRC be developed.

## 1.2 Conventional treatment for colorectal cancer

In conventional medicine, where possible, surgical resection is the primary treatment for CRC. Following resection, cytotoxic chemotherapy is often used to prevent the recurrence of cancer. This is known as adjuvant chemotherapy. In ACRC, when the tumour is not resectable, chemotherapy and/or radiotherapy may be used to downstage the tumour and potentially allow complete surgical resection. This is known as neoadjavant therapy. In advanced and progressive disease combinations of surgery, chemotherapy and/or radiotherapy may be used to maintain or improve quality of life and prolong survival. In late stage disease, palliative therapies are used to manage symptoms (American Cancer Society, 2008; Braun & Seymour, 2011). In recent years bio-targeted monoclonal antibodies have been developed into drugs that can be used in the more advanced stages of CRC (Braun & Seymour, 2011; Hirsch & Zafar, 2011).

Due to the cytotoxic actions of chemotherapy on fast-dividing cells, these drugs also have adverse effects on normal cells in the body, especially on normal fast-dividing cells such as the cells in hair, skin, the gastrointestinal tract and the bone marrow. As a result, a number of chemotherapy-related adverse events (AEs) may occur during and/or after chemotherapy, including nausea and vomiting, diarrhoea, bone marrow suppression, and neuropathy which impair quality of life and may lead to the cessation of the chemotherapy earlier than is optimal (DeHaven, 2007). A number of therapies are in current use to manage these adverse events but new therapies are still needed.

## 1.3 Herbal medicine and colorectal cancer

There is increasing international interest in complementary and alternative medicine (CAM) therapies, including herbal medicines (HMs) for CRC. Up to 75% of CRC patients in Western countries were reported to have used a form of CAM and nearly half of them claimed benefits (Sewitch & Rajput, 2010). A survey of European countries found nearly half of CRC patients (48.7%) used HM (Molassiotis et al., 2005). HMs have been used to treat many ailments including tumours for centuries in China and in other Asian countries, and Asian HMs are increasingly being used in Western countries (Dobos et al., 2005; Qi et al., 2010).

Chinese herbal medicine (CHM) is a major arm of Traditional Chinese Medicine (TCM) which is commonly combined with conventional therapy in hospitals in China for the management of a wide range of cancers (Dobos et al., 2005; Parekh et al., 2009; Saif et al., 2010). CHM typically involves the oral administration of multi-herb formulas as decoctions, tablets or capsules; and sometimes as enemas or intravenously (Gu et al., 2009; Wang et al., 1999).

HMs have been reported to: alleviate AEs induced by conventional cancer therapies and improve a patient's quality of life (Molassiotis et al., 2009); enhance cellular immunity of cancer patients

receiving chemotherapy or radiotherapy (Zhuang et al., 2009); reduce cancer pain (Xu et al., 2007); relieve cancer related fatigue (Jeong et al., 2010); and improve anorexia and cachexia (Lee & Lee, 2010).

Experimental studies have demonstrated that bio-active components in HMs possess anti-cancer activities including: inducing tumour cell apoptosis; inducing tumour cell differentiation; cellular transduction pathway regulation; suppression of tumour angiogenesis; inhibition of telomerase activity; regulation of immune function; and reversal of multiple drug resistance (Han & Li, 2009).

However, numerous issues remain unresolved. These include the complexity of the chemical components of HMs; limited understanding of their pharmacological mechanisms; variability in the quality of the HMs; potential for herb-drug interactions; the incidence of herbal toxicity, especially when used at high doses and with long term use; and the quality of existing randomised controlled clinical trials (RCTs). These issues are of concern to medical practitioners and patients (Fong, 2002).

## 1.4 Objectives of this research project

The primary objectives of this research project were to:

- evaluate the efficacy and safety of HMs in the clinical management of CRC;
- identify potentially effective HMs and combinations of HMs that warrant further experimental and clinical research;
- investigate the actions and mechanisms of action of promising HMs in experimental models of CRC, and
- determine directions for future experimental and/or clinical research.

This project involved a sequence of stages, each of which was designed to answer one or more of the following research questions.

## 1.5 Research questions

The following research questions were targeted in this study:

1. Can HM interventions, used either singly or in combination with conventional therapies, elevate tumour response rate and/or prolong the survival of CRC patients? (Chapters 4 and 5)
2. Can HM interventions alleviate the adverse events associated with conventional anti-cancer treatments for CRC? (Chapters 4 and 5)
3. Can HM interventions improve the quality of life of CRC patients? (Chapters 4 and 5)
4. How safe are HM interventions for CRC? (Chapters 4 and 5)
5. Which herbs and herbal combinations appear effective for CRC treatment and/or alleviation of adverse events associated with conventional CRC treatments? (Chapter 6)

6. What are the effects of specific herb-derived compounds in CRC cell lines? (Chapter 7)
7. What are the likely mechanisms of action of potentially effective HMs and their constituent compounds? (Chapter 8)
8. What questions could be addressed in future studies and how would a future study be implemented? (Chapter 9)

## 1.6 Thesis outline

The thesis includes nine chapters, including the present introductory chapter. The chapters are outlined below.

**Chapter 1: General introduction**

This chapter introduces the topic and and the general needs for this reseach. It summarises the features of CRC including epidemiology, incidence rates and mortality worldwide; the risk factors and prevention; the staging of CRC and survival rate. It introduces the conventional treatment and the HM management of CRC. The research questions are also presented in this chapter.

**Chapter 2: Literature review: physiology, aetiology, diagnosis and treatment of colorectal cancer**

This chapter reviews: the large intestine anatomical location and physiological functions; details CRC epidemiology, risk factors, prevalence and burden; outlines bowel cancer screening programs; explains the etiology and tumour immunology of CRC; outlines CRC diagnosis and staging systems; summarises the current management of CRC using conventional treatments; details the use of CHM in cancer treatments, especially for CRC; explains the integrative management of CRC; and introduces the literature on laboratory studies of CHM for CRC.

**Chapter 3: Research methodology**

This chapter details the research methods and procedures used in each stage of the project. This includes: 1. the procedures for systematic reviews and meta-analyses based on the Cochrane Handbook 5.1.0 (Higgins & Green, 2011); 2. the approach used for the selection of short-lists of potentially effective HMs based on sensitivity analysis of the clinical literature; 3. the methods for the experimental investigation of selected herb(s)/compounds using CRC cell-lines to determine cytotoxicity; 4. the approach to the literature on *in vitro* and *in vivo* studies that investigates the molecular mechanisms of action of selected herb(s)/compounds in the management of CRC.

**Chapter 4: Systematic review of herbal medicines in the management of colorectal cancer**

This chapter reports the results of a systematic review and a series of meta-analyses of the results of RCTs of HMs in the management of CRC. Meta-analysis was carried out into two major groups according to the intervention in the test arm: 1. HM alone versus chemotherapy, or placebo, or no treatment; and 2. HM combined with chemotherapy versus chemotherapy.

**Chapter 5: FOLFOX4 combined with herbal medicine for advanced colorectal cancer**

FOLFOX4 is a well-established and commonly used regimen for advanced CRC (ACRC). This chapter reports the results of a focussed series meta-analyses of RCTs of FOLFOX4 combined with HMs for advanced CRC in a palliative setting.

**Chapter 6: Contributions of specific plants to tumour response, neutropenia, nausea and vomiting**

This chapter provides results of a series of meta-analyses of oxaliplatin-based chemotherapies combined with HM for: 1. tumour response rate (tRR) (42 included studies); 2. neutropenia (29 included studies); and 3. nausea and vomiting (27 included studies). Each meta-analysis includes a sensitivity analysis aimed at selecting short-lists of HMs that are potentially effective for each of these outcomes. It also reviews the experimental literature on the short-listed herbs for tumour response rate, neutropenia and nausea and vomiting.

**Chapter 7: Colorectal cancer cell-line study of matrine from *ku shen* (*Sophora flavescens* root)**

The herb *ku shen*, which is from the root of the plant *Sophora flavescens*, was identified as having potential effects on tumour response in the meta-analysis of clinical trials in Chapter 6. This effect was supported by a review of the experimental literature. Chapter 7 reports the results of a series of experiments on matrine which a major alkaloid from *ku shen*. The experiments include: cell viability assay, cell cycle analysis, and apoptotic assay for matrine and the positive control drug oxaliplatin.

**Chapter 8: The molecular mechanisms of action of *Sophora flavescens* and its constituent compounds**

This chapter reviews and discusses the likely molecular mechanisms of action of the major constituent compounds of *Sophora flavescens*, including matrine, which are relevant to the management of CRC based on the results of *in vitro* and *in vivo* studies.

**Chapter 9: General discussion and directions for future research**

Chapter 9 discusses the approaches taken in this project, including the strengths and weakness, and provides directions for future clinical and/or experimental research into the effects and applications of herbal medicines in CRC management. It includes conclusions relating to each of the research questions.

# Chapter 2. Literature Review of the Epidemiology, Physiology, Aetiology and Management of Colorectal Cancer

## 2.1 Introduction to Chapter 2

In this chapter, the CRC literature was reviewed. This includes the anatomic location and physiological functions of the large intestine; CRC epidemiology, etiology, and tumour immunology; CRC diagnosis and staging systems; current management of CRC in conventional medicine; Chinese herbal medicine (CHM) in the treatment of cancer, especially for CRC; integrative management of CRC; and a summary of laboratory studies of CHMs for CRC.

## 2.2 Large intestine anatomic location and physiological functions

The colon and rectum are generally referred to as the large intestine, which is a muscular tubal organ. The colon is the first and longest portion of the large intestine. It absorbs water and mineral nutrients from food substances, then the residue (faeces) passes to the rectum, which is the final part of the large intestine. The small intestine is attached to the colon at the cecum. The colon is divided into four sections, the small intestine is attached to the ascending colon which then attaches to the transverse colon, the descending colon, and the sigmoid ('S' shaped) colon, which joins to the rectum (American Cancer Society, 2008).

The colon is also divided into the proximal colon and distal colon. The proximal colon includes the cecum, ascending colon, and transverse colon, whereas, the descending colon and sigmoid colon are parts of the distal colon. The proximal colon and distal colon have different embryologic origins. The proximal colon derives from the midgut, and its function involves nutrient absorption, while the distal colon derives from the hindgut, and is mainly for storage (Bufill, 1990).

The wall of the large intestine is structured as five main layers. From the inside lumen outwards are: the mucosa, submucosa, circular muscles, longitudinal muscles and serosa. The mucosa is composed of columnar glandular epithelium and muscles. The glandular epithelium secretes mucus to lubricate the movement of food along the colon and protect the colon from digestive enzymes. Underneath the epithelium is the lamina propria. This contains myofibroblasts, blood vessels, nerves, and immune cells. The crypts of Lieberkuhn are glands found in the epithelial lining of the large intestine, which secrete various enzymes. Mutative crypts initiate colorectal cancer. The submucosa contains nerves, lymphoid tissue, blood vessels and elastic fibres with collagen that construct the shape of the intestine. Longitudinal and circular smooth muscles help with continual peristalsis which moves digested material along and out of the colon. The serosa is a layer of thin loose connective tissue which secretes mucus as a lubricant to prevent friction damage from the intestine rubbing against adjacent organs (Williams & Warwick, 1980).

## 2.3 Colorectal cancer epidemiology review

Demographic and geographic data on CRC are reviewed in this section.

### 2.3.1 General incidence and mortality

According to the WHO 2012 Cancer Report (Ferlay et al., 2014), WHO estimated there were 14.1 million new cancer cases and 8.2 million cancer deaths worldwide in 2012. Among these, CRC accounted for 1.361 million cases, and 694,000 deaths (Table 1). CRC was the third most common cancer worldwide. Compared with the WHO 2008 Cancer Report (Ferlay et al., 2010), CRC increased in both incidence and mortality.

CRC incidence and mortality varies across world regions. Overall, it is ranked as the fourth most common cancer in developed regions and the fifth most common cancer in less developed regions (Ferlay et al., 2014). In China in 2011, CRC was ranked fifth in terms of incidence in men and third in women. It was the fifth leading cause of cancer death in both men and women (Chen et al., 2015). In Australia, Cancer Council figures showed that CRC was the second most common cancer in both sexes. Overall, CRC had the third highest mortality of the cancers. Translated to figures, there were 16,682 new cases in 2017, and 4,114 people died from CRC in 2017 (Australian Institute of Health and Welfare, 2017). Overall, the mortality rate due to CRC in Australia has declined from 28 to 16 per 100,000 between 1991 and 2010 (Australian Institute of Health and Welfare, 2012).

**Table 2.1: Colorectal Cancer Estimated Incidence, Mortality and Prevalence Worldwide in 2012**

| Estimated numbers (thousands)/ Regions | Men | | | Women | | | Both sexes | | |
|---|---|---|---|---|---|---|---|---|---|
| | Cases | Deaths | 5-year prev. | Cases | Deaths | 5-year prev. | Cases | Deaths | 5-year prev. |
| World | 746 | 374 | 1953 | 614 | 320 | 1590 | 1361 | 694 | 3544 |
| More developed regions | 399 | 175 | 1164 | 338 | 158 | 966 | 737 | 333 | 2130 |
| Less developed regions | 347 | 198 | 789 | 276 | 163 | 624 | 624 | 361 | 1414 |
| Africa region | 16 | 11 | 32 | 15 | 11 | 31 | 31 | 22 | 63 |
| Americas region | 125 | 57 | 362 | 121 | 55 | 342 | 246 | 112 | 705 |
| E. Mediterranean region | 18 | 12 | 40 | 15 | 10 | 33 | 33 | 21 | 73 |
| Europe region | 255 | 120 | 686 | 216 | 108 | 573 | 471 | 228 | 1258 |
| South-East Asia region | 68 | 48 | 122 | 52 | 37 | 93 | 120 | 85 | 216 |
| Western Pacific region | 264 | 125 | 711 | 195 | 100 | 518 | 460 | 225 | 1229 |
| IARC membership[1] | 418 | 187 | 1181 | 351 | 167 | 976 | 769 | 353 | 2157 |
| United States of America | 69 | 29 | 214 | 65 | 27 | 199 | 134 | 55 | 413 |
| China | 147 | 79 | 338 | 107 | 60 | 245 | 253 | 139 | 583 |
| India | 37 | 28 | 50 | 27 | 21 | 37 | 64 | 49 | 87 |
| European Union | 193 | 83 | 536 | 152 | 69 | 417 | 345 | 152 | 953 |

Data from International Agency for Research on Cancer (IARC): 24 countries (adapted from Ferlay et al., 2014)
prev.: prevalence

A recent study showed the trend towards increase in the CRC incidence rate has stabilised in the most developed countries, but it was increasing in economic transition countries across Eastern Europe, a

large part of Asia, and some parts of South America. From 1998-2002, the incidence rates in the Czech Republic, Slovakia, and Japan in male populations have exceeded the traditionally high CRC countries such as the United States, Canada, and Australia (Center et al., 2009).

### 2.3.2 **Incidence and mortality by age**

The incidence and mortality rates of colorectal cancer increase with age: 82.1% of new cases and 89.9% of deaths occur in people 55 and older for both genders. The median ages at diagnosis and death due to CRC were 70 for men and 75 years for women (Ries et al., 2008). In Australia, CRC is the most commonly diagnosed cancer and the third most common cause of death from cancer in people aged 65 years or over based on 2013 data (Australian Institute of Health and Welfare, 2017).

### 2.3.3 **Incidence and mortality by gender**

The 2012 global CRC incidence rates in men were estimated at 10.0%, which was higher than in women (9.2%), and the estimated mortality rates for men and women were 8.0% and 9.0% respectively. In men, CRC was the third most common cancer and the second highest cause of death due to cancer in developed regions, whereas, in less developed regions, it was the fifth most common cancer and the fourth cause of death from cancer. In women, CRC was the second most common cancer and the third highest cause of cancer death in developed regions, while it was the fourth most common cancer and the fifth cause of cancer death in less developed regions (Ferlay et al., 2014).

In Australia, the estimated CRC incidence in males was 9,127, and in females was 7,555 in 2013. In men the estimated incidence rate declined from 76 per 100,000 in 1991 to 73 per 100,000 in 2012. The incidence rate for women varied between 51 and 55 per 100,000 from 1991 to 2009 but it was about 20% lower than men during this time period. Overall, the mortality rate has declined from 28 to 16 per 100,000 between 1991 and 2010 (Australian Institute of Health and Welfare, 2012).

The number of cancer deaths from CRC in Australia was estimated at 2,136 for men and 1,978 for women in 2013 (Australian Institute of Health and Welfare, 2017). The mortality rate decreased from 1991 to 2010 for both men (from 34 to 20 per 100,000) and women (from 24 to 13 per 100,000) (Australian Institute of Health and Welfare, 2012). The reasons for the higher risk in men are unknown. It may be associated with the higher rate of obesity, smoking, and drinking in men, and hormonal therapy in women post-menopause or other factors (American Cancer Society, 2008).

### 2.3.4 **Incidence by cancer location**

The ratio of tumour initiation in the colon and rectum is different between high-risk countries and low-risk countries (Muir et al., 1987). In the USA, from 1975 to 2007 the proportion of colon cancer and rectal cancer in both sexes in all races among all colorectal cancers was 41.07 (colon) and 15.84

(rectum) per 100,000 population age-adjusted respectively (Altekruse et al., 2010). In Australia, CRC incidence projections estimated there were 9,753 new cases of colon cancer and 5,027 cases of rectal cancer in 2010 (Australian Institute of Health and Welfare, 2008).

In contrast, the ratio of patients with rectal cancer was larger than colon cancer in China. However, there is a trend towards an increasing proportion of colon cancer and a decreasing proportion of rectal cancer. In the 1980s, the proportions of colon cancer and rectal cancer were 27.4% and 71.2%, but that changed to 33.1% and 66.7% respectively in the 1990s (Ming & Gu, 2005).

In addition, many investigators worldwide have reported a shift in the location of CRC toward the proximal colon (Rhodes et al., 1977; Mamazza & Gordon, 1982; Bufill, 1990; Mensink et al., 2002; Takada et al., 2002; Ming & Gu, 2005). Tumours in the proximal colon and distal colon are different, in terms of embryologic origin, genetic changes, molecular and clinical characteristics, and biologic identity (Distler & Holt, 1997; Gervaz et al., 2001). Proximal tumours and distal tumours appear to develop through different pathogenesis mechanisms. Proximal tumours are more genetically stable and the possible genesis appears to be from the same mechanisms as for inherited nonpolyposis colon cancer. In contrast, distal tumours are associated with genetic instability and may share the same mechanisms with polyposis-associated colorectal cancer syndromes (Bufill, 1990).

### 2.3.5 **Incidence by ethnicity**

CRC incidence and/or morbidity also vary among ethnic populations within a country. In the USA, African American men and women had the highest CRC incidence and mortality. Compared with white Americans, African Americans had a 20% higher incidence rate and 45% higher mortality. These differences may reflect socio-economic differences – African Americans generally have higher amounts of fat in their diet, have lower physical activity, and have an increased obesity rate; while white Americans generally have had greater access to and use of recommended screening tests as well as proper treatments (American Cancer Society, 2008).

Similar phenomena have been evident in Singapore and Israel, where ethnic Chinese had higher CRC incidence compared to Malays, and the Jewish population had higher CRC incidence compared to the non-Jewish population. However, in both countries there has been a reversal of these trends in incidence rates over the last two decades, as Malays and non-Jews have almost doubled their incidence rates of CRC, while ethnic Chinese and the Jewish populations have only experienced a 10-30% increase (Center et al., 2009).

Even within the same ethnicity, populations living in different regions of the same country show variation in the incidence rates of CRC. An epidemiological study of four regions of Guangdong province in China found that the Pearl River delta area, a highly developed region, had the highest

incidence of CRC compared with other regions (Xu et al., 2010). In Japan, records from registries demonstrated that in the male population, the incidence rate has increased more than 90% in Yamagata and Miyagi prefectures (both rural areas) during the last two decades; in comparison, there was only a 35% increase in Osaka prefecture (an urban area) (Center et al., 2009). This suggests that CRC has been increasing faster in rural areas along with the modernisation of lifestyle.

McMichael et al. (1980) have studied migrant epidemiology in Australia for decades. From the 1960s to the 1980s, migrants from Europe (England, Scotland, Ireland, Poland, Yugoslavia, Greece, and Italy) had showed increasing CRC incidence with increasing duration of stay in Australia (McMichael et al., 1980). However, the migrant sub-group from southern Europe (Italy, Greece, Yugoslavia, and Malta) whose native risk of colon cancer was about half that of the Australian population, have had a consistently lower rate of incidence in comparison with their counterpart migrants over the same duration. This was probably related to this sub-group's adherence to their Mediterranean dietary habits (McMichael & Giles, 1988).

Studies have also demonstrated that Chinese and Japanese migrants increased their risks of CRC in the USA, and that this change was associated with a lifestyle lacking in physical activity and a diet rich in saturated fat compared with their compatriots in China and Japan (Whittemore et al., 1990; Maskarines & Noh, 2004).

Overall, the demographic and geographic evidence show that high-risk ethnicities or countries continue to lead the rate of incidence/mortality worldwide for CRC, but that there has been a slowing of the trend towards rising CRC incidence in these groups. On the other hand, the rates are sharply increasing in some former low risk ethnicities that migrated to the high CRC risk countries or in countries that are experiencing economic transition. This suggests that differences in genetics and culture, as well as lifestyle and environmental factors all influence the etiology and incidence of CRC. In addition, utilization of modern technology for screening and treatment in the precancerous stages may have contributed to a slowdown in the trend towards increasing incidence of CRC in high risk countries.

### 2.3.6 **Prevalence and burden**

GLOBOCAN 2012 estimated there were 3,544,000 people who had been diagnosed with CRC and had survived for 5 years or more in 2008 (Bray et al., 2013). In USA, 64.9% of people who had been diagnosed with CRC had survived 5 years or more in 2005-2011 and it was estimated that in 2012, 1,168,929 people who has been diagnosed with CRC were still alive (Howlader et al., 2015).

In Australia, the 5 years prevalence of CRC at the end of 2009 was 48,596 people (men: 26,700; women: 21,896) (Australian Institute of Health and Welfare, 2014). The total burden of disease and

injury was expressed as the disability-adjusted life year (DALY). The burden of CRC was estimated to be 38,800 DALYs for men and 30,700 DALYs for women in 2012 (Australian Institute of Health and Welfare, 2012). The direct cost of CRC was $427.35 million, which represented 9.4% of all cancer expenditure in 2008-09 (Australian Institute of Health and Welfare, 2013). Of all the cancers, in 2011 CRC accounted for the second highest cancer burden of disease in males and was the third highest in females (Australian Institute of Health and Welfare, 2017).

## 2.4 Risk factors for colorectal cancer

Besides increased risk by age, a number of lifestyle-related factors (excessive consumption of red meat, diet high in animal fats, lack of exercise, alcohol, smoking etc), obesity, hereditary factors and chronic inflammatory bowel diseases are commonly thought to be factors that initiate and promote CRC. In contrast, consumption of vegetables and fruit, exercise, vitamin D, calcium, folic acid, non-steroidal anti-inflammatory drugs (NSAIDs) and hormone replacement therapy (HRT) for postmenopausal women may reduce the risk of CRC (American Cancer Society, 2008).

### 2.4.1 **Diet**

A review of epidemiological studies found that high consumption of red meats, saturated fat, and processed meat were all associated with increased risk of CRC. Risk was correlated with ingestion of fresh red meat (17% increased risk for 100 g/day increase in consumption) and processed meat (18% increase for 50 g/day increase in consumption) (Chan et al., 2011). In particular, long-term high consumption of red and processed meat appears to increase the risk of carcinoma of the distal colon and rectum (Chao et al., 2005). Several carcinogenic factors that are generated during processing, cooking, and digesting may be associated with increased risk, for instance, high fecal bile acids from high-fat diets, heterocyclic amines and polycyclic aromatic hydrocarbons from high temperature cooking, N-nitroso compounds and heme iron in red meat (Santarelli et al., 2008).

Information from studies of the relation between the intake of fruits, vegetables and fibre, and CRC risk reduction are inconsistent. Several reports from prospective cohort studies and reviews found a diet with a higher intake of fruits, vegetables and grain fibre may reduce CRC risk (Fung et al., 2010; Gonzalez & Riboli, 201; Kirkegaard et al., 2010; Miller et al., 2010). Higher intake of wholegrain products (mainly insoluble, non-degradable fibre) decreased the chance of CRC in men, but not in women (Egeberg et al., 2010). However, a review found there was no strong evidence that supported the notion that high intake of fibre, including fruits and vegetables, has an influence on CRC risk (Doyle, 2007). The differences in these findings may be associated with study design, definition of which fruits, vegetables and other foods the study focussed on and the observation duration. Nevertheless, a balanced diet with more vegetables and fruits that contain nutrients such as carotenoids, folate, and ascorbate, and substances with anticarcinogenic properties, such as phenols,

flavonoids, isothiocyanates, and indoles are of benefit to health in general (Wattenberg, 1978; Steinmetz & Potter, 1991).

### 2.4.2 **Vitamin D, Calcium, Folate, Vitamin B6**

Epidemiological investigations have found that there was an inverse relationship between intake of vitamin D, calcium, folate vitamin $B_6$ and risk of CRC. It was estimated that every 100 microg/day increase in total folate intake will result in a 2% risk reduction (95% CI 0-3%) in colon cancer, based on a pooled analysis of 13 prospective cohort studies (Kim et al., 2010). A prospective investigation of 10 European countries on diet and cancer prevention that involved 519,978 participants (366,521 women and 153,457 men), who were mostly aged 35-70 years, found that high intake of dietary fibre, fish, calcium, and vitamin D were correlated with reduction of CRC risk (Gonzalez & Riboli, 2010). A randomised controlled trial suggested vitamin D and calcium probably act together to reduce risk of colorectal adenoma recurrence (Grau et al., 2003). Pyridoxal 5'-phosphate (PLP) is an active form of vitamin $B_6$. A meta-analysis of prospective studies that included nine studies on vitamin $B_6$ intake and 4 studies on blood PLP levels found up to 49% reduction in risk of CRC for every 100-pmol/mL increase in blood PLP levels (RR, 0.51; 95% CI, 0.38-0.69) (Larsson et al., 2010).

### 2.4.3 **Physical Activity, Body Mass Index (BMI), Smoking, Alcohol**

Evidence from various studies indicated physical inactivity, obesity, smoking, and high alcohol intake all increased the risk of CRC.

A meta-analysis of 19 cohort studies found physical activities could significantly reduce colon cancer in men, with occupational activities (RR=0.79, 95% CI 0.72–0.87) showing similar reductions to recreational activities (RR=0.78, 95% CI 0.68–0.91). Only recreational activities reduced colon cancer for women (RR=0.71, 95% CI 0.57–0.88). No effect on rectal cancer in either sex was seen in this study (Samad et al., 2005). The American Cancer Society recommends that people should engage in moderate activity for at least 30 minutes each day, 5 days per week, and it is even better if a person takes 45 to 60 minutes of intentional physical activity (American Cancer Society, 2008). Prolonged sitting, including television viewing and sitting at work has been linked to an increased risk of CRC with an increase in sitting time of two hours a day showing significant increases (Ma et al., 2017; Milne et al., 2017).

Larsson and Wolk (2007) conducted a meta-analysis of 30 prospective studies that investigated the correlation between obesity and CRC. The available evidence showed a 5-unit increase in body mass index (BMI; in $kg/m^2$) correlated with a 30% increase in risk of colon cancer and a 12% increase in risk of rectal cancer in men. There was a 12% increase in risk of colon cancer in women but no effect on risk of rectal cancer. There was also a statistically significant positive correlation between both

waist circumference (per 10-cm increase) and waist-hip-ratio (per 0.1-unit increase) and colon cancer risk in men and women (Larsson & Wolk, 2007).

A meta-analysis by Tsoi et al. (2009) which included a total of 1,463,796 participants who were involved in 28 prospective cohorts from America, Europe, and Asia, with a median follow-up of 13 years (range, 4-30 years) concluded that smoking was associated with a significantly increased risk of CRC. The authors found there was a modest but significantly higher risk of CRC in current smokers than never smokers (RR 1.20; 95% CI [1.10, 1.30]), and the risk of CRC among smoking men (RR 1.38; 95% CI [1.22, 1.56]) was significantly higher than in smoking women (RR 1.06; 95% CI [0.95, 1.19]). The risk of rectal cancer was associated more with smoking than colon cancer (RR 1.36; 95% CI [1.15, 1.61]). Former smokers still demonstrated a higher risk of CRC than never smokers. This increased risk of CRC was dose-related. Higher consumption of cigarettes, in terms of the number of cigarettes per day, or the number of years of smoking, or the number of total pack-years of cigarette smoking, was associated with a higher risk of CRC (Tsoi et al., 2009).

Based on evidence from the epidemical studies, the International Agency for Research on Cancer (IARC), has added CRC to the list of alcohol-related malignancies (Baan et al., 2007). Alcohol consumption of 30 g/day or more (approximately > or =2 drinks/day) was significantly associated with increased risk of CRC compared to non-drinkers (Cho et al., 2004).

### 2.4.4 **Heredity and medical history**

It was estimated that 5% to 10% of CRC patients have inherited gene alterations that predispose them to carcinogenesis. Familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC) are well known inherited diseases associated with CRC. Most of those with FAP and HNPCC will develop CRC by the age of 40 (Lynch & de la Chapelle, 2003).

People who have a family history of CRC, especially in those who are closely related, are at higher risk of having CRC, and 20% of CRC patients have a family history of the disease (Lynch & de la Chapelle, 2003). The cohort of high CRC risk also includes people who have had one or more adenomatous polyps, or chronic inflammatory bowel disease such as ulcerative colitis and Crohn's disease over a long duration (Schatzkin et al., 1994; Bernstein et al., 2001).

## 2.5 Chemopreventive medicines

Nonsteroidal anti-inflammatory drugs (NSAIDS) have been investigated for their chemoprevention of CRC. Pooled-data from RCTs found that aspirin 81-325 mg/day had a statistically significant effect with 21% reduction in the relative risk of adenoma recurrence in patients with a history of adenomas. The data also showed aspirin 300-1500 mg/day had a 26% reduction in CRC incidence over 23 years

of follow-up, but there was no effect in the first 10 years. However, high-dose aspirin has increased risk of gastrointestinal adverse reactions including peptic ulcers, gastrointestinal bleeding and hemorrhagic stroke. Celecoxib, a COX-2 inhibitor, at a dose of 400 mg/day showed a 34% decrease in the risk of adenoma recurrence in patients with a history of adenomas as well as a 55% decrease in the risk of advanced adenoma (generally defined as adenoma ≥ 1 cm in diameter, villous or tubulovillous adenoma, adenoma with severe dysplasia, or CRC). But given that celecoxib increased the risks of hypertension and renal toxicity, it is important to consider the risk-benefit effect before making a recommendation for using NSAIDS for chemoprevention (Cooper et al., 2010). The chemopreventive mechanisms of NSAIDs for colorectal neoplasia may be associated with restoration of apoptosis in APC-deficient cells and inhibition of angiogenesis (Thun et al., 2002).

Epidemiological studies have found that the CRC morbidity and mortality in men was higher than in women. Postmenopausal hormone therapy (PHT) may contribute to this difference. A study by Grodstein et al. (1999) found there was a 20% reduction in colon cancer and a 19% reduction in rectal cancer in women who had used postmenopausal hormones in the past compared with never users. In women who were currently using PHT there was a 34% decrease in the risk of CRC compared to those who had never used. However, there was no correlation between the duration of use of PHT and reduction of CRC risk (Grodstein et al., 1999).

## 2.6 Bowel cancer screening programs

CRC is a curable disease if detected at early stage. Adenoma polyps at each stage are detectable by an endoscope or on x-ray, and are removable by surgery. This can greatly prevent CRC onset. In the USA, the national Polyp Study found that periodic colonoscopy screening could prevent 76% to 90% of colon cancers (Winawer et al., 1993). People who have a strong family history of FAP or HNPCC or colorectal cancer or other risk factors for adenoma polyps or cancer should start screening at a younger age and/or get screened more frequently.

Based on data from four RCTs, a Cochrane review found that CRC screening programs in average-risk adults aged 50 years or older that used the modality of fecal occult blood testing (FOBT), including guaiac FOBT (gFOBT) and immunochemical FOBT (iFOBT) reduced the mortality of CRC by 16% (RR 0.84, 95% CI [0.78, 0.90]), and potentially decreased CRC incidence by removal of adenomas (Hewitson et al., 2007).

CT colonography (CTC) screening was of comparable sensitivity to colonoscopy for CRC and large adenomas (10 mm or larger). But it was of lower sensitivity for smaller polyps (6 mm or larger). Colonoscopy is the most accurate screening test for CRC. However, serious harms due to colonoscopy are about ten times more common (3.1 per 1,000 procedures) than harms due to fecal tests (3.4 per 10,000 procedures) (Whitlock et al., 2008).

The Australian Bowel Cancer Screening Pilot Program followed people aged 55 to 74 years in three cities (Mackay, Adelaide and Melbourne) between 6 November 2002 and 1 October 2004. The program used FOBT with follow-up colonoscopy for people with FOBT positive results. 45.4% of invitees (25,840 out of 56,907 invitations) responded by returning a completed FOBT. The positive predictive value that was defined by the authors as 'the proportion of FOBTs with cancers and adenomas detected out of all positive FOBTs that are followed up with a colonoscopy' was 19.4% for cancers and advanced adenomas across both tests (Stevenson & Hotstone, 2005). A recent study reported that the Australian National Bowel Cancer Screening Program (NBCSP) significantly reduced risk of CRC recurrence and death compared with patients with a symptomatic presentation (Ananda et al., 2016). Overall, an appropriate bowel cancer screening program can reduce CRC morbidity and mortality.

Clinical practice guidelines for the prevention, early detection and management of colorectal cancer were approved by the National Health and Medical Research Council (NHMRC) on 27 October 2017 (https://www.nhmrc.gov.au/guidelines-publications/cp62).

## 2.7 Etiology of colorectal cancer

Although the exact causes of CRC are unclear, epidemiological studies have demonstrated that environmental factors, lifestyle, hereditary factors and chronic inflammatory bowel diseases are common causes that initiate and promote CRC.

The formation of CRC has a long progressive duration associated with the accumulation of genetic mutations. Three main genetic pathways of CRC have been proposed:

1. The Chromosomal Instable (CSI) pathway: caused by accumulation of mutations in tumour suppressor genes and specific oncogenes (e.g. APC, KRAS, PIK3CA, BRAF, SMAD4, TP53, etc) which result in chromosomal abnormalities characterised by loss of heterozygosity. In 70% of CRC and FAP, carcinogenesis is via this pathway;
2. The Microsatellite Instability (MSI) pathway: initiated by mutation in mismatch repair (MMR) genes, including MLH1 and MSH. The mutation of MMR genes that is characterised by hypermethylation which leads to deficiency of DNA repair. MSI is found in HNPCC and 15% of sporadic CRC;
3. The CpG Island Methylation (CIMP) pathway: cytosine methylation inside genes that can silence the genes was found in most MIS colon tumours. BRAF mutations and MLH1 methylation, and KRAS mutations were related to CIMP.

However, the formation of CRC could be the result of multi-pathways (Bogaert & Prenen, 2014).

Most CRC is believed to initiate from adenoma and progress to carcinoma. The characteristics of an early adenoma are small in size and mildly dysplastic with a tubular architecture, while advanced adenoma will be large in size, highly dysplastic, and with a villous architecture (Hayat, 2009).

## 2.8 Tumour immunology

Tumour immunology specifically studies the immune response to tumours in which the cell-mediated immunity (CMI) response plays a major role. The immune responses express the dual actions of host-protection (tumour immunosurveillance) and tumour-sculpting (tumour immunoediting) on the processes of tumour development (Dunn et al., 2002). In this section the functions of the human immune system and its role in the immune response to tumours is briefly reviewed. In addition, since surgical procedures are important in CRC treatment, the immunosuppression induced by surgical procedures was also included in this section.

### 2.8.1 **Outline of the human immune system**

The human immune system consists of innate immunity and adaptive immunity. Both these types have cell-mediated and humoral components. The CMI response is a process that activates cytotoxic cells to cause death by apoptosis, and releases cytokines in response to antigens. Humoral immune response involves antibody secretion by immune cells, mainly B-type lymphocytes, and binding to antigens. The binding complex of antibody and antigen is ingested by phagocytic cells, including macrophages, neutrophils, and dendritic cells (DC), and the cell is destroyed (Janeway et al., 2001).

Innate immunity is the body's first line of defence. It involves the skin and mucous membranes as physical barriers, as well as innate immune cells and molecules (Scholl & Babu, 2012). The innate immunity is able to respond rapidly when pathogens or damaged cells are identified by pattern recognition receptors (PRRs) that are germline-encoded and expressed by innate immune cells. These cells include, phagocytes, mast cells, eosinophils, basophils, and natural killer (NK) cells (Medzhitov, 2007).

Adaptive immunity is activated by the innate response, and requires specific antigen presentation. In this process, antigens are caught by innate immune cells and marked to enable their recognition by lymphocytes, which are special types of leukocytes. The lymphocytes involved in adaptive immunity are classified as T cells and B cells. The T cell is a cell-mediated response cell, whereas the B cell is a humoral response cell (Janeway et al., 2001).

Both B cells and T cells clonally express a large repertoire of antigen receptor molecules that recognise specific antigens. When primitive T and B cells come across antigens in lymph nodes or the spleen, they differentiate into various effector cells (Vivier et al., 2011). These effector cells are

recruited to the site of the target, mediated by cytokines and chemokines that are secreted from the innate immune system (Medzhitov, 2007).

T cells can recognise antigens when they bind to the major histocompatibility complex (MHC), a cell surface molecule that mediates interactions of leukocytes. In humans, MHC is also called human leukocyte antigen (HLA) (Kuo & Hood, 1987). There are two sub-groups of MHC — class I and class II. A T cell that recognises antigens combined to a Class I MHC moleculeis called a T killer cell; and a T cell that recognises antigens combined to Class II MHC molecules is referred to as a T helper cell. T cell receptor (TCR) is a molecule on the surface of T cells that recognises antigens bound to MHC molecules. When a T cell approaches a specific antigen bound to a MHC, its TCR recognises and binds to the MHC assisted by a co-receptor called cluster of differentiation (CD), a transmembrane glycoprotein bound to MHC, so the T cell is activated and exerts its immune function (Medzhitov, 2007).

The anti-tumour immune responses are primarily dominated by cell-mediated immunity (CMI). NK, CD4+ and CD8+ T cells are the main types of lymphocytes in cell-mediated immunity and play a central role in the induction of efficient immune responses against tumours. There are two major cytotoxic mechanisms involved: 1. causing target cell lysis by releasing cytolytic granules such as perforin and granzymes; and 2. inducing cell apoptosis by expressing death-receptor ligands such as tumour necrosis factor–related apoptosis-inducing ligand (TRAIL), or Fas Ligand (FasL) (Dunn et al., 2002).

The CD4+ molecule is expressed on the surface of T helper cells (Miceli et al., 1993). CD4+ is a co-receptor that aids binding of TCR to Class II MHC. These cells do not kill target cells directly, but they are important to switch a B cell's production of antibodies from one class to another, to activate cytotoxic T cells, and for optimising the anti-bacterial activity of phagocytes such as macrophages (Janeway, 2005).

There are two major sub-types of T helper cells, referred to as Type 1 and Type 2 T helper cells. Type 1 T helper cells secrete gamma-interferon (IFN-γ), interleukin (IL)-2, and IL-12, and stimulate the cellular immune system to promote the killing effect of macrophages and the proliferation of cytotoxic $CD8^+$ T cells. Type 2 T helper cells secrete IL-4, IL-5, and IL-10, and stimulate the humoral immune system to promote proliferation of B cells and antibody production (Kidd, 2003). Each sub-type of T helper cell acts to preserve its own response by producing cytokines to inhibit another sub-type of T helper cell. The balance of Type 1 T helper cells / Type 2 T helper cells maintains the homeostasis of immunity (Abbas et al., 1996).

Since CD4+ T helper cells play an important role in optimizing CD8+ T cell activation, an adequate number of CD4+ T helper cells are required to sustain the cytotoxic function against tumour cells of CD8+ T cells (Gerloni & Zanetti, 2005).

Besides T helper cells, there is another subset of CD4+ T cells, the regulatory T cells (Treg cells). Once activated, T helper cells mediate the activation of CD8+T cells. Conversely, Treg cells attenuate the immune reaction to maintain immunotolerance and suppress autoreactive T cells (Buckner, 2010).

CD8+ is a co-receptor that aids TCR and is specific for Class I MHC. The CD8+ molecule is commonly expressed on the surface of killer T cells, but can express on NK cells, cortical thymocytes, and dendritic cells as well (Gao & Jacksen, 2000). The T cell that expresses CD8+ surface protein is called a CD8+ T cell. When an activated CD8+ T cell binds to a target cell that expresses Class I MHC molecule, it kills the target cell by releasing cytotoxins or granulysin, or induces the target cell to undergo apoptosis by the upregulation of the FasL pathway (Harty et al., 2000; Radoja et al., 2006).

NK cells are a type of cytotoxic lymphocyte that represents 5-20% of peripheral lymphocytes. Small portions of NK cells are also present in the thymus, lymph nodes, and bone marrow. In humans, NK cells bear a CD3-negative mark. NK cells also express CD56, CD16 and CD94 (Arina et al., 2007). NK cells play a critical role in the innate immune response, and can kill infected cells or transformed cells rapidly without previous antigen exposure or MHC binding. Studies have also found that NK cells can act as part of the adaptive immune response (Wallace & Smyth, 2005).

There are several pathways linked to the cytotoxicity of NK cells, including: 1. direct cytotoxic activity without initiation by any specific antigen recognition mechanisms; 2. antibody-dependent cell-mediated cytotoxicity (ADCC), in which the target cell with antigen-specific antibody expression activates NK cells through the CD16 receptor, causing release of cytolytic granules, as well as secretion of IFNγ, Tumour necrosis factor (TNF) α, or T cell recruiting chemokines which result in the apoptosis or lysis of the target cell; 3. secretion of T helper 1-type cytokines such as IFNγ and TNFα. Macrophages are activated by IFNγ and exert phagocytosis and lysis, whereas TNFα promotes direct killing of tumour cells by NK cells; and 4. NK cell interactions with dendritic cells, e.g. the cytokines IL-12 and IL-2 that are secreted by mature dendritic cells, induce the secretion of IFNγ by NK cells. Meanwhile the activated NK cells secrete T helper 1-type cytokines, such as IFNγ and TNFα, that promote the maturation and activation of dendritic cells (Terunuma et al., 2008).

### 2.8.2 **Tumour immunosurveillance**

The hypothesis of tumour immunosurveillance was proposed by Macfarlane Burnet and Lewis Thomas (Burnet, 1967). The major concept of this hypothesis is that the immune system, especially

the cell-mediated immunity (CMI), is able to detect and eliminate a tumour in its formation stage. This immunity involves both the innate and adaptive immune systems (Dunn et al., 2002).

Tumour-specific T cells (CD8+ cytotoxic T cells and CD4+ helper T cells) mediate immunity by detecting and eliminating tumour cells. They rely on recognition of the tumour associated antigen (TAA) that is presented by MHC-class molecules on the surface of tumour cells. Types of TAA are: 1. neoantigen, 2. self-antigen, and 3. modified self-antigen. Most tumours express self-antigen and modified self-antigen. Inactive T cells are activated by dendritic cells that have encountered dead tumour cells and present TAA with MHC class molecules to T cells. Thus, the T cells are activated and exert their protective effects (Topfer et al., 2011).

Dunn et al. (2002) summarised the process of immune surveillance in four phases: 1. the innate immune system (NKT, NK, γδ T cells, macrophages and dendritic cells) is alert to the formation of tumour cells and produces IFN-γ; 2. IFN-γ induces a series of innate immune system responses, such as releasing angiostatic chemokines to block new vessel growth in the forming tumour, recruiting innate immune effector cells to the tumour site and exerting cytotoxic effects. Tumour cells and debris of dead tumour cells are ingested by dendritic cells and are transported to the draining lymph node; 3. the tumour is continuously surveilled by innate immune effector cells, meanwhile, CD4+ and CD8+ T cells marked with specific tumour antigens are developing; 4. the matured CD4+ and CD8+ T cells are recruited to the tumour site, where they recognise and kill tumour cells that are bearing specific tumour antigens (Dunn et al., 2002).

Extensive studies support the tumour immune surveillance hypothesis. Laboratory studies have found that animals in which different components of immunity were deleted were more susceptible to tumour formation induced by chemicals or growth of transplanted tumours than wild-type controls (Dighe et al., 1994; van de Broek et al., 1996; Kaplan et al., 1998; Girardi et al., 2001; Shankaran et al., 2001; Street et al., 2001). In human studies, researchers found that organ transplant patients who used immune-suppressants, people who were inherently immune-deficient, and AIDS patients were more at risk of cancer development (Buell et al., 2005; Goedert & Bower, 2012; Kubica & Brewer, 2012). In addition, researchers have found that cancer patients who presented with tumour infiltrating lymphocytes (TIL) in tumour sites had better survival rates than those with an absence of TIL (Clemente et al., 1996; Naito et al., 1998; Kawai et al., 2008).

Recently, immunotherapies have shown promising results. Ipilimumab, the human antibody inhibiting CTLA-4, improved overall survival (OS) of patients with unresectable stage III or stage IV melanoma in a phase III trial. Thus, ipilimumab was approved by the FDA in 2011. Ipilimumab also demonstrated benefit in elevating treatment response in patients with metastatic renal cell carcinoma and patients with metastatic prostate cancer (Postow et al., 2011). This evidence from both animal and

human studies indicates that the immune system plays important roles in tumour formation, prognosis and treatments.

### 2.8.3 **Tumour immunoediting**

Although the immune system exerts host-protective functions in tumour formation, researchers have also found tumour selection and tumour escape effects which can explain why tumours can occur in immunocompetent individuals (Urban et al., 1982; Engel et al., 1997; Iwashiro et al., 2001). Tumour immunoediting is a concept that has been incorporated into tumour immunosurveillance theory to further describe the immune system's dual host-protecting and tumour-sculpting actions on the processes of tumour development. It comprises three processes: elimination, equilibrium and escape. The elimination process is inherent in the concept of tumour immune surveillance discussed earlier. The equilibrium process is characteristic of dynamic interactions of the host immune system and tumour cell variants that survived the elimination process. In this process, the host immune system continually selects and kills immunologically sensitive tumour cell variants, meanwhile, due to the genetic instability of tumour cells, they rapidly produce new mutated variants that allow them increased resistance to the host's immune system killing ability. The escape process refers to tumour cell variants that survive detection and elimination by the host immune system through various mutations that gain them immunological insensitivity. This results in clinical malignant disease (Dunn et al., 2002).

Based on laboratory and clinical studies, a review by Topfer et al. (2011) proposed the following five mechanisms of tumour escape from immune surveillance, mainly from T cells:

1. Recognition of tumours by activated T cells is defective due to inhibition of MHC I class molecule expression. There are several pathways that have been observed in various cancer studies: 1. mutations or deletions of heterozygosity on chromosome 6p21 of human lymphocyte antigen (HLA) (Maleno et al., 2002; Maleno et al., 2004); 2. mutations of β2-microglobulin (β2m) on MHC I class molecules damage the transport of MHC class I molecules to the cell surface (Chen et al., 1996); 3. hyper-methylated DNA of HLA class I causing transcriptional inactivation (Nie et al., 2001); 4. down-regulation of HLA mRNA transcription by inhibited expression of locus-specific transcription factors such as CCAAT (nucleotides) and Sp1-like sequence (Soong & Hui, 1992); 5. suppressing expression of transporters associated with antigen processing 1 and 2 (TAP1 and TAP2) and the proteasome subunits of low-molecular mass polypeptides 2 and 7 (LMP2 and LMP7) (Korkolopoulou et al., 1996; Seliger et al., 1998); and 6. impairment of IFN-γ signalling or deficiencies in interferon regulatory factor-1 (IRF-1) resulting in reduced MHC class I expression (White et al., 1996; Hobart et al., 1997; Respa et al., 2011). In addition, inhibition of cell adhesion molecule expression helps cancer cells escape host immune attack (Madhavan et al., 2002).

2. T Cell-mediated killing activity is resisted by cancer cells. Cancer cells can resist apoptosis via a number of pathways: 1. T cells can induce cancer cell apoptosis by the calcium-dependent 'perforin /granzyme pathway' but cancer cells can resist T cell attack by expression of serine protease inhibitor PI-9/SPI-6 that blocks apoptosis via the perforin /granzyme pathway (Medema et al., 2001); 2. the TRAIL-mediated apoptosis pathway can be blocked by cancer cells' expression of the anti-apoptotic regulator FLICE inhibitory protein (c-FLIP) (Griffith et al., 1998; Dutton et al., 2006); 3. via the calcium-independent 'death receptor pathway' cancer cells can down-regulate or inactivate death receptors CD95/FAS and TRAIL through oncogenic Ras expression (Peli et al., 1999 ), somatic mutations in Fas (Apo-1/CD95) gene (Park et al., 2001) and/or mutations of TRAIL-R1 and -R2 genes) (Shin et al., 2001); also, 4. cancer cells can release soluble decoy receptors that competitively inhibit death receptor or Fas ligand signaling (Pitti et al., 1998).
3. Suppression of cytotoxic effects of T cells. Activated T Cells are suppressed by tumour cells expressing inhibitory co-stimulatory B7 molecules, CD80 (B7.1), CD86 (B7.2), and B7-homolog 1 (B7-H1), which are types of extracellular membrane proteins that can bind to T cell inhibitory co-receptors of cytotoxic T lymphocyte antigen-4 (CTLA-4) and program death-1 (PD1) in the surface of the T cell to decrease T cell activity (Zou & Chen, 2008). In addition, tumour growth factor β (TGF-β) suppresses CD8+ T cells' cytotoxic effect in the tumour microenvironment (di Bari et al., 2009). Increased cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2) expression is associated with reduction of T cells and dendritic cells in cancer patients (Pockaj et al., 2004).
4. Active T cells are counter attacked by tumour cells. Active T cells are regulated by the process of T cell activation-induced cell death (AICD) that is mainly mediated by CD95/FAS death receptor. Tumour cells were found to express Fas ligand (FasL) that may induce apoptosis of antigen-specific T cells. This proposal was supported by an animal study that demonstrated there was delayed growth of FasL positive melanoma cells in lpr-positive mice (lpr: lymphoproliferation gene; which encodes mutated non-functional CD95/FAS in these mice) when compared to wild-type controls (Hahne et al., 1996). However, this theory is in contradiction to a study that found FasL can also induce pro-inflammatory and anti-tumour effects *in vivo* (Arai et al., 1997).
5. Promoting expression of immunosuppressive FoxP3+, CD4+CD25+Tregs and myeloid-derived suppressor cells (MDSCs). CD4+ CD25+ Tregs expression of the transcription factor forkhead box P3 (FoxP3) is important to maintain immune homeostatic peripheral tolerance (Thompson & Powrie, 2004). The expression of CD4+ CD25+ Tregs is higher in cancer tissues and peripheral blood of cancer patients than in healthy cohorts. Over-expression of FoxP3+, CD4+ CD25+ Tregs suppressed tumour-specific T cell immunity in cancer patents, and was correlated with poorer survival (Curiel et al., 2004). MDSCs are immature myeloid cells, including macrophages and DCs. MDSCs over-expression in the peripheral blood of

cancer patients is associated with DC dysfunction in immune surveillance. MDSCs also directly suppress tumour-specific T cell response (Almand et al., 2001).

### 2.8.4 **Immunosuppression induced by surgical procedures**

Surgery is the primary treatment for CRC, but surgical procedures can suppress the immune system, especially the cell-mediated immunity (CMI) (Ogawa et al., 2000). After reviewing animal studies and human studies, Neeman (2012) proposed several surgery-related factors that promote cancer metastasis:

1. surgical procedures increase the chance of cancer cells entering into the blood and lymphatic circulation by manipulating the tumour and its vasculature;
2. surgery promotes cancer cell proliferation and resistance to apoptosis;
3. surgery enhances residual cancer cell invasion by up-regulating matrix metalloproteinases (MMP) and adhesion-molecules on cancer cells;
4. surgery reduces the expression of tumour-related anti-angiogenic factors (e.g. angiostatin and endostatin), and promotes the expression of pro-angiogenic factors (e.g. VEGF);
5. surgical wounds stimulate secretion of growth factors (e.g. EGF) that promote local and distant recurrence;
6. finally, the most important factor is surgery suppresses cell-mediated immunity (CMI) that is associated with the neuroendocrine system, especially the hypothalamo-pituitary-adrenal axis and the autonomic nervous system through increasing secretion of immune suppressing hormones (e.g. cortisol), decreasing the numbers and activity of NK, Th1 and CTL cells, and reducing the pro-CMI type-1 cytokines (e.g. IL-12 and IFN-γ).

As a consequence of these factors, residual cancer cells in the peripheral blood system could potentially escape the host's immune surveillance. It may improve the prognosis if the cell-mediated immunity (CMI) is quickly restored from the immusuppressed state in the peri-operative period (Neeman & Ben-Eliyahu, 2012).

However, researchers have also found that reducing tumour burden by surgical resction could reduce tumoural immunosuppressive status, and restore patients' cell-mediated immunity (CMI) (Wang et al., 1999). Thus, surgical resection is a double-edged sword. It is important to consider the risk-benefit effect before making a recommendation for patients.

## 2.9 Diagnosis and management of colorectal cancer

In this section the literature on CRC diagnosis and staging systems, current management of CRC in conventional medicine, the use of CHM in cancer treatment, especially for CRC, the potential anti-cancer mechanisms of HMs, and integrative management of CRC were reviewed.

### 2.9.1 **Symptoms and diagnosis**

Early stage CRC often has no signs and symptoms. For people, especially those over 50 years old with symptoms of progressive constipation, blood in the stool, changing shape of stool, loss of appetite, unexplained weight loss or unexplained anaemia are warning signs (Astin et al., 2011). The diagnosis of CRC relies on pathological tests of samples taken from the suspicious area of the large intestine often via colonoscopy or sigmoidoscopy. The cancer cell type and grade are determined by pathology tests. A CT scan is often used to determine the extent of CRC. Other tests such as MRI or PET scan can be used if necessary (Cunningham et al., 2010).

### 2.9.2 **Staging**

The staging of CRC is critical to predict prognosis and determine treatment in CRC management. It describes the extent to which the CRC has developed, in terms of the size of the tumour, how deep (how many layers) into the wall of the large intestine it has penetrated, and whether it has invaded adjacent organs or metastasized to lymph nodes (if so, how many) and to distant organs.

CRC staging initially used the Dukes stage system, which was invented by Dr. Cuthbert Dukes in 1932 (Dukes, 1932), and has since been developed into several modified versions (Astler & Coller, 1954). The Dukes system involved four stages from A (local tumour) to D (metastasis). The current American Joint Committee on Cancer (AJCC) staging system classifies CRC from stage 0 to stage IV with stage 0 indicating no carcinoma and stage IV indicating the most severe stage of the disease (Frederick et al., 2002). The AJCC stages I to IV are roughly equivalent to Dukes stages A to D (AJCC, 2010).

The TNM staging system, originally created by Pierre Denoix (Denoix, 1946), was developed by the International Union Against Cancer, which was later renamed Union for International Cancer Control (UICC). Today, the TNM staging system is widely used for CRC staging in clinical practice internationally. ‘T’ for tumour, denotes the extent of invasion of the intestinal wall, ‘N’ for lymphatic nodes, indicates the amount of lymphatic node involvement, and ‘M’ refers to metastasis. The AJCC also uses the TNM classification, which has the same definitions for each stage and sub-group as the UICC. The two systems were unified into a single system in 1987 (Sobin, 2003).

The TNM system has been modified several times over the decades as scientists learn more about cancer. The latest edition was published in 2009 (Sobin et al., 2009). A recent review showed the 7$^{th}$ edition is better than the previous edition in predictive capacity for CRC (Gao et al., 2013). However, this system does not cover all survival discrepancies, especially with stage II of CRC. Therefore, other prognostic factors should be considered for decision making with regard to therapy (Hari et al., 2013). The modifications between the 6$^{th}$ edition and the 7$^{th}$ edition are shown in Table A in Appendix A. The

major staging principles in the 6th edition were retained in the 7th edition, but additional sub-groups in stage II, III, and IV were added.

**Table 2.2: AJCC-6 stages with corresponding TNM system and descriptors**

| AJCC-6 stage | TNM | TNM stage criteria for colorectal cancer |
|---|---|---|
| Stage 0 | Tis N0 M0 | Tis: Tumour confined to mucosa; cancer-in-situ |
| Stage I | T1 N0 M0 | T1: Tumour invades submucosa |
| Stage I | T2 N0 M0 | T2: Tumour invades muscularis propria |
| Stage II-A | T3 N0 M0 | T3: Tumour invades subserosa or beyond (without other organs involved) |
| Stage II-B | T4 N0 M0 | T4: Tumour invades adjacent organs or perforates the visceral peritoneum |
| Stage III-A | T1-2 N1 M0 | N1: Metastasis to 1 to 3 regional lymph nodes. T1 or T2. |
| Stage III-B | T3-4 N1 M0 | N1: Metastasis to 1 to 3 regional lymph nodes. T3 or T4. |
| Stage III-C | Any T, N2 M0 | N2: Metastasis to 4 or more regional lymph nodes. Any T. |
| Stage IV | Any T, any N, M1 | M1: Distant metastases present. Any T, any N. |

From: Frederick et al., 2002. AJCC cancer staging manual, 6th edition

More than 96% of CRCs are adenocarcinomas (Stewart et al., 2006). Survival rates vary according to the stage of the carcinoma. The 5-year relative survival rate of CRC greatly depends on the stage at initial diagnosis, with up to 90% for stages I and II, 68% for stage III, and 11% for stage IV. In addition, 57% of CRC patients have developed regional lymph node or distant metastasis at diagnosis, and about 35% of CRC patients die within 5 years (Ries et al., 2008). In the 42% of post adjuvant treatment patients who relapsed within 5 years, 80% of recurrences occurred in the first three years and 91% of them died within five years. The median survival time was 12 months for this cohort (Sargent et al., 2005).

### 2.9.3 **Conventional treatment of colorectal cancer**

The conventional treatment of CRC mainly includes surgical resection, chemotherapy, and radiotherapy. In recent years, bio-targeted monoclonal antibody medicines have been used in addition to chemotherapy for ACRC treatment. In this section, chemotherapy and bio-targeted monoclonal antibody therapy is reviewed.

#### *2.9.3.1 Chemotherapy drugs*

Chemotherapy employs a cytotoxic drug or a combination of such drugs to kill cancer cells and reduce tumour size. Cancerous tumours have the characteristics of uncontrolled rapid growth of cells, with invasion of adjacent tissues or organs and metastasis to distant organs (Fenton & Longo, 2010). Most chemotherapeutic drugs aim at killing fast-dividing cells, such as cancer cells, by causing DNA damage or disruption of normal RNA processes and functions, thereby causing impaired mitosis (cell division) and inducing cells to undergo apoptosis (programmed cell death) (William & Kastan, 1994; Johnstone et al., 2002). However, due to this specific feature of killing fast-dividing cells, cytotoxic drugs also have adverse effects on normal cells in the body, especially on normal fast-dividing cells

such as the cells in hair, skin, the gastrointestinal (GI) tract and bone marrow (DeHaven, 2007). Therefore, the common side effects of chemotherapy include myelosuppression, gastrointestinal distress, fatigue, and hair loss. Thus, the adequate management of the chemotherapeutic side effects can improve patients' quality of life and assist in the delivery of optimal treatment. Chemotherapeutic drugs can be used singly or in combinations that aim to enhance their anti-cancer effects. The following are the main chemotherapy agents that are commonly used for CRC treatments.

*5-Fluorouracil (5-FU)*

5-fluorouracil (5-FU) was introduced over 40 years ago and has remained a backbone of treatment regimens for colorectal cancer (CRC), both alone and in combination with other agents (Hirsch & Zafar, 2011). 5-FU inhibits thymidylate synthase (TS), which is important for methylation of deoxyuridine monophosphate (dUMP) into thymidine monophosphate (dTMP) (Carreras & Santi, 1995). dTMP is essential for DNA synthesis and repair (Longley et al., 2001). When 5-FU is transferred intracellularly, it is converted to a number of metabolites: fluorodeoxyuridine monophosphate (FdUMP), fluorodeoxyuridine triphosphate (FdUTP), and fluorouridine triphosphate (FUTP) (Longley et al., 2003).

FdUMP and 5,10-methylenetetrahydrofolate (CH2THF) together bind to thymidylate synthase, to form a ternary complex, and block thymidylate synthase binding to dUMP. Thus, it disrupts dTMP synthesis (Santi et al., 1974). Without CH2THF presence, the FdUMP binding to TS is reversible (Danenberg & Danenberg, 1978). FdUTP is incorporated into DNA cleavage and repair, and eventually leads to DNA damage and cell death. FUTP is also incorporated into RNA, and disrupts normal RNA process and function (Longley et al., 2003). However, after administration 5-FU will be rapidly catabolised by dihydropyrimidine dehydrogenase (DPD) and converted to dihydrofluorouracil (DHFU) thereby losing its bioactivity (Santi et al., 1974).

The common side effects of 5-FU include granulocytopenia, diarrhoea, stomatitis, and vomiting (Leichman et al., 1995). The incidences of these side effects are dose-dependent, i.e., the higher the systemic exposure to 5-FU, the higher the incidence of 5-FU toxicity (Boisdron-Celle et al., 2002).

5-FU is administered intravenously and has been tested in a variety of different schedules to optimise the clinical effect and minimise toxic events in CRC. The single-agent 5-FU infusion regimen has demonstrated an overall survival (OS) that was two months longer than 5-FU bolus therapy (i.e. rapid administration of a large dose) with fewer high-grade toxic events of granulocytopenia and diarrhoea (Leichman et al., 1995).

*Leucovorin (LV)*

Leucovorin (folinic acid) is not cytotoxic but it can enhance the effect of 5-FU. Intracellularly, leucovorin (LV) converts to CH2THF, which is then polyglutamated by folylpolyglutamate synthetase. The polyglutamation increases the concentration of CH2THF in the cell and increases the stability of the thymidylate synthase ternary complex, thereby enhancing the effect of 5-fluorouracil in inhibiting DNA synthase (Radparvar et al., 1989).

*Capecitabine*

Capecitabine is an oral fluoropyrimidine, which converts to fluorouracil in tumour cells through three sequential enzymatic reactions (Walko & Lindley, 2005). The first enzymatic reaction is in the liver by carboxylesterase to 5'-deoxy-5-fluorocytidine (5'-dFCyd); the second is by Cyddeaminase to 5'-deoxy-5-fluorouridine (5'-dFUrd) in the liver and tumour tissues; and the third is by thymidine phosphorylase (TP) to 5-FU in tumours (Miwa et al., 1998). In a comparison of the toxic profiles of capecitabine versus 5-FU/LV, the most common adverse events for both capecitabine and 5-FU/LV were fatigue (21.1% vs. 25%) and vomiting (23.3% vs. 27%). Capecitabine had a higher rate of hand-foot syndrome (HFS) (53.3% vs. 6.2%), whereas 5-FU/LV caused more stomatitis (24.3% vs. 61.6%), alopecia (6.0% vs. 20.6%), neutropenia (1.2% vs. 10.3%), diarrhoea (47.7% vs. 58.2%), and nausea (37.9% vs. 47.6%) (Walko & Lindley, 2005).

*Oxaliplatin (OXA)*

Oxaliplatin is a third-generation platinum drug, which contains a 1,2-diaminocyclohexane (DACH) carrier ligand. The cytotoxicity of oxaliplatin is effected through the DACH carrier ligand combining with DNA to form adducts that inhibit DNA repair and replication, resulting in cell death (Raymond et al., 1998). Adverse events associated with oxaliplatin treatment include: neuropathy, hematologic toxicities, and gastrointestinal (GI) tract toxicities. However, unlike other platinum derivatives, nephrotoxicity has not been reported in oxaliplatin regimen trials (Cassidy & Misset, 2002).

Hematologic toxic events clinically present as anaemia, neutropenia, and thrombocytopenia. Anaemia and thrombocytopenia usually occur as grade 1 or 2 events (Cassidy & Misset, 2002). The anaemia incidence was found to be similar in both the oxaliplatin and control arms in phase III trials. Cassidy et al. (2000) suggested the incidence is probably an implication of the disease state.

The incidence of neutropenia and thrombocytopenia was associated with the method of 5-FU/LV and oxaliplatin administration. In a phase III trial (Giacchetti et al., 2000), using chronomodulated administration of 5-FU/LV and oxaliplatin (FOLFOX) induced 32% neutropenia and 21% thrombocytopenia. These rates were lower than those from a FOLFOX4 continuous-infusion which had 70.3% neutropenia and 76.2% thrombocytopenia (de Gramont et al., 2000). The chronomodulated administration method is based on the biologic rhythms of DNA synthesis to reduce bone marrow

sensitivity to cytotoxic chemotherapy along the 24-hour scale (Smaaland et al., 1991). However, the effects of the chronomodulated administration of 5-FU/LV and oxaliplatin on gastrointestinal toxic events were not better than in the FOLFOX4 regimen.

Oxaliplatin combined with 5-FU increases the incidence of neutropenia. In monotherapy using oxaliplatin (130 mg/m$^2$, 2-hour infusion on day 1, every 21 days), there was 5.2% of grade 3/4 neutropenia (Becouarn et al., 1998), whereas a FOLFOX4 regimen induced 41.7% incidence (de Gramontet al., 2000). This may be associated with the oxaliplatin reducing 5-FU plasma clearance by inhibiting 5-FU catabolism (Boisdron-Celle et al., 2002).

Gastrointestinal tract toxicity clinically appears as nausea, vomiting, diarrhoea, and mucositis. Nausea and vomiting is mild to moderate and can be controlled by standard antiemetic treatment. In the de Gramont et al. (2000) phase III trial, grade 3/4 nausea and vomiting was experienced in 11.5% of ACRC patients with FOLOFOX4. Other grade 3/4 events were 11.9% for diarrhoea, and 5.8% for mucositis (de Gramont et al., 2000). However, these gastrointestinal tract toxicities were manageable by dose modification and generally did not cause discontinuation of treatment (Cassidy & Misset, 2002).

Oxaliplatin related neurologic toxicity describes two different types of neurological symptoms. The first type is reversible acute paresthesia and dysesthesia that occurs during or immediately after the end of the infusion. Patients experience transient peripheral sensory disorder in the hands, feet and perioral region with jaw tightness, sometimes accompanied with muscular contractions. The symptoms can be triggered or enhanced by exposure to cold or increased by repeated administration. The second type is chronic neuropathy that is usually late-onset deep sensory loss, sensory ataxia and functional impairment, jaw pain, eye pain, ptosis, leg cramps and visual and voice changes (Pasettoet al., 2006). It correlates with the cumulative-dose of oxaliplatin (over 540 mg/m$^2$) (Cersosimo, 2005). In the de Gramont et al. (2000) phase III trial, 68% of patients in the FOLFOX4 group experienced oxaliplatin-related neurologic toxicity (all grades), but only 18% of this group of patients had grade 3 events (de Gramont et al., 2000). Moreover, the acute neuropathy was usually mild and completely reversible after discontinuation of the treatment. Chronic neuropathy related to the cumulative-dose of oxaliplatin may be reversible with a median time to recovery from grade 3 neurotoxicity of 13 weeks (Grothey & Goldberg, 2004).

*Irinotecan*

Irinotecan is a topoisomerase I inhibitor anti-cancer drug that is a derivative of camptothecin (CPI). Camptothecin is a quinoline alkaloid isolated from the bark and stem of *Camptotheca acuminata* (*xi shu*) that was used traditionally for cancer treatment in CHM (Efferth et al., 2007). Topoisomerase I (Top 1) breaks down single DNA strands that allow DNA replication and transcription (Wang, 2002).

Irinotican prevents DNA unwinding and replication by inhibiting topoisomerase I (Liu et al., 2000). Diarrhoea and myelosuppression are common adverse effects of irinotecan (Saad & Hoff, 2005). It is also called hydroxycamptothecine (HCPT).

*Methotrexate (MTX)*

MTX inhibits purine synthesis, which is the precursor of CH2THF that is essential for dTMP synthesis (Gorlick & Bertino, 1999). In methotrexate-treated cells, MTX leads to accumulation of cellular 5-phosphoribosyl-1-pyrophosphate (PRPP) (Sant et al., 1992), which correlates to increased concentrations of intracellular FUTP that inhibits RNA synthesis by incorporating into RNA process and function. This accounts for the MTX-5FU synergism (Cadman et al., 1979).

*Vincristine*

Vincristine is a vinca alkaloid that was originally isolated from *Vinca rosea* (*Catharanthus roseus*). It induces tumour cell death by suppressing microtubule dynamics, blocking mitosis at the metaphase/anaphase transition. Its toxic side effects are: peripheral neuropathy, hyponatremia, constipation, and hair loss (Jordan, 2002)

*Doxorubicin*

Doxorubicin (ADM) is an anti-tumour antibiotic. It binds to DNA-associated enzymes, to produce a range of cytotoxic effects. The side-effects of ADM are mainly in the brain, liver, kidney and heart (Tacar et al., 2013).

*Semustine (MeCCNu)*

Semustine is a chloroethyl nitrosoureas that causes DNA damage by inducing DNA interstrand cross-links (Agarwal et al., 2015). The side effects are nausea and vomiting, myelosuppression, stomatitis, alopecia, anaemia, anorexia, hepatotoxicity, and neurotoxicity (Wasserman et al., 1975).

*Mitomycin (MMC)*

Mitomycin is a natural antibiotic that derives from *Streptomyces caespitosus* or *Streptomyces lavendulae*. It belongs to the family of aziridine, and exhibits anti-tumour effects that induce DNA damage, DNA interstrand and intrastrand cross-links and alkylation of DNA (Tomasz & Palom, 1997). Its toxicity includes delayed bone marrow toxicity, hemolytic uremic syndrome (HUS), lung fibrosis and renal damage. Permanent bone marrow damage may occur with prolonged use (Saif et al., 2013).

#### *2.9.3.2 Clinical trials of chemotherapy*

Chemotherapy is classed in three settings based on the aim of treatment in CRC: adjuvant, palliative, and neoadjuvant (Braun & Seymour, 2011). This section is focussed on adjuvant and palliative chemotherapy.

*Adjuvant chemotherapy*

Clinical trials indicate that adjuvant chemotherapy reduces risk of CRC recurrence and prolongs survival for stage III CRC and confers a modest benefit for stage II after curative surgery when compared to observation (no treatment) alone. A 5-FU/LV regimen as adjuvant chemotherapy has demonstrated significant improvement in both disease free survival (DFS) (63% vs. 47% over 3½ years) and overall survival (OS) (71% vs. 55% over 3½ years) for stage III CRC (Moertel et al., 1990). In another study of 5-FU/LV, the 3-year recurrence rate for stage III CRC was 35.5% and the 3-year survival rate was 71% (Kerr et al., 2000). However, the 5-FU/LV treatment benefits are modest for stage II CRC, with only 3.6% increase in survival for patients who received chemotherapy, and almost no difference between the chemotherapy group and observation group in 2 years recurrence [144/1127 (12.8%) vs. 132/1040 (12.7%), p=0.94] (Gray, 2007).

Addition of oxaliplatin to the 5-FU/LV regimen has proven more effective for treating stage II /III CRC compared to control groups which received 5-FU/LV alone. Significant improvements in 3 years DFS were consistent in two important international trials (78.2% for MOSAIC trial, 76.1% for NSABP trial). MOSAIC also reported significant improvements in OS in the stage III cohort of patients who received oxaliplatin (72.9% vs. 68.7%, p=0.023), but there was no difference in stage II patients. However, the regimens were not the same in these two trials (MOSAIC - FOLFOX: 5-FU 4000mg/$m^2$, LV 800mg/$m^2$ bolus plus infusion, OXA. 340mg/$m^2$ per cycle; NSABP - FLOX: 5-FU 3000 mg/m2, LV 3000mg/$m^2$ bolus, OXA. 255mg/$m^2$, per cycle).

The incidence of grade 3/4 neurotoxicity was higher in the MOSAIC trial than in the NSABP trial (12.4% vs. 9.6%) due to the higher cumulative dose of oxaliplatin in MOSAIC. Grade 3/4 gastrointestinal toxicity, including diarrhoea, vomiting and nausea, was significantly higher in NSABP patients. This indicated the infusional 5-FU schedule (used in MOSAIC) had less GI toxicity than the bolus schedule of 5-FU (used in NSABP). FOLFOX regimens have been recommended as standard first line adjuvant chemotherapy for CRC (Kuebler et al., 2007; Sharif et al., 2008; Andre et al., 2009; Gravalos et al., 2009).

*Palliative chemotherapy*

For ACRC, where the disease is so locally advanced that curative surgery becomes impossible or there is distant metastasis, chemotherapy aims to downgrade ACRC in order to enable potentially curative

surgery, or to palliatively treat ACRC to improve the quality of life (QoL) or to prolong survival time. 5-FU/LV monotherapy or plus oxaliplatin or irinotican has been used extensively in ACRC (Hind et al., 2008).

A meta-analysis study based on individual patient data, that compared various 5-FU based palliative chemotherapy regimens versus supportive care alone for ACRC found that the median overall survival (mOS) was improved by 3.7 months (11.7 vs. 8 months), and the median progression free survival (mPFS) was calculated at 10.0 months for the chemotherapy group but only 4.0 months for the supportive care group (Simmonds, 2000).

In a multi-centre trial that compared a monthly schedule of low-dose LV and 5-FU bolus with a bimonthly schedule of high-dose LV and 5-FU bolus plus continuous infusion in patients with ACRC, the bimonthly infusion regimen was more effective and less toxic than the monthly bolus regimen. The mPFS was 22 weeks for monthly bolus regimen and 27.6 weeks for bimonthly infusion regimen ($P = 0.0012$). The mOS was 56.8 weeks for the monthly bolus regimen and 62 weeks for the bimonthly infusion regimen ($P = 0.067$). Grade 3-4 toxic events occurred in 23.9% of patients in the monthly arm compared with 11.1% of those in the bimonthly arm ($P = 0.0004$) (de Gramont et al., 1997).

Hind's review of 11 RCTs of ACRC demonstrated that the OS varied from 15 to 20.6 months for oxaliplatin plus 5-FU/LV and 14.8 to 21.1 months for irinotecan plus 5-FU/LV as first line treatments; PFS was 7.9 to 9.0 months for the oxaliplatin regimen and 7.5-8.8 months for the irinotican regimen. The tumour response rate (tRR) was between 31-54% for the two regimens when 5-FU was administered by infusion. Different toxicity profiles appeared in the two regimens. Based on grade 3 and 4 toxicity, diarrhoea, vomiting/nausea, stomatitis and febrile neutropenia were more prevalent in patients who received irinotican plus 5-FU/LV, whereas patients who received oxaliplatin plus 5-FU/LV generally had a higher incidence of neuropathy and neutropenia. However, there was no significant difference between the two regimens in overall, quality of life (QoL) (Hind et al., 2008).

Oral administration of capecitabine has been used to replace intravenous 5-FU in treating CRC. When used as monotherapy or in combination with oxaliplatin, capecitabine has been demonstrated to be as effective as the 5-FU/LV intravenous setting in terms of tRR, DFS/PFS and OS. The common severe toxicities (grade 3/4) of capecitabine are hyperbilirubinemia, hand and foot syndrome, diarrhoea, nausea/vomiting, and neutropenia (Twelves et al., 2005; Cassidy et al., 2008).

The staging of chemotherapy strategies (starting with a single drug and modifying to combination regimens upon progression) and intermittent chemotherapy have not shown significant impacts on overall survival (OS), when compared with initial combination chemotherapy, but have shown lower toxicity profiles (Braun & Seymour, 2011).

#### *2.9.3.3 Bio-targeted monoclonal antibody therapy*

Bio-targeted monoclonal antibody therapy refers to the use of monoclonal antibodies to bind monospecifically to certain cells or proteins in order to stimulate the patient's immune system to attack those cells. Bio-targeted monoclonal antibody medicines used in addition to chemotherapy for ACRC include: cetuximab and panitumumab, which target epidermal growth factor receptors (EGFR), and bevacizumab, an anti-vascular endothelial growth factor (VEGF). The addition of cetuximab to an irinotecan based regimen (i.e. FOLFIRI) demonstrated improvement in PFS in patients with wild-type KRAS gene but not in patients with mutant KRAS gene. However, there was an increased rate of adverse events in the cetuximab plus FOLFIRI group (Van Cutsem et al., 2009). Cetuximab plus an oxaliplatin based regime (i.e. FOLFOX) found an improvement in tRR for the wild-type KRAS but not for the mutant KRAS gene groups. However, there was no difference in overall survival (OS) or PFS between the cetuximab plus FOLFOX and the FOLFOX groups (Maughan et al., 2011). As in the previous study, the skin and gastrointestinal toxicity were greater in the cetuximab group (Van Cutsem et al., 2009; Maughan et al., 2011). Although the addition of EGFR agents showed benefits for wild-type KRAS CRC, the potential for increased toxicities needs to be considered when applying these agents (Braun & Seymour, 2011).

In two studies, the addition of bevacizumab to FOLFOX regimes increased the mOS and mPFS by 1-2 months for metastatic CRC as first line or second line treatment. Adverse events associated with bevacizumab included hypertension, bleeding, and vomiting, which appeared to be tolerable (Giantonio et al., 2007; Saltz et al., 2008). Therefore, the decision to add bevacizumab for ACRC should depend more on the effectiveness rather than on the toxicities (Braun & Seymour, 2011).

Overall, with advances in technology, which include early detection via screening programs, development of novel drugs, and new combination chemotherapy regimes, CRC has become a curable disease when detected at an early stage.

### 2.9.4 **Herbal medicine in cancer therapy**

Herbal medicine (HM) has been used to treat cancers and many other ailments for centuries in China and in other Asian countries. Chinese HM (CHM) alone or in combination with conventional therapy is accepted by both oncologists and patients in China as an approach to treating a range of cancers including CRC (Saif et al., 2010). In accordance with Traditional Chinese Medicine (TCM) etiology and pathology, tumours belong to the categories of '*zhengjia*' (癥痂), and '*jiju*' (积聚). These result from the combination of '*zhengxu*' (正虚) - asthenia of healthy vital energy 'qi', and '*xieshi*' (邪实) – excess of unhealthy energy (*xie* 邪). In TCM, *zhengxu* refers to deficiency of vital qi and blood, whereas *xieshi* refers to constitutional qi stagnation, blood stasis, phlegm coagulation, or gathering

toxins. *Zhengxu* and *xieshi* both can be induced by unhealthy mental state, malnutrition/poor diet, and adverse environmental conditions (Huang, 2004).

The TCM principle of treatment for cancer is referred to as '*fuzheng quxie*' (扶正驱邪) which means 'support the normal and dispel the perverse', since the typical features of a cancer patient's constitutional state are '*zhengxu xieshi*' (正虚邪实) which means that the normal energy of the patient is empty or weak while the perverse disease, i.e. the cancer, is abundant. CHM aims to address the emptiness of the patient's normal energy which impedes their ability to resist the progression of the cancer whilst dispelling the invasion of the cancer. Therefore, the CHMs used for cancer are mainly composed of herbs in the categories of *fuzheng* 扶正 and *quxie* 驱邪 (Huang, 2004).

In TCM, *fuzheng* herbs are those that can tonify the internal organs, invigorate the vital qi and enrich the blood in order to support the normal functioning of the patient's body. *Quxie* herbs, from a TCM perspective, are those that disperse pathogenic phlegm, remove blood stasis, detoxify the body and resolve masses (Bensky & Gamble, 2004).

Treatment typically involves the administration of multi-herb formulas orally as decoctions, tablets or capsules, and/or the intravenous use of extracts. The composition of a herbal formula is based upon the methodology of TCM pattern differentiation (*bian zheng* 辩证) and determined using TCM principles at each consultation. The formula may or may not be modified by the practitioner according to the patient's constitution and response over the course of the treatment.

CHMs are also used for the local treatment of cancers. For instance, in CRC treatment, herbal liquid from CHM decoctions can be administered via enema to relieve symptoms such celialgia, tenesmus, or blood and pus in the faeces (Li & Li, 1999). Javanica oil injected through transcatheter arterial chemoembolization (TACE) is used in treating metastatic liver cancer from CRC (Zhang & You, 2008).

Experimental studies have demonstrated that bio-active components in a number of single Chinese HMs and multi-herb formulas possess anti-cancer potential. Based on multiple studies, the anti-cancer mechanisms of Chinese HMs have been summarised as having multi-actions and multi-targets, in terms of induction of tumour cell apoptosis and tumour cell differentiation; mediation of cellular transduction pathways; suppression of tumour angiogenesis; inhibition of telomerase activity; regulation of immunofunction; and reversal of multiple drug resistance (Han & Li, 2009, Parekh et al., 2009).

### 2.9.5 **Integrative medicine**

Integrative medicine (IM) refers to combining conventional medicine with complementary and alternative medicine (CAM), in order to obtain optimal health and healing outcomes. The western model of IM requires complementary and alternative medicine to be based on scientific evidence. CAM research and educational institutions have been established in western countries including Australia, USA, Germany, Great Britain. Two-thirds of medical schools in the USA had introduced CAM methods into medical education curricula by 1999, and since 2003 German medical students have been required to include integrated CAM programs into their curricula by law (Dobos & Tao, 2011). Some conventional medical centres in USA and Germany have introduced CAM care into their established therapeutic programs (Dobos et al., 2012; Chan et al., 2010).

CAM products and therapies include dietary therapy, exercise, stress reduction, mind/body therapy, manual and massage therapy, reflexology, acupuncture, vitamin and mineral therapy, and HM. Chinese and Western forms of HM are popular among patients including cancer patients. For many patients, the option of both CAM and conventional medicine is preferable. CAM use in conjunction with conventional treatments without the knowledge of medical doctors is common. A challenge for IM in the west is how safe and effective this form of healthcare is. Many CAM therapies lack sufficient scientific evidence to support their use, and herbal products have the potential for herb-drug interactions. Consequently, IM may not be suitable for widespread use in western countries until there is sufficient research evidence (Robinson, 2011).

In China, the IM approach in cancer is typically a combination of TCM, which includes CHM, acupuncture, massage (*tuina*), diet, nutrition and exercise (including *tai chi*), and conventional Western medicine. Since 1949, TCM has been an essential part of health care in China and plays an important role in the health care system (Robinson, 2011). A review showed more than 60% of cancer patients in China used IM for cancer treatment, and among them, 98.5% of patients used CHM treatment (Liu et al., 2011). In China, CHM is used as an adjuvant therapy to chemotherapy in order to alleviate adverse reactions induced by conventional cancer therapy, improve the effectiveness of cancer treatment, improve quality of life (QoL), and eventually prolong overall survival (OS) (Konkimalla & Efferth, 2008; Molassiotis et al., 2009).

Due the popularity of herbal medicines among cancer patients, the potential for herb-drug interaction is of concern to the medical profession. Currently, herb-drug interaction research is mostly based on the pharmacokinetic interaction between the active constituents of herbal medicines and drugs. The majority of potential herb-drug interactions are associated with metabolising-enzyme cytochrome P450 (CYP), ABC transporters, and p-glycoprotein (P-gp), each of which mediates the metabolism and disposition of drugs. CYP3A4, CYP3A5, CYP2D6, CYP2C19 are critical members in CYP superfamily (Cheng et al., 2018). Active constituents of herbal medicines can inhibit or induce activity

of CYP, drug transporters and P-gp protein, so there is potential for interaction with drugs, at least theoretically.

In addition to these pharmacokinetic factors, other factors such as pharmacodynamics, the patients' individual condition, use of narrow therapeutic index drugs (e.g. anticancer drugs, anti-HIV drugs) should all be considered when interpreting the potential for herb-drug interactions in clinical practice (Hermann and Richter, 2012).

#### *2.9.5.1 Clinical studies of integrative medicine for colorectal cancer*

In this section, studies of CHM integrated with conventional chemotherapy for CRC were reviewed.

Zhong et al. (2012) systematically reviewed RCTs of CHM as an addition to chemotherapy for CRC. In comparison to chemotherapy alone, the meta-analysis results showed the combination of CHM and chemotherapy significantly increased 1-year survival rate, and 3-year survival rate (OR 2.40, 95% CI [1.49, 3.87]) based on 5 studies of 396 patients. The combined therapy significantly improved tumour response rate (tRR) (OR 1.89, 95% CI [1.26, 2.88]) based on 7 studies with 475 patients and quality of life (QoL) (OR 3.43, 95% CI [2.35, 5.02]) based on 9 studies with 649 patients. The integrative medicine therapy also had positive effects in immunoregulation (Zhong et al., 2012).

A Cochrane review pooled data from four RCTs (342 CRC patients) of CHM as adjuvant therapy to chemotherapy compared to the same chemotherapy alone in the control. The results showed the addition of the CHM alleviated the chemotherapy side effects of nausea and vomiting and leucopenia. The CHM also increased the proportions of T-lymphocyte subsets: CD3; CD4 and CD8. No adverse effect was reported from the CHM interventions (Wu et al., 2005).

Another systematic review assessed the combination of *jianpi* 健脾 'strengthen spleen' type CHMs with chemotherapy in CRC. Six RCTs which included 334 patients were pooled. This review found that the integrative approach significantly reduced the incidences of grade I and grade II leucopenia (grade I: RR 0.50 [0.31, 0.80]; grade II: RR 0.37 [0.21, 0.66]), and grade II nausea and vomiting (RR 0.51 [0.31, 0.84]) in patients who were received CHM treatment concurrently with chemotherapy, compared to patients who had chemotherapy alone. There was a trend favouring the integrative interventions in reduction of the incidence of neurotoxicity reactions, but this was not statistically significant for grade I: (RR 0.84 [0.57, 1.24]); grade II: (RR 0.73 [0.45, 1.19]; or grade III: (RR 0.40 [0.13, 1.25]) (Liu & Zhu, 2009).

In a retrospective study, 103 CRC patients, who mostly were ACRC (4 stage II, 7 stage III, 92 stage IV), were divided into a group that was treated with CHM plus conventional treatment which included chemotherapy and/or surgical resection, and a control group that was treated with conventional

treatment alone. The baselines were balanced between groups in terms of age, gender, pathology, stage, distant metastasis, and history of surgery. 1-, 3-, 5- years survival rates and median survival times were compared. The results showed there was a significant improvement in median survival times in the combination treatment group (27 months vs. 16 months, p=0.03), and better overall survival rates at 1-, 3-, and 5-years (82%, 32%, 6% vs. 64.2%, 13.2%, 1.9% respectively) (Ren et al., 2010).

In a consecutive case series study with a 10-year follow-up conducted in the USA, 193 colon cancer patients who used HM and vitamins combined with standard chemotherapy were compared to controls who only used standard chemotherapy, based on data from the California Cancer Registry (n=1,1678) and Kaiser Permanente Northern California (n=1987). The Kaplan-Meier survival curve and traditional Cox regression showed motality was reduced in the combination treatment group by 95% for stage I, 64% for stage II, 29% for stage III, and 75% for stage IV, when compared with matched controls from the cancer registries who received standard chemotherapy alone (McCullock, 2011).

Overall, despite the possibility of methodological weaknesses in some studies, the results from many of these studies suggest that integrative HM may be able to provide an alternative approach to the care of CRC.

### 2.9.6 **Laboratory studies of HM for colorectal cancer**

The following CHMs have been studied for their effects on CRC cell-lines or CRC-bearing animals.

#### *2.9.6.1 Astragalus saponin extracts*

An astragalus saponin extract (AST), derived from *Astragalus membranaceus,* was found to inhibit proliferation of HT-29 human colon cancer cells. AST treated HT-29 cells were arrested at S phase and G2/M, through up-regulation of cyclin-dependent kinase inhibitor protein p21 expression and inhibited cyclin-dependent kinase activity *in vitro*. AST also induced apoptosis in HT-29 cells, in which DNA fragmentation and nuclear chromatin condensation was observed in a time and dose dependent manner. It was suggested that apoptosis was through activation of the caspase-3 signaling pathway and cleavage of poly (ADP-ribose) polymerase (PARP). In the same study, the effect of AST on tumour growth in xenografts of HT-29 cells in nude mice was investigated. The tumour–bearing mice were randomly assigned into a control group with phosphate-buffered saline (PBS) alone and various treatment groups, including: AST alone group, AST+5-FU group, and 5-FU+OXA group. AST alone treatment reduced tumour volume by 35-38%, which was similar to the effect of 5-FU monotherapy. When AST + 5-FU was compared to 5-FU + OXA, the results for shrinkage of tumour volume were 66% for AST + 5-FU and 61% for 5-FU + OXA at day 21. Importantly, treatment with 5-FU + OXA caused 33%

mortality in the animals and significant loss of body weight, but no deaths were recorded in the AST + 5-FU treatment group, and there was a minor drop in body weight (Tin et al. 2007).

#### *2.9.6.2 Ganoderma lucidum*

The medicinal mushroom *Ganoderma lucidum* (Curtis) P. Karst is regarded as a tonic and has been used for millennia in China. Today, it is a popular CHM in adjuvant cancer treatment in the Chinese community. A *G. lucidum* triterpene extract (GLT) was found to suppress proliferation in HT-29 cells and GLT inhibited tumour growth in a xenograft model of colon cancer HT-29 cells in nude mice through cell cycle arrest at G0/G1, and induced programmed cell death type II – autophagic through the inhibition of p38 mitogen-activated kinase (p38 MAPK) (Thyagarajan et al., 2010).

#### *2.9.6.3 Ginseng saponins*

Ginseng saponins (known as ginsenosides) are derived from *Panax ginseng* C.A Meyer, *P. quinquefolium* L (American ginseng) and *P. notoginseng* (Burkill) F.H.Chen (*sanqi*). These are the main compounds with anti-cancer effects in the ginseng family. Among them, ginseng Rg3, Rh2, IH-901 (compound K) and protopanaxadiols (PPD) have shown inhibition of the growth of different cancer cells (Nag et al., 2012).

American ginseng (which mainly contains ginsenosides Rg3 and Rh2), induced apoptosis in SW-480 human colorectal cancer cells mainly via mitochondrial pathways (Wang et al., 2009). 20S-ginsenoside Rg3 (20S-Rg3) induced apoptosis in HT29 colon cancer cells and suppressed cell proliferation. Proteomic analysis found that 20S-Rg3 up/down regulated proteins associated with apoptosis. 20S-Rg3 mediated anti-proliferation by inhibition of mitosis, DNA replication and repair, and growth factor signalling in HT29 cells (Lee et al., 2009).

#### *2.9.6.4 Isoliquiritigenin*

Isoliquiritigenin from liquorice, combined with tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), induced apoptosis in TRAIL resistant colon cancer HT29 cells by accumulation of DR5 protein among TRAIL receptors (Yoshida et al., 2008).

#### *2.9.6.5 Tanshinone I*

Tanshinone I isolated from *dan shen* (*Salvia miltiorrhiza* Bunge) has been shown to induce apoptosis through both p21 mediated cell differentiation arrest at G0/G1 phase, and mitochondrial-mediated intrinsic cell-death pathways in human colon cancer Colo 205 cells *in vitro* (Su et al., 2008).

#### *2.9.6.6 Pseudolaric acid B*

Pseudolaric acid B (PAB) is a natural diterpenoid extracted from *Pseudolarix kaempferi* Gordon (*jin qian song* 金钱松). In HT29 cells, PAB has been reported to induce apoptosis and suppress cell cycle progression which was related to cell cycle arrest at G2/M phase, modulate cyclin expression and down-regulate the proto-oncogene c-myc. PAB concomitantly increased the protein and gene expression of the nonsteroidal anti-inflammatory drug-activated gene (NAG-1), and inhibited cyclooxygenase-2 (Cox-2) (Ko et al., 2007).

#### *2.9.6.7 Farnesol and Geranylgeraniol*

Farnesol (FOH) and geranylgeraniol (GGOH) are two isoprenoids (terpenes) that are commonly present in the essential oils of herbs and fruits. They have both induced apoptosis in HT-29 and HCT116 colon cancer cells *in vitro* through caspase-3 activation, and PARP cleavage (Kim et al., 2005; Au-Yeung et al., 2008).

#### *2.9.6.8 Chinese mistletoe lectin-55*

In one study, BALB/c mice were subcutaneously inoculated with the colon cancer cell line CT26 (5 x 10 (5) cells). The mice were orally administered Chinese mistletoe lectin-55 (ACML-55), an active compound from *Loranthus parasiticus* (L) Merr., at a daily dose of 200 μL/mouse (2 mg/mL) or phosphate-buffered saline (PBS) at a daily dose of 200 μL/mouse as the negative control, for 2 weeks. The investigators found that compared to PBS treatment, ACML-55 significantly inhibited tumour formation and reduced tumour size in xenograft mice. An immunology study demonstrated that ACML-55 intervention promoted both activation and proliferation in CD4+, CD8+ T cell subsets, and increased production of CD8+ T cells in response to IFN-γ. Moreover, ACML-55 intervention increased gammadelta T cells (γδT cells), a specific subset of T cells that are important for tumour immune surveillance through furnishing an early source of IFN-γ. This study indicated that ACML-55 has anti-cancer potential by promoting both specific T cells and IFN-γ, which are essential to tumour immune surveillance (Ma et al., 2008).

### 2.10 Chapter 2 summary

Overall, CRC incidence and mortality are increasing worldwide. CRC is a curable disease if it is diagnosed in early stage using the advancing technologies of early detection. For advanced CRC, the tumour response rate and overall survival rate are improving since the development of new chemotherapeutic drugs, bio-targeted monoclonal antibody medicines, and cancer immunotherapy technology. HMs are used in China and in other Asian countries as part of integrative medicine (IM) for CRC treatment, particularly as adjuvant therapy to chemotherapy, since the IM approach appears to improve cancer treatment response, improve patient's quality of life, and eventually prolong

survival time. Constituent compounds in certain HMs have demonstrated inhibition of CRC proliferation *in vitro* and *in vivo*. However, many HM therapies lack sufficient scientific evidence and herbal products have the potential for herb-drug interactions. Consequently, the efficacy and safety of HMs require further research.

# Chapter 3. Research Methodology

## 3.1 Introduction to Chapter 3

This chapter details the research methods and procedures used in each stage of the project. These are linked with the specific research questions, which the methods are designed to answer. This chapter presents the methods and procedures for:

- The systematic reviews of clinical trials by using Cochrane collaboration method
- Sensitivity analysis for the selection of specific herbs for further research
- Laboratory studies of compounds from promising herbs
- The review of experimental studies of the mechanisms of action of the compounds in promising herbs

The methods in 3.1 to 3.3 were used for Chapters 4, 5 and 6 are included in the following published papers:

1. Chen M, May BH, Zhou IW, Xue CC, Zhang AL. (2014). FOLFOX 4 Combined with herbal medicine for advanced colorectal cancer: A systematic review. *Phytother Res*, Jul, 28(7): 976-91. doi: 10.1002/ptr.5092. DEpub 2013 Dec 17.
2. Chen MH, May BH, Zhou IW, Zhang AL, Xue CCL (2016b). Integrative Medicine for Relief of Nausea and Vomiting in the Treatment of Colorectal Cancer Using Oxaliplatin-Based Chemotherapy: A Systematic Review and Meta-Analysis. *Phytother Res*, 30(5):741-53 doi: 10.1002/ptr.5586. Epub 2016 Feb 23.
3. Chen MH, May BH, Zhou IW, Xue CCL, Zhang AL (2016a). Meta-analysis of oxaliplatin-based chemotherapy combined with traditional medicines for colorectal cancer: contributions of specific plants to tumor response. *Integr Cancer Ther*, Mar;15(1):40-59. DOI: 10.1177/1534735415596424
4. Chen M, May BH, Zhou IW, Sze DM, Xue CC, Zhang AL (2016c). Oxaliplatin-based chemotherapy combined with traditional medicines for neutropenia in colorectal cancer: A meta-analysis of the contributions of specific plants. *Crit Rev Oncol Hematol*, Sep;105:18-34. doi: 10.1016/j.critrevonc.2016.07.002. Epub 2016 Jul 7

## 3.2 Search methods for identification of clinical studies

Three approaches were used to identify clinical trials for consideration in the systematic reviews:

1. Online database searches;

2. Hand searches of journals; and

3. Searches of reference lists in review articles and clinical studies.

### 3.2.1 **Search strategies for electronic databases.**

Searches were conducted of the major international biomedical databases: PubMed, Cochrane CENTRAL, CINAHL, ScienceDirect and PsycINFO; and the major Chinese language databases: China Academic Journals (CNKI) and Chinese Sci &Tech Journals (CQVIP).

Search terms were divided into three groups:

1. Disorder: colorectal cancer and related terms;
2. Intervention: Chinese medicine, herbal medicine and related terms;
3. Study type: controlled trial, randomised and related terms.

Individual terms were linked by the Boolean operator "OR". Then, the three groups of terms were combined using the "AND" operator to limit the retrieved articles to those that were related to clinical trials, colorectal cancer and HM.

No limits were imposed. Publication dates were from the inceptions of the respective databases to the present. The search strategies for each of the electronic databases are detailed in Appendix B.

**Hand searches of printed journals and reference lists of published articles**

Hand searches were conducted of journals in RMIT library: Australian Journal of Acupuncture and Chinese medicine 2008-2009; Chinese Journal of Integrated Traditional and Western Medicine 1995-2002; Journal of Traditional Chinese Medicine 1991-2009; American Journal of Chinese Medicine 1993-2002; and the Journal of Chinese Medicine 1991-2009, since these journals are relevant to HM but were not indexed in PubMed. The reference lists of articles obtained (including those from previously published systematic reviews) were checked to identify any additional relevant reports.

### 3.2.2 **Inclusion and exclusion criteria**

Retrieved citations were combined in an Endnote library. The 'find duplicate' function in the Endnote Library was used to find duplicated copies which were then excluded. The titles and abstracts of the remaining citations were then filtered according to the following inclusion and exclusion criteria.

*Inclusion criteria*

1. Study type: Randomised controlled trials (RCTs) of HM interventions alone or combined with conventional interventions in a test arm, regardless of blinding were included. No restrictions were placed on language or publication year;

2. Types of participants: Patients (aged 18 to 80 years) diagnosed with primary colorectal cancer based on histological/pathological results. There was no restriction on pathological sub-group, stage, new or recurrent;
3. Types of interventions: 1. herbal medicine combined with conventional medicine versus conventional medicine alone; 2. herbal medicine versus conventional medicine; 3. herbal medicine versus placebo or no intervention;
4. Types of outcome measures: The study provides data on at least one of the CRC related primary or secondary outcome measures detailed below;

Provides the identities of the key herbs used in the study.

*Exclusion criteria*

1. Study subjects with benign colorectal tumour or secondary malignant tumour without primary CRC;
2. *In-vitro* and *in-vivo* studies;
3. Case reports and case series studies;
4. Clinical trials other than RCTs;
5. Interventions that are not considered as primarily herbal – such as purified compounds.

### 3.2.3 **Outcome measures**

#### *3.2.3.1 Primary outcome*

Tumour response rate (tRR). Assessment criteria referred to the WHO standard for solid tumour response rate (Miller et al., 1981), or the Response Evaluation Criteria In Solid Tumours (RECIST), which was firstly published in 2000 by an international collaboration including the European Organisation for Research and Treatment of Cancer (EORTC), National Cancer Institute of the United States, and the National Cancer Institute of Canada Clinical Trials Group (Eisenhauer et al., 2009). For details of these two assessments see C1 and C2 in Appendix C.

#### *3.2.3.2 Secondary outcomes*

- Overall survival rate (OS): defined as the percentage of people who survive for a certain period of time after they were diagnosed with or treated for a disease. The overall survival rate is often referred to as a five-year survival rate in clinical trials;
- Progression free survival (PFS): in clinical trial, it is defined as ‘the time interval from the randomization date to the date of disease progression or, if the patient died without evidence of progression, to the date of death’ (de Gramont et al., 2000, p 2940);

- Time to progression (TTP): the difference between PFS and TTP is that TTP is only interested in the event of disease progression. Both PFS and TTP in a clinical study have often been used as surrogate end points (Beauchemin et al., 2014);
- Disease free survival (DFS): in a clinical trial, it was defined as 'the time from randomisation to the first event of either recurrent disease or death' (Sargent et al., 2005, p. 8665);
- Alleviation of the side effects of chemotherapy: mainly including neutropenia, anaemia, thrombocytopenia, diarrhoea, nausea and vomiting, and neuropathy. The criteria for assessment of chemotherapy toxicity are found in WHO Recommendations for Grading of Acute and Subacute Toxicity (Miller et al., 1981). These are detailed Table C1 in Appendix C;
- Improvement in a validated quality of life (QoL) measure: Karnofsky performance status (KPS) (Yates et al., 1980) or other validated measure. See details Table C2 in Appendix C;
- Improvement in immune function (CD3, CD4, CD4/CD8, NK, TNF-α);
- Adverse events from the HMs.

The full texts of all potentially included studies were obtained through RMIT library for further scrutiny to determine whether the inclusion criteria were satisfied. Records were kept of the numbers of studies at each stage in the search and selection process. These were summarised in the form of a flowchart according to the PRISMA guidelines for systematic reviews (Moher et al., 2009). Eligible studies were included in the review. Excluded studies were categorised into sub-groups for future reference.

### 3.2.4 **Data extraction**

Data were extracted from each included study using pre-designed data collection forms. The following items were extracted:

- Authors and year of publication;
- Participant's baseline information: source of participants, number of participants, gender, age (mean/median), TNM/Duke's stages, performance status, etc;
- Diagnostic criteria: pathological/histological;
- Intervention: name of herbs/regimen, dosage, routes of administration, period of intervention, etc;
- Methodological information: generation of allocation sequence, allocation concealment, blinding, drop-outs, follow-up;
- Outcomes: tRR, OS, PFS/TTP, Karnofsky performance status (KPS), immune function, side-effects of chemotherapy, etc;
- Adverse events for HM interventions; and
- Name of each HM in the included studies.

Data were checked for accuracy by a second researcher (Dr Jing Cui and/or Dr Iris Zhou).

### 3.2.5 **Risk of bias assessment*:***

Risk of bias was assessed using the Cochrane Risk of Bias assessment method which includes the following categories of bias: selection bias, performance bias, detection bias, attrition bias, reporting bias, and other bias. The approach was according to the Cochrane Handbook version 5.1.0 (Higgins & Green, 2011). This assessment approach emphasizes the risk of bias in outcomes for which the results have possibly overestimated or underestimated the true treatment effects. Each domain was labelled as 'low risk' or 'high risk' or 'unclear risk', with the last category indicating that there was insufficient information to judge the potential for bias. The judgment criteria for the six domains of risk of bias are presented Table D in Appendix D.

The risk of bias assessments were conducted by two reviewers independently (Meng Chen & Iris Zhou). Any differences were mediated by Anthony Zhang or Brian May. The results of assessment were presented as a 'Risk of bias graph' figure that illustrates the percentage of studies with each of the judgements, and as a 'Risk of bias summary' table that shows all of the judgements as a cross-tabulation for each study.

### 3.2.6 **Assessment of the quality of reporting**

The quality of reporting was assessed by using a modified version of the CONSORT checklist (total of 39 items) based on the 'CONSORT 2010 Explanation and Elaboration' (Moher et al., 2010). Each item was given a 'yes' or 'no' depending on whether the item was included in the study report. This method is detailed the following published paper:

Chen, M., Cui, J., Zhang, A.L., Sze, D.M., Xue, C.C., May, B.H. (2018). Adherence to CONSORT Items in Randomised Controlled Trials of Integrative Medicine for Colorectal Cancer Published in Chinese Journals. *Journal of Alternative and Complementary Medicine*, 24(2), 115-24. doi: 10.1089/acm.2017.0065.

### 3.2.7 **Analysis and presentation of results*:***

The included studies were categorised into two major groups according to the HM intervention in the test arm:

1. HM alone versus chemotherapy, or placebo, or no treatment;
2. HM combined with chemotherapy versus chemotherapy.

All included studies were narratively synthesized with regard to their characteristics, findings, adverse events, and validity of outcomes. The characteristics of the studies also were summarised in tabular form.

RevMAN 5.1 was used for meta-analysis when there were more than two studies using the same outcome measure. The aim of the meta-analysis was to statistically express whether there was evidence of an effect of the intervention, to estimate the size of the effect and to investigate whether the effect was consistent across studies. The Z-test was used to test the overall effects. The differences between the test groups and the control group were considered to be statistically significant when $p < 0.05$.

Risk ratio (RR) and risk difference (RD) was used for dichotomous outcomes, which included the data for tRR, KPS, BW and chemotherapy adverse events. The RR is the proportion of clinically effective cases/events in the test groups divided by the proportion of clinically effective cases/events in the control groups. The RD is the risk in the test groups minus the risk in the control groups. The RR represents the relative effect between the two groups, whereas RD is the absolute effect. Time-to-event data, such as survival rate, were analysed as dichotomous data. Mean difference (MD) was used for continuous outcomes. This is the absolute difference of the mean value between the test and control groups. A Confidence interval (CI) of 95% was selected and a Fixed-effect or a Random-effect model was used according to the degree of heterogeneity (Higgins & Green, 2011).

Where possible, missing data were analysed as 'intent to treat' (ITT) regardless of which treatment they actually received, time of drop-out, or whether data on the outcome were actually collected. Irretrievable missing data were imputed and sensitivity analysis (worst case/best case scenarios) were undertaken where possible. Heterogeneity of data between studies was examined by $chi^2$ and $I^2$ (%) tests. Large $chi^2$ and small p-values indicate that there is more heterogeneity than explicable by chance. When $p<0.10$ it is statistically significant. $I^2$ is the proportion of heterogeneity. The guideline for the interpretation of $I^2$ was adopted from Cochrane Handbook 5.1.0 (Higgins & Green, 2011).

When $I^2$ was more than 50%, a sensitivity analysis was undertaken and/or the analysis model was changed from Fixed-effect to Random-effect. Publication bias was tested by a funnel plot test if there were more than 10 studies in a meta-analysis group. Overall, if studies were highly heterogeneous i.e. clinically diverse, or poor in methodological quality, or there was serious publication and/or reporting bias, meta-analysis was considered inappropriate.

The results of the meta-analyses were presented in both narrative form and graphically as Forest plots and tables.

## 3.3 Construction of the Chinese herbal medicine short-list

In clinical practice, CHM is commonly applied as a formula that contains several herbs. These herbs are rationally combined to synthesise the efficacy of herbs that have the same or similar clinical effects, and to minimize unwanted side effects (Scheid et al., 2009). The aim of this section was to

identify particular herbs that were the most likely to have contributed to the significant meta-analysis results.

### 3.3.1 **Data collection**

Each single herb that was used in the included studies was entered into a database along with the key features of the study and its outcomes. Frequency of usage was calculated for each herb included in all the studies that contributed to a particular meta-analysis pool. In addition, for the higher frequency herbs, their combinations with each other and with other herbs were calculated.

### 3.3.2 **Sensitivity analysis**

A series of meta-analyses were conducted of studies that contained higher frequency herbs. Studies of multi-ingredient orally administered HM interventions which had plant-based ingredients in common are identified. Then analyses were conducted for groups of studies that employed HM interventions that contained the same herbal ingredients.

The hypothesis was that, if a particular herb was effective (or ineffective) in improving a certain outcome, this would be reflected in the pooled RR outcomes of the studies that employed this herb as an intervention. Therefore, by investigating the pooled RR of all studies that used the same HM in the intervention, specific HMs that showed potential for further research could be identified and shortlisted. Similarly, combinations of herbs could also be identified.

These sensitivity analyses were only conducted for the multi-ingredient oral HM interventions since these are the most comparable in terms of dosage and bioavailability. Injections were excluded since these were likely to vary according to manufacturer and administration.

The following method was used to identify groups of studies that used comparable oral interventions and to assess the effects of individual herbs on the RR for the outcome measure.

Firstly, herbs that were present in two or more of the multi-ingredient oral HM interventions used in the RCTs were identified. Then all combinations of two, three and more herbs present in the HM interventions used in two or more RCTs were identified. Meta-analyses of each of these sub-groups of RCTs were conducted to determine which combinations of HMs produced greater or lesser changes in RR values for the outcome measure. The following multi-stage procedure was used:

1. Pooled RR was calculated for each group of studies that contained the same herb as an ingredient in the intervention. The pooled RRs were listed in ascending order and any significant results were noted.

2. Pairs of herbs present in two or more studies were identified. The pooled RRs were calculated, listed in ascending order, and any significant results were noted.
3. The same procedure was conducted for groups of 3, 4 and more herbs as the data set allowed. This produced a matrix of results for RR, with 95% CI. The heterogeneity also was calculated for each pool.

In assessing the combinations of HMs used in the RCTs, only independent combinations were included. For example, although herb 1 might be paired with herb 2 in the data matrix, all the HM interventions that contained herb 1 + herb 2 may also include herb 3. In such cases, the RR of this group of RCTs reflected the combination of all 3 herbs (as well as any other herbs present), so no independent contribution from herb 1 + herb 2 as a pair could be assessed. Therefore, in this case only the group herb 1 + herb 2 + herb 3 was included in the RR matrix. This procedure eliminated spurious combinations.

### 3.3.3 **Criteria for herbal medicine identification**

To identify HMs for further research, the following criteria were used:

1. Significantly improved (decrease/increase) RR relative to controls;
2. The RR was equal or greater/lesser than the total pooled RR for all multi-ingredient oral HM interventions;
3. Lack of important heterogeneity ($I^2$ not greater than 30%);
4. Consistent RR results at multiple levels of combination i.e. as single herbs, in pairs with other herbs, in triplets with other herbs.

When combinations of herbs produced RRs that were greater/lower than those of the herbs individually, these were identified as possible examples of a synergistic effect.

### 3.3.4 **Searches of experimental literature research on selected herbal medicines**

For the herbs identified as promising for further research based on their use in the clinical trials identified in the systematic reviews, additional searches were conducted of online databases to identify the following types of data:

- *In-vitro* studies of the herb's pharmacological actions;
- *In-vivo* studies of the herb's activity with regard to cancer; and
- Analytical studies of the constituents of the herbs.

In selecting herbs for further research the following two criteria were used:

1. The herb appeared to make a significant contribution to the meta-analysis results based on the sensitivity analyses;

2. There was evidence of effect as well as plausible mechanism(s) of action in terms of the proteins regulated by the herbal extracts or active constituents *in-vitro* and/or *in vivo*.

Herbs that satisfied these conditions were considered for further analysis.

## 3.4 Experimental *in vitro* studies of herbal medicine compounds

Experimental studies to explore the potential anti-tumour effects of HM compounds and the underlying mechanisms were conducted at the Sun Yat Sen University, China. We tested a chemical compound derived from the short-listed of herbs that showed potential anti-cancer effects in CRC to determine its effects in CRC cells *in vitro*. No human or animal model was used and thus ethics application was not sought. The results will be beneficial to inform future studies (outside of the scope of this thesis) on its anti-cancer effects *in vivo* and its molecular mechanisms.

The study was designed with three arms:

1. Test compound arm (matrine, 4 concentrations);
2. Negative control arm (growth medium); and
3. Positive control arm (oxaliplatin, 4 concentrations).

Qxaliplatin (OXA) is a chemotherapy drug known to have an effect in CRC. The comparison with the negative control was to identify whether the test compound had an effect, or no effect; whereas the comparison with the positive control aimed to assess the relative effect against a valid CRC drug.
The following steps were carried out:

### 3.4.1 **Cell line culture**

The human colorectal adenocarcinoma cell lines were cultured in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum in a humidified incubator at 37 °C in a humidified atmosphere (45-65% humidity) with 5 % $CO_2$.

### 3.4.2 **Maintenance of cell lines**

Cells were maintained in cell culture flasks and kept in a humidified atmosphere (45-65% humidity) with 5% $CO_2$ at 37°C inside an incubator. During the culture of the cells, the morphology of the cells was examined (i.e., their shape and appearance) every 1 to 2 days using an optical microscope. The culture medium was pre-warmed to 37$^{o}$C before medium renewal. The medium was changed to fresh medium 2-3 times per week. When the cells' confluence reached 80-90%, they were trypsinized and harvested. Then, the cells were sub-cultured into fresh medium once again to yield a consecutive passage number.

### 3.4.3 **Detaching cell lines and sub-culturing**

The culture medium was removed and discarded. The cell layer in the petri dish was rinsed with phosphate buffered saline (PBS) (approximately 2 mL per 10 $cm^2$ culture surface area) twice to remove any traces of serum, calcium, and magnesium that would inhibit the action of trypsin. Approximately 0.5 mL per 10 $cm^2$ of 0.25% (w/v) trypsin solution was added to the dish. The dish was incubated at room temperature for 5-7 minutes, with checking for dissociation every 30 seconds, until more than 90% of cells were detached. The detached cells were washed with 10-30 mL of PBS and transferred to a 15 mL conical tube. The cells were centrifuged at 400 x g for 5 min using benchtop centrifuges. The supernatant was discarded, and the cells were re-suspended in a minimal volume of pre-warmed fresh culture medium and counted for the total number of cells and percent viability using a hemacytometer. Appropriate aliquots of cell suspension were seeded to new cell culture dishes. The cells were incubated at 37$^{o}$C in a humidified atmosphere containing 5% $CO_2$.

### 3.4.4 **Cell viability assays**

Cytotoxicity activity was assayed by Cell Counting Kit-8 (CCK-8) according to the manufacturer's instructions. Briefly, cells were collected in the phase of logarithmic growth and seeded into 96-well plates at a density of $5\times10^3$ cells/well, and cultured in the DMEM with a 5% (v/v) FBS for 24h. Then they were divided into: vehicle groups (negative control) that contained the same medium with 0.9% saline solution or 5% GS (100 μl/well); OXA groups as positive controls in series of concentrations (100μl/well); and matrine groups in series of concentrations (100μl/well). After incubation for 24, 48 and 72h, the medium was replaced with 10μl of 10% CCK-8 solution dissolved in 90μl PBS and all the cells were incubated for another 1h at 37°C in the dark. Absorbance at 450nm was measured with a microplate reader. All the experiments were repeated three times in sextuplicate wells. To calculate the cell viability, the absorbance readings were plotted and analysed. Results were expressed as a percentage of the control. The inhibition rate was calculated based on the percentage of cell viability for each cell line (means of 6 wells) by the following formula:

$$\text{Inhibition rate(IR)} = (1-\frac{\text{Sample solution OD value}}{\text{Vehicle OD value}})\times 100\% \quad \text{(Formula 1)}$$

### 3.4.5 **Morphological changes of colorectal cancer cells**

CRC cells were seeded into 6-well plates ($1\times10^5$ cells/well, 2 mL/well). Low-dose and high-dose matrine groups were set for each cell line according to the $IC_{50}$ calculated by the CCK-8 assay. OXA was set as the positive control for the CRC cell lines and concentrations were also set by $IC_{50}$. The vehicle group was the negative control. Morphological changes in each cell line were observed by optical microscope every 12h and pictures were taken after 48 h.

### 3.4.6 **Cell cycle analysis**

Flow cytometry (FCM) was used to measure DNA content for cell cycle phases. CRC cells were seeded into 6-well plates ($1\times10^5$ cells/well, 2 mL/well) and allowed to attach for an additional 12h. Then the medium was replaced with matrine (low-dose and high-dose) or OXA. The cells were incubated in humidified atmosphere with 5% $CO_2$ at 37°C for 24 h. The cells were collected, washed twice with cold PBS, and incubated with 500 μL RNase A (50 μg/mL) for 30 min at 37 °C, then the cells were washed again with PBS and fixed in 70% cooled ethanol at 4°C overnight. Finally, they were stained with propidium iodide (PI) at room temperature in the dark for 30min. After the staining, the fluorescence intensity of all the cells were analysed using a FCM. All the experiments were repeated three times.

### 3.4.7 **Apoptosis analysis**

The Annexin V-FITC/PI double staining assay was used to observe cellular apoptosis. CRC cells were seeded into 6-well plates ($1\times10^5$ cells/well). They were exposed to DMEM (control), matrine or OXA (2 mL/well) for 24h. According to the manufacturer's instructions, the cells were harvested by centrifugation, washed twice with cold PBS, then the collected cells were resuspended in 200μl binding buffer and incubated with RNase A (50 μg/mL) at 37°C for 30 min. Then cells were incubated with Annexin V- FITC (5μl) and PI (5μl) for 15min at room temperature in the dark followed by the FCM analysis. All experiments were repeated three times. The apoptotic rate was calculated as the percentage of both early apoptotic cells and late apoptotic cells:

$$\text{Apoptosis \%}=\frac{\text{Early apoptotic cells + Late apoptotic cells}}{\text{Total cell count}}\times100\% \qquad \text{(Formula 2)}$$

### 3.4.8 **Statistical analysis**

Results were expressed as the mean ± standard deviation (SD) using Student's t-test and one-way analysis of variance (ANOVA) with the Dunnett correction. Mean values were calculated from experimental data that were measured in triplicate. The $IC_{50}$ in the CCK-8 assay was calculated by probit regression. All statistical analyses were performed using the SPSS 13.0 software (SPSS Inc., Chicago, IL). P values were two-tailed, and a P value $<0.05$ was considered statistical significance.

The above results are documented and analysed in Chapter 7.

## 3.5 Review of experimental studies of the selected herb in colorectal cancer

This review aimed to comprehensively investigate the literature on the molecular mechanisms of the potential anti-cancer effects in CRC of the selected herb (i.e. *ku shen – Sophora flavescens*). The results will direct future studies.

PubMed and CNKI were searched (to December 2016) to locate experimental studies of the selected herb, its extracts and its principal compounds when tested in CRC cell lines or animal models of CRC.

The search terms included:

- names of the selected herb in Chinese characters, Chinese pinyin, botanical names; or
- the principal constituent compounds in the herb (species); and
- colorectal cancer, or colon cancer, or bowel cancer; and
- *in vitro* or *in vivo* study.

Studies that tested the selected herb, herb extract or its compounds for anti-cancer properties in CRC models *in vitro* or *in vivo* were included. Studies published in Chinese and English were included.

Retrieved citations were combined in an Endnote library. The 'find duplicate' function in the Endnote Library was used to find duplicated copies, which were then excluded. The titles and abstracts of the remaining citations were filtered according to the inclusion criteria. The full texts of all potentially included studies were obtained through RMIT library for further scrutiny to determine whether the inclusion criteria were satisfied.

Data were extracted from each included study using pre-designed data collection forms.

The following items were extracted:

- Authors and year of publication;
- Study design: *in vitro* or *in vivo*;
- Name of cell line, animal model, assay name;
- Name of compound, extract;
- Test compound dose, treatment time, method of experimention, targeted protein(s);
- Experimental results including:
    - anti-proliferative activity;
    - induction of apoptosis;
    - induction of cell cycle arrest;
    - anti-angiogenic activity;
    - anti-metastatic activity;
    - anti-multi-drug resistance activity;

- activities related to cell migration and/or adhesion, and
- any proposed metabolic pathway and any adverse reactions.

# Chapter 4. Systematic Review of Herbal Medicines in the Management of Colorectal Cancer

## 4.1 Introduction to Chapter 4

This chapter presents the results of the search strategy described in Chapter 3 and the meta-analyses of clinical outcomes. Firstly, the characteristics of included studies, characteristics of participants, types of interventions, outcome measures, risk of bias assessments and quality reporting are summarised narratively and tabled. Secondly, meta-analyses were performed where possible for major outcome measures to statistically express whether there was evidence of an effect, estimate the size of the effect, the certainty surrounding the effect, and to investigate whether the effect was consistent across studies. Meta-analysis was carried out in two major groups according to the intervention(s) in the test arm:

1. HM alone versus chemotherapy (CMT), placebo, or no treatment;
2. HM combined with chemotherapy (CMT) versus the same chemotherapy alone.

## 4.2 Summary of search results

The initial systematic search of biomedical databases was conducted in 2011. The numbers of potential citations were as follows: PubMed 280, Cochrane Central 284, ScienceDirect 41, CINAHL 70, PsycINFO 57, CNKI 878, and CQVIP 317. A follow-up search was conducted in December 2013. The above databases were searched with the same search terms and EMBASE was searched as an additional database. The additional citations were: PubMed 32, Cochrane Central 89, ScienceDirect 29, CINAHL 37, PsycINFO none, CNKI 346, CQVIP 34 and EMBASE 20. In addition, 54 potential citations were identified by checking reference lists and print journals in the RMIT University library. Thus, 2,514 potentially relevant citations were identified.

The screening procedures followed the PRISMA Statement flow diagram 2009 (Moher et al., 2009). After the removal of duplicates, non-RCTs and studies that did not satisfy the selection criteria, 88 studies were included in this review (see the flow diagram Figure 4.1). Eighty-four studies were conducted in China and were published in Chinese journals. Eighteen of the studies conducted in China were funded by state or provincial Chinese governments. Four studies were published in English: two were carried out in Japan (Torisu et al., 1990; Kono et al., 2013), one in Germany (Schink et al., 2007), and one in Romania (Cazacu et al., 2003). The publication year of the studies ranged from 1990 to 2013.

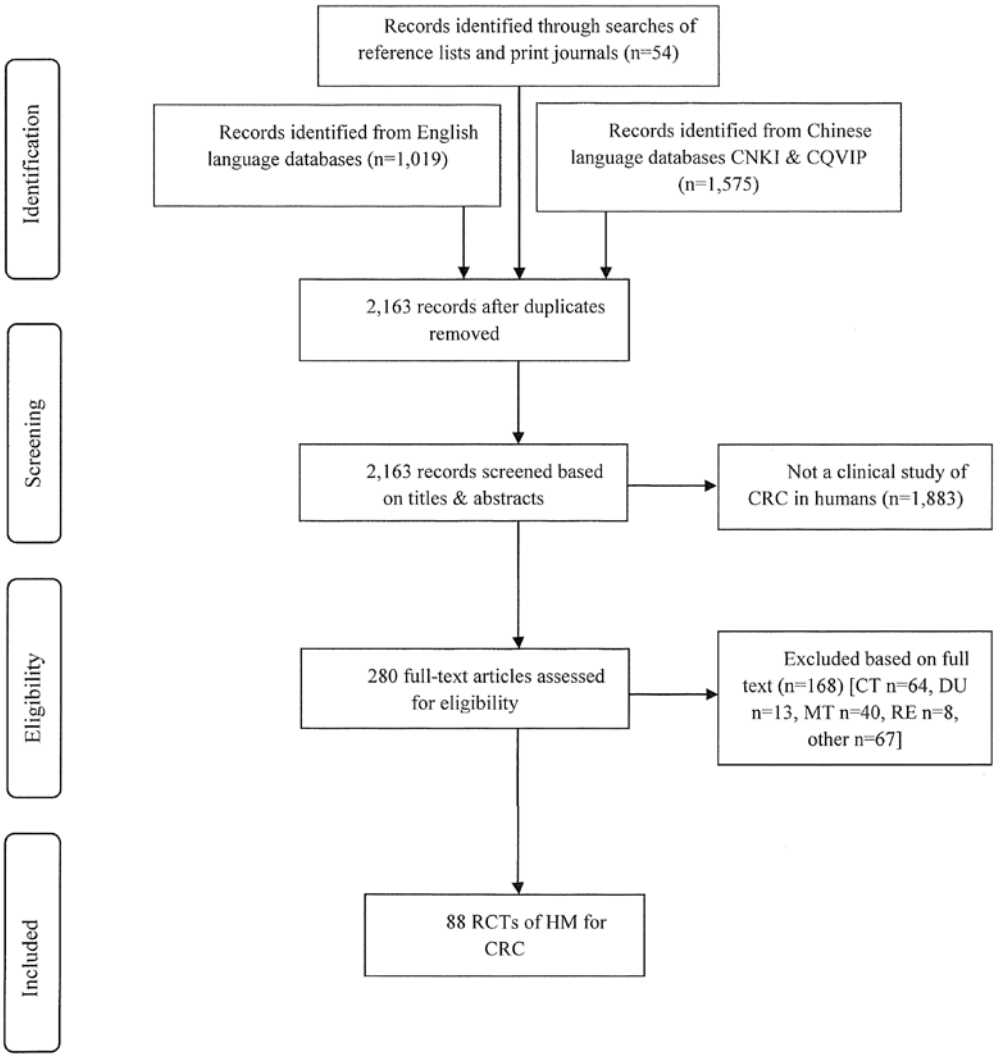


**Figure 4.1: Flow diagram of the search and selection process of RCTs of herbal medicine (HM) for colorectal cancer (CRC)**

## 4.3 Characteristics of study participants

Two out of 88 studies (Wang et al., 2000; Li et al., 2000) employed three arms that compared HM vs. HM + CMT vs. CMT, thereby providing the comparisons HM vs. CMT and HM + CMT vs. CMT. Therefore, there were 90 comparisons in this review. The 88 studies enrolled 6,385 participants with 3,357 participants in the test groups and 3,028 participants in the control groups. All participants were diagnosed with primary CRC by pathology. While most of the participants were in-patients, three studies included some out-patients (Mao & Huang, 2005; Xiao et al., 2003; Yang et al., 2005). Two studies did not provide information about the in- or out- patient status of the participants (Kono et al., 2013; Wang et al., 2007), and one study (Torisu et al., 1990) conducted a long-term trial, so it was

probable that the setting was out-patients. The included studies were mostly at a single centre. Six studies were conducted at two or more centres (Dong et al., 2011; Liu et al., 2013; Wand et al., 2011; Yang et al., 2007; Cao et al., 2011; Kono et al., 2013). Torisu et al. (1990) did not provide information on the site. The characteristics of the 88 included studies are presented in Table E1 and Table E2 in Appendix E. In Table E1, studies in group one investigated HM alone versus chemotherapy (CMT), or placebo, or no treatment, and in Table E2, studies of group two compared HM combined with CMT with the same CMT.

Dukes staging system and the Union for International Cancer Control (UICC) TNM staging system were the most commonly used diagnostic criteria in the included studies. Thirty-six studies used the UICC staging criteria, twenty-eight studies applied Dukes staging criteria, four studies adopted the 1978 Chinese National CRC Staging Standard, which is the same as the Dukes system. Participants were simply categorised as 'advanced CRC' in 16 studies, and no staging information was reported in 4 studies (Mao & Huang, 2005; Zhang et al., 2007; Tang, 2009; Zhang et al., 2010) (Table E1; Table E2 in Appendix E).

Seventy-five studies provided information on participants' ages as means or medians. Participants in 8 studies were under the mean/median age of 50 years; in 69 studies the mean or median age ranged from 50 to 70 years; 2 studies (Li et al., 2007; Schink et al., 2007) enrolled at least some participants who were over 70 years old. Six studies only provided the age of participants as a range without a mean or median. Three studies (Zhang et al., 2010; Wang, 2013; Qin & Zhu, 2011) did not specify participants' ages (Table E1; Table E2 in Appendix E). No studies were excluded based on the age of participants.

For 417 patients from five studies, gender was not specified (Torisu et al., 1990; Cazacu et al., 2003; Zheng & Sun, 2005; Zhang et al., 2010; Qin & Zhou, 2011). Of the remaining 5,968 patients in 83 studies, 3,584 (60.1%) were male and 2,384 (39.9%) were female (Table E1; Table E2 in Appendix E).

Nineteen of the studies did not provide information on participants' physical performance status in the inclusion criteria. Two studies (Cao et al., 2011; Kono et al., 2013) used the ECOG measurement; and sixty-seven studies used the KPS measurement system. For details of participants' characteristics see (Table E1; Table E2 in Appendix E).

### 4.4 Study design and types of interventions

Herbal medicine (HM) alone as the test arm versus chemotherapy (CMT), or placebo, or no treatment was used in 11 studies (Table E1 in Appendix E). These included the two studies that involved three

arms (Li et al., 2000; Wang et al., 2000). In these two studies the comparisons were separated for analysis i.e. HM vs. CMT, and HM plus CMT vs. CMT.

HM combined with CMT as an integrative medicine (IM) intervention was used in the test arm, and was compared to the same CMT regimen as the control group in 79 studies (Table E2 in Appendix E), which included the two studies that involved three arms (Li et al., 2000; Wang et al., 2000).

#### 4.4.1 **Herbal medicine interventions**

Orally administered multi-herb decoctions were the most common interventions and were used in 46 of the 88 studies. Eighteen studies used manufactured orally administered HM tablets; 21 studies used a HM extract that was injected intravenously; enema interventions were investigated in two studies (Li et al.,1999; Wang et al.,1999); and treatment using Javanica oil transcatheter arterial chemoembolization (TACE) for liver metastasis was reported in one study (Zhang & You, 2008) (Table E1; Table E2 in Appendix E).

#### 4.4.2 **Chemotherapy interventions**

Intravenous infusions of combinations of chemotherapeutic agents were the most common CMT interventions. This type of CMT was in two main groups:

1. Oxaliplatin-based regimens: 5-FU+LV+oxaliplatin (FOLFOX), or capecitabine + oxaliplatin (XELOX/CAPOX) were reported in 56 of the included studies; and 16 of these 56 studies used the FOLFOX4 regimen (Table 4.2; Table 4.6).
2. 5-FU regimens administered by intravenous infusion were used in 19 studies. These included: 5-FU + LV regimen (n=8); 5-FU + MMC (n=2); 5-FU + MMC + ADM (n=1); 5-FU + LV + MeCCNu (n=3); 5-FU + MeCCNu + vincristine (n=1); 5-FU + LV + HCPT (n=2); 5-FU + cisplatin (n=1); and single agent 5-FU (n=1). Others included HCPT administered by intravenous infusion (n=1), and orally administered capecitabine (n=1) (Table E1; Table E2 in Appendix E).

Besides intravenous infusion, other chemotherapeutic methods used were: 5-FU enema (n=1), 5-FU+cisplatin intraperitoneal hyperthermic perfusion (n=1), and 5-FU transcatheter arterial chemoembolization (TACE) (n=2). Six studies provided supportive care without any chemotherapy (Table E1; Table E2 in Appendix E). Overall, HMs combined with the FOLFOX regimen in both adjuvant and palliative settings for CRC was the most common combination in the test groups.

### 4.5 Assessment of risk of bias and the quality of reporting for included studies

This section includes three parts: assessment of risk of bias, assessment of the quality of reporting, and a discussion of methodological reporting issues.

### 4.5.1 **Assessment of risk of bias**

Included studies were assessed using the Cochrane Risk of Bias (selection bias, performance bias, detection bias, attrition bias, reporting bias, and other bias) approach in the *Cochrane Handbook Version 5.1.0* (Higgins & Green, 2011). The method is described in Chapter 3. For the summary of risk of bias see Figure 4.2 that is based on the review authors' judgements about each risk of bias item presented as percentages across all 88 included studies, and Table E3 in Appendix E that is based on the review authors' judgements about each risk of bias item for each of the eighty-eight included studies.

All studies claimed to be randomised, but only 27 studies (30.7%) stated a proper method for the generation of a randomisation sequence and the risk of bias was judged to be low. One study used an inadequate method of randomisation and was judged to be high risk (Wang et al., 2000). The other studies did not describe what method was used to generate the randomisation sequence, so the risk of bias was judged to be unclear. Two of the studies (Cao et al., 2011; Kono et al., 2013) described the procedure for allocation concealment and were therefore judged to be low risk. Other studies did not describe a method of allocation concealment and were judged as unclear (Figure 4.2; Table E3 in Appendix E).

A placebo group was used in four of the studies (Torisu et al., 1990; Yang et al., 2007; Cao et al., 2011; Kono et al., 2013) and these studies reported using a double-blinding method. One study (Deng & Shen, 2010) claimed to be a single-blind trial. These five studies were judged to be low risk for blinding of participants. There is generally no blinding in oncology trials (Hind et al., 2008) so the other studies were assumed to be open to participants and personnel. Thus, in these studies there was possible performance bias and detection bias for subjective outcomes such as Karnofsky Performance Status (KPS). Therefore, a high risk of bias was judged for blinding of outcome assessment for subjective outcome measures. Radiologists and pathologists measured the objective outcomes, such as objective tumour response, and laboratory tests. Outcome data on survival rate, and time to progression were obtained from medical records that were unlikely to have been influenced by any lack of blinding. So, the judgement of low risk of bias was made for blinding of outcome assessment of these objective outcomes (Figure 4.2; Table E3 in Appendix E).

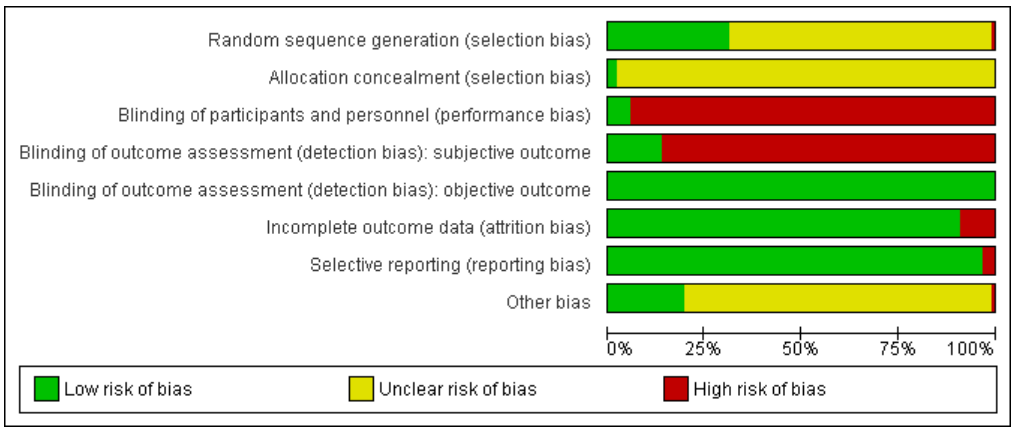


**Figure 4.2: Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies**

Eight studies reported the number of participants who dropped out, were excluded during the trial or were lost to follow-up (Guo et al., 1999; Hou et al., 2009; Li et al., 2007b; Pan et al., 2003; Qin & Zhou, 2011; Xu et al., 2006; Yang et al., 2007; Zhang et al., 2010; Zhang et al., 2010a) but these missing data were not analysed as intent-to-treat. Therefore, a high risk of attrition bias was judged (Figure 4.2; Table E3 in Appendix E).

Only one of the studies (Kono et al., 2013) had published a study protocol. In 83 of the studies that did not publish a protocol, the study objectives and outcome measures were stated in the study method section and were reported in the results section. These studies were judged to have a low risk of selective reporting. In the other three studies (Wang et al., 2000; Li et al., 1999; Qin & Zhou, 2011) there were no pre-statements of the measured outcomes, and these were judged to be high risk of selective reporting (Figure 4.2; Table E3 in Appendix E).

The assessment of ‘other bias’ was based on the source of funding for the study. If a study was funded by an organisation with a vested interest in the outcome, the outcome is more likely to be in favour of the interests of the organisation (Lundh et al., 2012). For studies conducted in China, there is evidence that if the study was funded by an official state source, the study was more likely to be an authentic RCT (Wu et al., 2009). Twelve studies conducted in China claimed that the studies were funded by an official state source and were judged to be low risk of bias in this domain. One study was partly funded by a pharmaceutical company (Cazacu et al., 2003), so there was possible bias related to funding source and it was judged to be high risk of bias. The other studies were judged to be unclear because no funding source had been reported by the authors (Figure 4.2; Table E3 in Appendix E).

### 4.5.2 **Assessment of the quality of reporting**

The issue of whether the conduct of randomised controlled trials reported in Chinese journals have been in accord with international standards was raised in a study by Wu et al. (2009) who reported that only 6.8% of studies published in Chinese medical journals adhered to established RCT methodology, however they found there was no difference between trials of conventional and of traditional medicine

in terms of methodological quality. In their stratification analysis, all pre-market drug trials were authentic RCTs and 51.6% of government funded studies were considered authentic. In China, hospitals are categorised into three grades according to their level and size, the highest level being level 3 which are hospitals affiliated to medical universities and provincial hospitals, other hospitals are classified as level 2 or below. The authors found that hospitals affiliated to medical universities were better in conducting RCTs than other level 3 hospitals and level 2 hospitals.

Since 84 out of the 88 studies included in this chapter were reported in Chinese publications, to further investigate this issue, we focussed on the studies published in Chinese and published in Chinese medical journals and assessed the quality of reporting in each study using the modified CONSORT 2010 checklist (Moher et al., 2010). This section is based on the published paper (Chen et al 2018).

CONSORT 2010 contains 25 items but a number of these include sub-items, so there are 37 items in total. Items 1 to 22 were included in CONSORT 2001 (Moher et al., 2001) and items 23 to 25 (other information) were added in CONSORT 2010. Two sub-items were modified to facilitate assessment and scoring as follows: item 6a was split into 6a (1) Primary & secondary outcomes, and 6a (2) End points (time of data measured); and 13a Flow of participants was divided into 13a (1) Flow diagram as figure, and 13a (2) Flow of participants (verbal) (Table 4.1). For each study the number of items reported was calculated (items 1 to 25, maximum score=39).

Of the 84 studies that were published in China, one study was written in English and two were Chinese theses published in CNKI. These three studies were excluded from the main analysis which focussed on articles in medical journals written in Chinese. In the remaining 81 studies, the total mean score of the CONSORT 2010 check-list items was 10.44 (SD ± 2.40) out of a maximum score of 39. Two items were reported by all studies (items 6a and 22) and 12 items were not reported at all (items 3b, 6b, 7a, 7b, 9, 10, 12b, 13a (1), 14b, 17a, 17b, 23, and 24) (Table 4.1). Reporting rates were above 70% for ten items (1b, 2a, 4a, 4b, 5, 6a (1), 6a (2), 12a, 14a, and 22).

For the items on RCT methodology, item 9 (allocation concealment), and item 10 (implementation of randomisation) were not reported in any study. Item 8a (sequence generation) was reported by 29.6% of studies, item 13b (losses and exclusions) was reported by 12.35% of studies, item 16 (intent-to-treat analysis) was reported by 4.94% of studies, and item 11a (blinding) was reported by 2.47% of studies. These items were the main contributors to the analyses. Eighteen studies mentioned a form of public funding, none mentioned commercial funding, and no studies mentioned published protocols or trial registration (Table 4.1).

**Table 4.1: Scores on Consort 2010 checklist (modified)**

| CONSORT checklist Item no., Criteria | This review (n=81) 1998-2013, n (%) |
|---|---|
| **Title/Abstract** | |
| 1a. Identification as a RCT in the title | 2(2.47) |
| 1b. Structured summary of trial design, methods, results, and conclusions | 69(85.19) |
| **Introduction** | |
| 2a. Background and objectives | 67(82.72) |
| 2b. Specific objectives or hypotheses | 10(12.35) |
| **Methods** | |
| ***Trial design*** | |
| 3a. Description of trial design | 6(7.41) |
| 3b. Changes to methods after commencement | 0(0) |
| ***Participants*** | |
| 4a. Eligibility criteria for participants | 58(71.60) |
| 4b. Setting and locations of data collections | 80(98.77) |
| ***Interventions*** | |
| 5. Details of interventions in each group | 67(82.72) |
| ***Outcomes*** | |
| 6a (1) Primary & secondary outcomes | 81(100) |
| 6a (2) End points (time of data measured) | 79(97.53) |
| 6b. Any changes to trial outcomes after the trial commenced, with reasons | 0(0) |
| ***Sample size*** | |
| 7a. Sample-size determination | 0(0) |
| 7b. Explanation of any interim analyses and stopping guidelines (if applicable) | 0(0) |
| ***Randomisation*** | |
| 8a.* Method used to generate the random allocation sequence | 24(29.63) |
| 8b.* Type of randomisation with details of any restriction (such as blocking) | 2(2.47) |
| 9.* Allocation concealment | 0(0) |
| 10.* Implementation of random-allocation sequence | 0(0) |
| 11a.* Blinding of participants (and others) to group assignment | 2(2.47) |
| 11b.* Description of the similarity of interventions (if relevant) | 1(1.23) |
| 12a. Statistical methods | 75(92.59) |
| 12b. Methods for additional analyses (e.g. sub-group analyses) | 0(0) |
| **Results** | |
| 13a (1). Flow diagram as figure | 0(0) |
| 13a (2). Flow of participants (verbal) | 2(2.47) |
| 13b.* For each group, losses and exclusions after randomisation, with reasons | 10(12.35) |
| 14a. Dates of recruitment and follow-up | 78(96.3) |
| 14b. Why the trial ended or was stopped | 0(0) |
| 15. Baseline and demographic clinical characteristics (table) | 13(16.05) |
| 16.* For each group, number of participants (denominator) included in each analysis and whether used intent-to-treat | 4(4.94) |
| 17a. Outcomes and estimated effect size with 95% confidence interval | 0(0) |
| 17b. For binary outcomes, presentation of both absolute (RD) and relative (RR) effect sizes | 0(0) |
| 18. Sub-group analyses/ancillary analyses | 1(1.23) |
| 19. Adverse events/harms | 5(6.17)[1] |
| **Discussion** | |
| 20. Trial limitations, sources of potential bias, imprecision, etc. | 9(11.11) |
| 21. Generalisation (external validity) of findings | 2(2.47) |

| | |
|---|---|
| 22. Interpretation consistent with results, balancing benefits and harms, and other relevant evidence | 81(100) |
| **Other information** | |
| 23. Registration number, name of trial registry | 0(0) |
| 24. Protocol | 0(0) |
| 25. Funding(public) | 18(22.22) |

* Eight items closely related to RCT methodology. Reference: Moher et al., 2010

### 4.5.3 **Discussion of issues relating to risk of bias and reporting of the RCTs**

Assessment of the quality of a clinical trial depends on whether the design, conduct, and analysis are adequately reported in the publication. There is no uniform international guideline for reporting the results of clinical trials but the CONSORT statement is broadly endorsed by international journals and several editorial groups. It comprises checklists that provide guidance for authors to report their trials clearly and completely (Moher et al., 2010). In addition, the Cochrane collaboration's risk of bias tool provides a convenient method of assessing key aspects of the reporting of RCT methodology which could compromise the results if these methods were not properly adhered to (Higgins & Green, 2011).

An analysis of 70 Cochrane reviews of TCM interventions published during or before 2008 concluded that the results of most of the TCM reviews were inconclusive due to the poor methodological quality of the included studies and the high heterogeneity in the meta-analyses (Manheimer et al., 2009). This judgement mainly relied on the quality of the reporting of these studies in published journal articles. Wang et al. (2007) have shown that only 39.4% of the 30 items on the modified CONSORT checklist were reported across all trials and a number of key components of RCTs were incompletely reported. For instance, sample-size calculation was reported in 1.1% of RCTs; 7.9% reported randomisation sequence; 0.3% mentioned allocation concealment; 0% described the implementation of the random allocation sequence; and 0% conducted analysis using intention to treat. This finding was based on 6,093 identified RCTs of TCM studies from 13 TCM journals published from 1999 to 2004 in mainland China. However, this study also found the quality of reporting of RCTs according to the mean Jaded score improved during this time (1999: $0.85 \pm 0.53$ versus 2004: $1.20 \pm 0.62$, $p<0.001$). Similarly, incomplete reporting of key components of RCTs was found in a subsequent study of 6994 articles that published in Chinese medical journals from 2005 to 2012, using CONSORT 2010 items for scoring (Li et al., 2014).

A major factor in poor reporting is the low level of endorsement of CONSORT in China. Li et al. (2012) reported that 6 of 195 high-impact medical journals (3.08%) mentioned CONSORT in the author guidelines (Li et al., 2012) and Song et al. (2015) reported that 7 of 1221 (0.57%) medical journals endorsed CONSORT (Song et al., 2015). Only 1 of 90 journals of Chinese traditional medicine (the English version only) and one specialist oncology journal endorsed CONSORT.

In this review, the 81 studies that were published from 1990 to 2013 in 53 Chinese journals in China were assessed using a modified CONSORT checklist. No endorsement of CONSORT in the author guidelines was found in any of the 53 journals. Overall, only 5.1 % (2/39) of items were reported by all studies. The incomplete reporting of key components of RCTs, such as 'sample-size calculation', 'randomisation sequence', 'allocation concealment', 'implementation of the random allocation sequence', and 'intention to treat' were similar to the proportions in Wang et al.(2007) and Li et al. (2014).

However, the incomplete reporting of clinical trial information is an international issue. For instance, in 2006, 34% of 616 RCTs indexed in PubMed reported information on the method of assigning participants to comparison groups, 54% defined a primary endpoint, and 45% reported a sample size calculation (Hopewell et al., 2010).

In the assessment of risk of bias there is the issue of whether a study was a real RCT. Proper randomisation that includes adequacy of sequence generation and allocation concealment are critical components of a high quality RCT (Altman, 1991). All the studies included in this review were reported to be randomised trials, but only 24 (29.63%) studies reported a proper method of randomisation sequence generation and only one study reported allocation concealment. This raises the question of whether the remaining studies were inadequately reported RCTs or not really RCTs at all.

Nevertheless, internationally, the reporting of the method of randomisation of participants to interventions is generally inadequate (Moher et al., 2010). For instance, only 9% of 206 reports of supposed RCTs in obstetrics and gynaecology journals described both randomisation sequence generation and allocation concealment (Schulz et al., 1994). Up to 81.3% of RCTs reported in pediatric complementary and alternative medicine journals had unclear allocation concealment (Moher et al., 2002). In a study of oncology RCTs in ten well-known international journals, allocation concealment was reported in 51% of articles, while 31% adequately described randomisation sequence generation (Peron et al., 2012).

Blinding of personnel and participants was also not implemented in all but one study, so the risk of bias for this aspect must be judged as high. However, the lack of blinding appears partly due to the nature of cancer studies (Hind et al., 2008) as well as due to the difficulty of blinding the administration of HMs, especially of a HM decoction which has a very distinct colour and taste. Nevertheless, the use of objective outcomes, such as tumour response and other outcomes that rely on laboratory test results, were considered to warrant a judgement of low risk of bias, because these outcomes were in the form of x-ray images or laboratory test results, which were assessed by specialists who are usually remote from the trial. Therefore, bias in outcome assessment was

considered unlikely and would not be expected to have influenced these objective outcomes. Outcome data on survival rate and time to progression was obtained from medical records, which were also unlikely to have been influenced by lack of blinding.

A low dropout rate was found in most of the 81 studies. All participants were hospital in-patients who were cared for by professional nurses and doctors. In China, these patients tend to be compliant and follow doctors' orders, especially over the relatively short period of these trials. Other factors (e.g. on-time administration of medicine, transportation issues, and the timely treatment of the side effects of chemotherapy) all influence a participant's acceptance of the treatment and reduce the chance of dropouts. Therefore, small numbers of dropouts are not unusual in trials conducted in Chinese hospitals, but the issue of unreported attrition bias remains.

## 4.6 Approach to meta-analyses

Studies were grouped according to the main comparisons and sub-grouped according to the chemotherapy type as follows:

Group 1: HM vs. chemotherapy or no treatment or placebo (11 studies)

Group 2: HM + chemotherapy vs. chemotherapy (79 studies), and sub-groups:

HM combined with local chemotherapy (4 studies)

HM combined with systemic chemotherapy (75 studies)

## 4.7 Results for Group 1: Herbal medicine vs. chemotherapy or no treatment or placebo

In this section, all the studies employed a HM intervention alone as the test group and compared it with a control group that received no specific intervention (4 studies) or a placebo (2 studies) or chemotherapy (5 studies). These studies were sub-grouped into:

1. The influence of HM therapy on immunity in the peri-operative period (3 studies);

2. HM alone as adjuvant therapy for stage II/III CRC patients after radical surgery (3 studies);

3. HM vs. CT for advanced CRC (ACRC) (4 studies);

4. Javanica oil emulsion versus chemotherapeutic drugs for liver metastatsis (1 study).

In sub-groups 1, 2 and 4 the outcomes are presented as a narrative summary with significance tests performed where possible for individual studies. In group 3, meta-analyses were conducted on the pooled data where possible. The characteristics of this group of studies are presented in Table E1 in Appendix E.

### 4.7.1 **Influence of herbal medicine therapy on immunity in the peri-operative period**

Three studies reported on measures of cell-mediated immunity (CMI) in the peri-operative period including NK cell killing activity, the percentages of CD3+, CD4+ and CD8+ cells and the ratio of CD4+/CD8+ cells. In general, improvement in CMI is indicated by increases in NK cell killing activity, and the percentages of CD3+ and CD4+ cells, while increase in the percentage of CD8+ cells and a decrease in the ratio of CD4+/CD8+ cells are indicative of suppression of CMI.

Iscador® M special injection, which is an extract of a fermented aqueous preparation of *Viscum album* L. (mistletoe); *Xuebijing* injection, an extract of a combination of herbs; and *Chang'ai Kangfu* oral decoction, are three different HMs that are used as immune-regulators in peri-operative patients. These HMs were investigated in three separate studies in CRC patients, who had just undergone tumour surgical resection procedures (Schink et al., 2007; Gu & Huo, 2009; Li et al., 2000). The main outcomes were measures of cell-mediated immunity (CMI).

In the study by Schink et al. (2007) patients in the test group received Iscador® M special infusion and general supportive care during the operation, whereas the control group only received general supportive care. Immune status was assessed pre-operation, post-operation and at seven days post-operation (Table E1 in Appendix E). The results showed that the NK cell killing activity (%) pre-operation was not significantly different between two groups (MD -0.10, 95% CI -3.25-3.05, p=0.95). In both groups, the NK cell killing activity dropped at day one post-operation. At day seven, NK cell killing activity in the test group was higher than the pre-operation level, in the control group, NK cell killing activity remained lower than the pre-operation level. Overall, NK cell killing activity was significantly higher in the test group than in the control group at both day one (MD 5.50, 95% CI [1.37, 9.63], p=0.009) and day seven (MD 5.30, 95% CI [0.25, 10.35], p=0.04) after the operation.

In the study by Gu et al. (2009), the effect of *Xuebijing* injection on regulation of cell immunity in peri-operative CRC patients was investigated. In addition to general supportive care, the test group (n=24) received *Xuebijing* injection at one to five days post-operation day, whereas the control group (n=23) only received the same general supportive care as the test group. In both groups, the percentages of T cell subsets (CD4, CD8, CD4/CD8) in peripheral venous blood were measured at one and five days post-operation and compared to the pre-operation values (Table E1 in Appendix E). CD4+ expression in both the test and the control groups was lower at day one and day five post-operation compared to the pre-operation values within the groups. However, CD4+ expression in the test group was significantly more than in the control group at post-operation day one and day five. CD8+ expression was suppressed during the observation period in both the test and the control groups and there was no significant difference between the two groups at both time points. The ratio of CD4/CD8 in both the test and the control groups had decreased at day one and day five post-operation,

compared with the pre-operation values within both groups. The ratio of CD4/CD8 was significantly higher in the test group than in the control group at day one and day five post-operation (Table 4.2).

**Table 4.2: Gu et al. (2009) comparison of T cell subsets and NK cell between test and control group**

| End-point | **CD4 + / % (MD)** | **CD8 + / % (MD)** | **CD4 + / CD8 + (MD)** |
|---|---|---|---|
| Pre-operation | -1.30 [-3.19, 0.59], p=0.18 | -0.61 [-2.73, 1.51], p=0.57 | 0.05 [-0.03, 0.13], p=0.19 |
| Post-operation day1 | 1.87 [0.23, 3.51], *p=0.03 | 1.40 [-0.15, 2.95], p=0.08 | 0.07 [0.01-0.13], *p=0.02 |
| Post-operation day5 | 3.40 [1.36, 5.44], *p=0.001 | -1.11 [-2.41, 0.19], p=0.10 | 0.16 [0.07, 0.25], *p=0.0003 |

*statistically significant; (-): favours control group, (+): favours test group, $p<0.05$: statistically significant, MD: mean difference

The study by Li et al. (2000) tested the effect of oral administration of *Chang'ai kangfu* decoction on CMI for Dukes B/C CRC patients who had undergone curative surgery. In this study, CHM was compared with CMT. The CHM group (n=16) received *Chang'ai kangfu* decoction, while the CMT group (n=17) was treated with 5-FU+MMC as adjuvant CMT. The CMI parameters included T cell subsets (CD3+, CD4+, CD8+, CD4+/CD8+), and NK cell activity in serum at different time points (Table E1 in Appendix E). In addition, thirty healthy adults were recruited as a reference group and blood samples were collected. The results of the T cell subsets and NK cells were referred to as the normal parameters based on this healthy reference group (Li et al., 2000).

The CMI pre-operation parameters in both the CHM group and the CMT group were both lower than in the healthy group. This indicated the cancer patients had lower than normal immunity at baseline. At first week after operation, CD3+, CD4+, and NK cell expression and the CD4+/CD8+ in both the CHM and the CMT groups were lower than the pre-operation values within these groups. Conversely, the CD8+ was increased and higher than in the pre-operation period in both groups. This indicated the host's immunity, especially the CMI, was in a suppressed state after the operation. There was no significant difference between the two groups in the above CMI parameters. At the first month post-operation, CD3+, CD4+, and NK cell expression as well as the ratio CD4+/CD8+ recovered and were higher than pre-operation values within the CHM group. The CMT group recovered slowly and CMI parameters still remained lower than the pre-operation level within this group at this end-point. There was a significant difference between two arms at the first month after operation. The CMI of participants continuously recovered in both the CHM and CMT groups until the third month post-operation. At this point there was no significant difference between the two groups in CMI parameters, except for CD3+ cell which remained higher in the test group. At three months after the operation, the parameters in the test group were not different to the healthy group, but the control group was still slightly below the healthy group (Table 4.3). This study showed the restoration of the CMI was quicker in the CHM group than in the CMT group during the post-operative period.

**Table 4.3: Li et al. (2000) comparison of T cell subsets and NK cell between test and control group**

| End-point | CD3+ | CD4+ | CD8+ | CD4+/CD8+ | NK cell |
|---|---|---|---|---|---|
| Pre-operation | -1.10 [-7.05, 4.85], p=0.72 | -0.80 [-5.78, 4.18], p=0.75 | 1.40 [-3.23, 6.03], p=0.55 | -0.03 [-0.31, 0.25], p=0.83 | 0.70 [-2.81, 4.21], p=0.70 |
| Post-operation first week | -1.10 [-7.05, 4.85], p=0.72 | -0.90 [-6.23, 4.43], p=0.74 | -1.40 [-7.88, 5.08], p=0.67 | 0.17 [-0.22, 0.56], p=0.40 | -0.70 [-4.19, 2.76], p=0.69 |
| Post-operation first month | 8.2 [3.49, 12.91], *p=0.0006 | 8.70 [2.40, 15.00], *p=0.007 | -2.90[-8.63, 2.83], p=0.32 | 0.41 [0.06, 0.76], *p=0.02 | 5.20 [1.73, 8.67], *p=0.003 |
| Post-operation second month | 7.60 [2.27, 12.93], *p=0.005 | 3.40 [-0.21, 7.01], p= 0.06 | -3.80 [-9.88, 2.28], p= 0.22 | 0.24 [-0.16, 0.64], p=0.24 | 1.60 [-1.35, 4.55], p=0.29 |
| Post-operation third month | 9.3, [3.66, 14.94], *p=0.001 | 1.5, [-3.31, 6.31], p=0.54 | 1.7 [-3.08, 6.48], p=0.49 | 0.05 [-3.4, 0.44, p=0.80 | 1.70 [-2.05, 5.45], p=0.37 |

*statistical significant; (-): favours control group, (+): favours test group, p<0.05: statistically significant, MD: mean difference

### 4.7.2 **Discussion of effects of herbal medicines on immunity after surgical resection in colorectal cancer**

Surgery is the primary treatment for loco-regional CRC (Saif et al., 2006). Also, up to 76.6% of patients who have distant metastasis of CRC receive surgical resection of the primary tumour (Lin et al., 2011). Although surgical removal of the primary tumour has a curative intent or aims to stop disease progression, the surgical procedure itself and the physical and psychological response to the surgical stress could suppress the patient's immunity, especially the CMI. This immunosuppression response during the peri-operative period is one of the important risk factors for inducing micrometastases that can initiate new metastases. Thus, up-regulating the patient's immunity, especially the CMI, during the peri-operative period may be critical to long-term cancer prognosis (Neeman & Ben-Eliyahu, 2012).

In these three studies, the investigators observed the suppression of CMI induced by the surgical procedure. They found the suppression of NK cell activity and T cell subsets in the test groups were less than in the control groups after the operation. The NK+ cells, CD4+ cells and the ratio of CD4+/CD8+ recovered more quickly in the HM groups than in the CMT groups. These outcomes suggested that the HM interventions in each of these studies may have up-regulated CMI during the peri-operative period and post-operative period. The results were consistent with other studies (Ogawa et al., 2000; Neeman et al., 2012).

### 4.7.3 **Herbal medicine alone as adjuvant therapy for stage II/III colorectal cancer patients after radical surgery**

Three studies made comparisons between HM interventions and control groups that received no treatment or placebo in stage II and III CRC patients who had received radical surgery. The outcomes included overall survival (OS) and disease-free survival (DFS).

In Shen et al. (2003) (n=101), the authors investigated the effect of *Changbian* capsule on the three-year survival rate compared to patients who received no specific treatment as the control group. The data were treated as ITT (Table E1 in Appendix E). The follow-up rates for the test group and control group were 78.43% and 80.00% respectively (Shen et al., 2003). The three-year survival rate for Dukes B patients was 74.07% (20/27) for the test group and 76.92% (20/26) for the control group (RR 0.96, 95% CI [0.71, 1.31], p=0.81); for Dukes C it was 70.38% (17/24) for the test group and 41.67% (10/24) for the control group (RR 1.70, 95% CI [0.99, 2.91], p=0.05). Therefore, the three-year survival rate was significantly higher in the test group than in the control group in these Dukes C patients.

The result of the three-year disease-free survival (DFS) showed there was no significant difference between the two treatment groups (all stages) (RR 1.31, 95% CI [0.96, 1.78], p=0.09). The stratified analysis found the *Changbian* capsule treatment significantly prolonged the three-year DFS for Dukes C patients (RR 2.13, 95% CI [1.14, 3.96], p=0.02), but no benefit was found for Dukes B patients (RR 0.96, 95% CI [0.69, 1.35], p=0.83). No obvious adverse reactions due to *Changbian* capsule were found (Shen et al., 2003).

Yang et al. (2007) studied the influence of *Quxie* Capsule on median time to progression (TTP), KPS, and immunity, in 23 participants who received *Quxie* Capsule for 6 months consecutively. The control group (n=21) only received an identical placebo capsule for the same period (Table E1 in Appendix E). All participants were TNM II or III. They were followed up for three years. The results showed a statistically significant improvement (MD 12.5, 95% CI [5.75, 19.22], p=0.0003) in the mean TTP for the test group (31.500 ± 7.778 months) compared with the control group (19.000 ± 13.856 months). After six months treatment, the test group showed greater improvement in B-lymphocytes (MD 2.54, 95% CI [0.92, 4.15], p=0.02) compared with the control group, as well as an improved CD4 to CD8 ratio (MD 0.44, 95% CI [0.03, 0.86], p=0.04). There was no difference between treatment groups in CD3+ (MD 1.03, 95% CI [-8.78, 10.84], p=0.84), CD4+ (MD 2.62, 95% CI [-6.46, 11.70], p=0.57), CD8+ (MD 2.57, 95% CI [-3.07, 8.22], p=0.37), NK cell activity (MD -2.64, 95% CI [-9.26, 3.98], p=0.43), or in KPS scores (MD 1.23, 95% CI [-2.39, 4.86], p=0.50). No obvious adverse events (AEs) due to *Quxie* Capsule were found (Yang et al., 2007).

Torisu et al. (1990) studied 111 post-surgical participants (Dukes C) who were randomly allocated with 56 in the test group and 55 in the control group. The test group received polysaccharide K powder. The control group received an identical placebo powder. The DFS and OS were examined and compared during the eight years follow-up (Table E1 in Appendix E). The Kaplan-Meier curves showed that the test group had higher disease-free survival (DFS) and overall survival (OS) than the control group, and the authers claimed that the differences between the two groups were significant ($p$<0.05), but the authors did not provide the numeric values (Torisu et al., 1990).

### 4.7.4 **Discussion of results for herbal medicine versus placebo or no treatment post-surgery**

These three studies compared different HMs to placebo or no treatment (Shen et al., 2003; Yang et al., 2007; Torisu et al., 1990). The results showed that the HMs significantly improved DFS and OS for Dukes C CRC patients who were post curative surgery. The results were consistent with a study that analysed data from a ten-year follow-up case-control study which found that HM had benefit for overall survival for stage III CRC patients after surgery compared with the surgical treatment alone (McCulloch et al., 2011). In a large international RCT, adjuvant FOLFOX4 was found to only benefit overall survival and disease-free survival in stage III CRC patients after radical surgery. However, the benefit was unclear in stage II patients (Andre et al., 2009).

In the above studies, Shen et al. (2003) found a similar effect with improved disease-free survival for the sub-group of Dukes C patients only, and the study by Torisu et al. (1990) reported improved overall survival in Dukes C patients. Yang et al. (2007) found improved TTP for HM treatment of stage II/III CRC patients post radical surgery. However, these results were based on single studies of different HMs. These HMs require further evaluation in future studies.

### 4.7.5 **Herbal medicine vs. chemotherapy or supportive care for advanced colorectal cancer**

Four RCTs provided data for comparisons between Chinese HMs and CMT or supportive care in advanced CRC (ACRC).

Two studies (Xion et al., 2003; Yang et al., 2005) examined the efficacy of *Changfukang* capsule compared with 5-FU in ACRC treatment (Table E1 in Appendix E). The pooled results at the end of treatment showed there was no statistical difference in tumour response rate (tRR) between the treatment groups (RR 1.15, 95% CI [0.74, 1.78], p=0.55, $I^2$=0%). For carcinoembryonic antigen (CEA), there was no difference between groups (MD -9.61, 95% CI [-27.74, 8.51], p=0.30, $I^2$=25%). After treatment, the test group was significantly higher in KPS scores than the control group (MD 22.46, 95% CI [19.81-25.11], p<0.00001, $I^2$=27%).

Mutouhui Glycoside Pill (an extract of *Patrinia heterophylla* root) was compared with CMT (5-FU+LV) for ACRC (Wang et al., 2000). The outcomes include: tRR, one-, two- and three-year OS, KPS, T cell subsets and CEA (Table E1 in Appendix E). There was no significant difference between the two treatment groups in tRR (RR 0.67, 95% CI [0.30, 1.48], p=0.32); one-year OS (RR 0.96, 95% CI [0.68, 1.36], p=0.82); two-year OS (RR 0.90, 95% CI [0.57, 1.42], p=0.65); or three-year OS (RR 0.82, 95% CI [0.43, 1.59], p=0.56). The incidence of increase in KPS scores of 10 points or more was significantly higher in the CHM group than in the CMT group (RR 4.45, 95% CI [1.84, 10.74], p=0.0009). The immune function measures showed no significant differences between the two groups before treatment but were significantly increased in the CHM group after treatment (Table 4.4). CEA readings were not significantly different between the two arms before treatment (MD 3.40, 95% CI [-17.88, 24.68], p=0.75) or after treatment (MD 5.10, 95% CI [-15.69, 25.89], p=0.63).

**Table 4.4: Wang et al. (2000) comparison of T cell subsets and NK cell between test and control group**

| Cell type | Before treatment | After treatment |
|---|---|---|
| NK cell activity (%) | 3.00 [-1.32, 7.32], p=0.17 | 14.00 [10.08, 17.92], *p<0.00001 |
| CD3+ (%) | -1.50 [-4.88, 1.88], p=0.38 | 8.2 [5.31, 11.09], *p<0.00001 |
| CD4+ (%) | -3.30 [-7.10, 0.50], p=0.09 | 7.80 [3.58, 12.02], *p=0.0003 |
| CD8+ (%) | 0.60 [-2.08, 3.28], p=0.66 | -5.6 [-7.53, -3.67], *p<0.00001 |

*statistically significant; (-): favours control group, (+): favours test group, p<0.05: statistically significant, MD: mean difference.

Hou et al. (2009) investigated the efficacy of *Fuzhengxiaoai* Decoction I in ACRC patients who could not accept chemotherapy due to poor performance status. The control group only received the best supportive care. All patients were followed up for two to ten months. Outcome measures were tRR, OS, QoL and CHM toxicity (Table E1 in Appendix E). There was no significant difference between groups for tRR. The median overall survival was not significantly different between the two groups (test group 6.5 months vs. control group 5.5 months $p>0.05$). For KPS score, the control group was higher at baseline and there was no difference between groups after treatment. However, in the test group, the KPS score was not significantly different before treatment versus after treatment (MD 1.14, 95% CI [-1.57, 3.85], p=0.41), but it significantly decreased in the control group (MD -7.73, 95% CI [-2.06, -13.40], p=0.008). The AEs due to *Fuzhengxiaoai* Decoction I were mild with two cases of vomiting reported, and no serious AEs observed.

### 4.7.6 **Discussion of Chinese herbal medicine vs. chemotherapy or supportive care**

The two CHM tablets (*Changfukang* capsule, *Mutouhui* Glycoside Pill) did not demonstrate greater benefits in improved tRR, decreased serum CEA, or prolonged overall survival for ACRC compared with 5-FU regimens. But they appeared not to be significantly inferior to the 5-FU regimens in treating ACRC, and they improved ACRC patients' KPS which is correlated with improvement of quality of

life (QoL) (Granda-Cameron et al., 2008). Patients in the *Mutouhui* Glycoside Pill group also showed better immune status than patients in the chemotherapy group.

These CHM treatments may be an option for ACRC patients who are intolerant of chemotherapy. In the terminal stage of ACRC, alleviation of cancer symptoms and maintaining or improving quality of life (QoL) are treatment priorities. In terms of KPS, all the CHMs showed improvement relative to the controls. Therefore, further studies in advanced and terminal stage patients should be considered.

### 4.7.7 **Javanica oil emulsion versus chemotherapeutic drugs for liver metastasis**

In one study, CRC patients who had liver metastasis were treated with transcatheter arterial chemoembolization (TACE). In the test group, Javanica oil emulsion was used alone, whereas a combination of chemotherapeutic drugs (oxaliplatin, 5-FU and pirarubicin) was used in the control group. Patients in both groups had the procedure an average of 2.5 times (Table E1 in Appendix E). The results showed an improvement of KPS scores of ten points or more was significant in favour the test group compared with the CMT control group (RR 2.00, 95% CI [1.19, 3.36], p=0.009). The incidence of medium to severe post-interventional symptoms were significantly lower in the test group compared to the control group: fever (over 38 ºC) (RR 0.50, 95% CI [0.31, 0.80], p=0.004); abdominal pain (needing treatment) (RR 0.42, 95% CI [0.22, 0.81], p=0.009); and nausea and vomiting (over 3 times/day) (RR 0.38, 95% CI [0.21, 0.67], p=0.0008). Neither tumour response rate (tRR) nor overall survival (OS) were reported (Zhang & You, 2008).

### 4.7.8 **Discussion of Javanica oil emulsion**

TACE is a medical procedure that restricts a tumour's blood supply and is commonly used for liver tumour treatment (Lo et al., 2002). Javanica oil is extracted from *Brucea javanica* seed. Its bioactive components are oleic acids and linoleic acids. Javanica oil emulsion has been found to reverse multidrug resistance and inhibit DNA topoisomerase in several sensitive and resistant tumour cells such as K562/A02 (drug resistant human erythroleukemia cells), MCF 7/ADM (drug resistant human breast adenocarcinoma cells) and KB/VCR (drug resistant human oral squamous carcinoma cells) *in vitro* (Tang et al., 2001). In China, Javanica oil emulsion has been used by by intravenous drip or TACE for the treatment of several cancers including liver cancer, stomach cancer, lung cancer, esophageal cancer, bladder cancer, ovarian cancer and prostate cancer (Zhao et al., 2014).

Nearly 50% of metastasis in CRC is in the liver and TACE is an alternative therapy for patients whose liver tumours are unresectable, or show no reponse to systemic chemotherapy or to local liver therapies as adjuncts to systemic chemotherapy (Belinson et al., 2012). The severity of post-interventional symptoms induced by CMTs used in TACE is one of the important factors affecting quality of life. According to Zhang (2008), the Javanica oil emulsion TACE for CRC with liver

metastasis showed a significant alleviation of post-interventional symptoms, and eventually elevated KPS. In other RCTs, Javanica oil emulsion TACE was reported to be not inferior to TACE using conventional drugs, in terms of tumour response rate (Tian et al., 2008) and overall survival (Yang et al., 2011) in treating primary liver tumours. It appears that Javanica oil emulsion is a potential surrogate for conventional drugs for primary or secondary liver tumours, especially for patients who are older or cannot tolerate conventional drugs. Large RCTs are needed to test these findings and determine the long-term toxicity profile of Javanica oil emulsion TACE.

## 4.8 Results for Group 2: Herbal medicine plus chemotherapy vs. chemotherapy

In this section, all the 79 included studies employed a Chinese HM intervention (CHM) in combination with chemotherapy (CMT) as the test group, and the control group received the same CMT intervention. Four of the 79 studies (Meng et al., 2003; Zeng et al., 2010; Wang et al., 1999; Li et al., 1999) employed local CMT, rather than systemic CMT administered by intravenous drip. Therefore, these four studies were analysed separately below. For the characteristics of the group 2 studies see Table E2 in Appendix E.

### 4.8.1 **Chinese herbal medicine combined with local delivery of chemotherapy**

In one RCT, participants in the test group were treated with a CHM decoction (500 mL) mixed with 5-FU (20mg/kg) via enema and the mixture was retained in the rectum for two hours, once a day for seven days before surgical resection. Paticipants in the control group were treated with 5-FU (20mg/kg) combined with 500 mL saline via enema using the same method (Table E2 in Appendix E). Histological studies of the resected tumours, tumour response rate (tRR) of unresectable tumours, immune response, and one-, three-, and five- year overall survival (OS) were reported. According to the *Chinese Guidelines for the Diagnosis and Treatment of Commonly Encountered Malignancies* (The Ministry of Health PRC, 1991), the histological appearance of resected tumour tissues is classified from grade 1 to 3. When more immune cells appear in tissues surrounding the tumour, a higher grade is awarded. Thirty-five out of 46 participants in the test group, and 31 out of 40 participants in the control group had their tumours resected by surgery. This histological study found that 18 out of 35 resected tumour tissue samples were classed as grade 3 in the test group compared with five out of 31 in the control group. This difference was significant (RR 3.19, 95% CI [1.34, 7.57], p=0.009) (Wang et al., 1999).

There was no statistical difference between the two treatment groups for one-year overall survival (OS) (RR 1.13, 95% CI [0.70, 1.80], p=0.62), three-year OS (RR 1.16, 95% CI [0.63, 2.15], p=0.64), and five-year overall survival (OS) (RR 1.16, 95% CI [0.44, 3.06], p=0.77). The ratio of CD4+ to CD8+ and the NK cell activity were significantly increased in the test group compared with the control group. There were 20 participants whose tumours were unresectable, 11 in the test group and none in

the control group. The tumour response rate was not significantly different between the two groups (RR 2.05, 95% CI [0.51, 8.16], p=0.31). The authors concluded that the pre-operation combination enema treatment improved the participants' immunity, and would reduce the risk of tumour residue spreading during the operation.

Meng et al. (2003) investigated the effect of CHM combined with chemo-therapeutic drugs in TACE for the treatment of CRC liver metastasis. The outcomes for tumour response rate (tRR), quality of life (QoL), side effects, one-, two-, and three- year overall survival (OS), and disease progression (i.e. the number of other organs that showed further metastasis during the study period) were assessed (Table E2 in Appendix E). The results found there was no significant difference between the two groups for: tRR (RR 1.90, 95% CI [0.55, 6.54], p=0.31); one-year OS (RR 1.02, 95% CI [0.67, 1.56], p=0.92); two-year OS (RR 1.27, 95% CI [0.54, 2.97], p=0.59); and three-year OS (RR 1.43, 95% CI [0.23, 7.61], p=0.68). The median OS was 18.6 months for the test group and 14.3 months for the control group (no range data available). The European Organisation for Research Treatment of Cancer (EORTC) quality of life questionnaire QLQ-C30 was used. The total improvement rate was 40.5% for the test group, and 31.8% for the control group but it was not possible to test the statistical significance. For disease progression, in the test group, metastases in other organs were observed in two participants; in the control group, five participants showed metastasis occurrence in other organs (Meng et al., 2003).

The combination of TACE treatment appeared to produce a better outcome in terms of quality of life, fewer metastases occurring during the trial, and extended median overall survival for the test group compared with the control group. However, there was no statistically significant benefit for improved tumour response rate or for one-year, two-year, or three-year overall survival. For each of these measures there was a trend towards improvement but the small sample size may have resulted in a lack of statistical power.

Zeng et al. (2010) investigated an oral CHM decoction combined with 5-FU plus cisplatin intraperitoneal hyperthermic perfusion chemotherapy for post-operation ACRC. The control group only received the intraperitoneal hyperthermic perfusion chemotherapy. The outcomes for quality of life (QoL), adverse events (AEs), immune response, and one-year- and three-year overall survival (OS) were reported (Table E2 in Appendix E). For QoL, 34 out of 54 participants in the test group gained more than 10 points on KPS compared with 14 out of 50 in the control group (RR 2.38, 95% CI [1.47, 3.86], p=0.0004). The HM intervention significantly alleviated the following AEs compared with the control group: neutropenia (RR 0.42, 95% CI [0.23, 0.77], p=0.005); thrombocytopenia (RR 0.11, 95% CI [0.04, 0.35], p=0.0001); diarrhoea (RR 0.46, 95% CI [0.26, 0.82], p=0.009); nausea and vomiting (RR 0.46, 95% CI [0.28, 0.75], p=0.002); and neurotoxicity (RR 0.45, 95% CI [0.20, 1.02], p=0.05). The addition of the CHM significantly improved CMI as measured by the ratio of CD4+ to

CD8+ cells (MD 0.32, 95% CI [0.23, 0.41], p<0.00001); and NK cell activity (MD 9.40, 95% CI [6.04, 12.76], p<0.00001), compared to the control group. The addition of the CHM also significantly prolonged the one-year OS (RR 2.67, 95% CI [1.25, 5.68], p=0.01) and the three-year OS (RR 3.26, 95% CI [1.10, 9.64], p=0.03), compared to the control group (Zeng et al., 2010).

The study by Li and Li (1999) enrolled 96 patients who had advanced CRC. All patients received 5-FU intraperitoneal infusion plus mitomycin intravenous infusion. The test group of 60 patients additionally received a CHM decoction via retention enema. The outcomes of clinical symptoms, tRR, and one-year, two-year, and three- year OS were observed after 10 weeks of treatment (Table E2 in Appendix E). Clinical symptoms (abdominal ache, tenesmus, and sepsis) were alleviated for 73.3% of the test group, and 47.2% of the control group. The tRR was not statistically different between groups (RR 1.54 [0.98, 2.44], p>0.050, but the OS was significantly better in the test group at one-year (RR1.49 [1.11, 1.99], p<0.01), two-years (RR 2.03 [1.28, 3.22], p<0.01), and three-years (RR1.93 [1.04, 3.61], p<0.05) (Li & Li, 1999).

### 4.8.2 **Discussion of local chemotherapy plus Chinese herbal medicine**

Three types of local administration of chemotherapeutic agents combined with CHM interventions were compared to local chemotherapy alone: intraperitoneal hyperthermic perfusion chemotherapy, TACE and enema. Both intraperitoneal perfusion chemotherapy and TACE have been reported to improve oncological outcomes in CRC management (Belinson et al., 2012; Sloothaak et al., 2014).

The combination treatments improved quality of life in all four studies, but there were no differences in tumour response rate for test groups compared with the control groups in three studies. There were inconsistent results for overall survival (OS), with two studies reporting the combination treatments prolonged OS, but the other two studies found no difference in OS between the two groups. Factors such as stage of the disease, integrative intervention methods, and selection of chemotherapy agents may have influenced the outcomes. In the two studies that investigated local administration of HM via enema, the treatments appear to have relieved local symptoms and increased the number of immune cells surrounding the tumour tissues. The available information on these therapeutic methods is limited, so further studies are needed.

### 4.8.3 **Chinese herbal medicine combined with systemic chemotherapy**

The 75 studies of HM combined with systemic chemotherapy all employed Chinese HM (CHM) (Table E2 in Appendix E). Studies were grouped for meta-analysis according to the major outcomes as follows:

- Tumour response (48 assessible studies)
- The bio-marker, carcinoembryonic antigen (CEA) (9 assessible studies)

- Survival rate (31 assessible studies)
- Quality of life (53 assessible studies)
- Effect on adverse events associated with chemotherapy (47 assessible studies)
- Effect on immunity (26 assessible studies)

Within each of these main outcomes, studies were further sub-grouped for meta-analysis according to the chemotherapy regimen or other factors as appropriate. Meta-analysis methods were described in Chapter 3.

#### *4.8.3.1 Effect on tumour response rate and complete response*

All studies that reported numerical results for tumour response rate (tRR) and complete response (CR) were meta-analysed as a whole group. They were then divided into sub-groups based on the chemotherapy regimen and whether the patients had not been previously treated with CMT or whether they had previously received CMT since these factors were likely to influence tumour response. The bio-marker CEA, which was reported in nine studies, was analysed separately.

In total, 48 studies reported and evaluated tRR. Forty-two of these studies used the WHO solid tumour response criteria (Miller et al., 1981), which used the following categories: complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). The other six studies (Qin & Zhou, 2011; Wang et al., 2011; Wang, 2013; Wang & Peng, 2012; Zeng et al., 2013; Zhang et al., 2010) used the Response Evaluation Criteria in Solid Tumours (RECIST) criteria, which uses similar categories (Park et al., 2003) so these were combined. In the palliative treatment setting, the tRR is an important outcome measurement that is used to assess the effectiveness of the anti-tumour treatment. The tRR is the sum of the CR plus PR, and was analysed as dichotomous data. Two sub-groups were based on CMT type: oxaliplatin group and non-oxaliplatin group.

When the Risk Ratio (RR) is more than 1 it favours the test group. The RR translated to a percentage, (RR-1) % provides the relative improvement rate (IR), which is also called the relative risk reduction (RRR). The Risk Difference (RD) is the difference in risk of the event in the test and control groups, which is also called ‘absolute risk difference’.

**Table 4.5: Meta-analysis results of tumour response rate for herbal medicine combined with systemic chemotherapy (48 studies)**

| **Outcomes** | **No. studies (participant No.)** | **RR (95% CI, FE), $I^2$; IR%** | **RD (95% CI, FE), $I^2$** |
|---|---|---|---|
| Total group (tRR) | 48 (3487) | 1.33 [1.23, 1.45], *p<0.00001, $I^2$=0%; 33%. | 0.12 [0.09, 0.15], *p<0.00001, $I^2$=0% |
| Total group complete remission (CR) | 47 (3367) | 1.81 [1.32, 2.47], *p=0.0002, $I^2$=0%; 81%. | 0.02 [0.01, 0.03], *p=0.005, $I^2$=0% |

| Outcomes | No. studies (participant No.) | RR (95% CI, FE), $I^2$; IR% | RD (95% CI, FE), $I^2$ |
|---|---|---|---|
| Oxaliplatin regimens sub-group (tRR) | 42 (3083) | 1.31 [1.20, 1.42], *p<0.00001, $I^2$=0%; 31%. | 0.11 [0.08, 0.14], *p<0.00001, $I^2$=0% |
| Oxaliplatin regimens sub-group (CR) | 41 (2963) | 1.80 [1.29, 2.51], *p=0.0005, $I^2$=0%; 80%. | 0.01 [0.00, 0.03], *p=0.03, $I^2$=0% |
| Non-oxaliplatin regimens sub-group (tRR) | 6 (404) | 1.73 [1.31, 2.28], *p<0.0001, $I^2$=0%; 73%. | 0.19 [0.10, 0.27], p<0.0001, $I^2$=0% |
| Non-oxaliplatin regimens sub-group (CR) | 6 (404) | 2.31 [1.01, 5.29], *p=0.05, $I^2$=0%; 131%. | 0.04 [0.00, 0.08], *p=0.03, $I^2$=0% |
| tRR (previously untreated patients) | 7 (475) | 1.25 [1.01, 1.55], *p=0.04, $I^2$=0%; 25%. | 0.10 [-0.01, 0.18], *p=0.03, $I^2$=0% |
| CR (previously untreated patients) | 5 (281) | 1.98 [0.81, 4.87], p = 0.13, $I^2$ = 0% | 0.02 [-0.02, 0.06], p=0.30, $I^2$=0% |
| tRR (previously treated patients) | 3 (188) | 1.65 [0.94, 2.91], p=0.08, $I^2$=0%. | 0.14 [0.02, 0.25], *p=0.02, $I^2$=0% |
| tRR (Aidi injection) | 2 (191) | 1.31 [0.91, 1.88], p=0.15, $I^2$=0%. | 0.11 [-0.03, 0.25], p=0.12, $I^2$=0% |
| tRR (Kang'ai injection) | 2 (103) | 1.07 [0.71, 1.61], p=0.76, $I^2$=0%. | 0.03 [-0.16, 0.22], p=0.76, $I^2$=0%. |
| tRR (Jianpi huoxue decoction) | 2 (142) | 1.16 [0.75, 1.82], p=0.50, $I^2$=0%. | 0.06 [-0.11, 0.23], p=0.49, $I^2$=0%. |
| CR (Jianpi huoxue decoction) | 2 (142) | 0.74 [0.12, 4.37], p=0.74, $I^2$=0%. | -0.01[-0.08, 0.06], p=0.75, $I^2$=0%. |

*statistically significant; RR: risk ratio; IR: improvement rate; RD: risk difference; FE: fixed effect; $I^2$: the proportion of heterogeneity.

The 48 studies that reported on tRR included 3,487 participants who were at various stages of the disease and had received different chemotherapy regimens. The pooled result for the tRR (measured as RR and RD) showed that integration of CHM with chemotherapy produced a significant improvement in the tRR (RR 1.33, 95% CI [1.23, 1.45), $I^2$=0%) without heterogeneity (Table 4.5; Figure 4.3). The relative improvement rate (IR) between groups was 33% in favour of the combined therapy groups and there was absolute increase of 12% in the incidence of tRR in the combined therapy groups (RD 0.12, 95% CI [0.09, 0.15], $I^2$=0%) without heterogeneity. In other words, we can be 95% sure that between 9% and 15% (average 12%) of the people who received combination therapy experienced increased tRR.

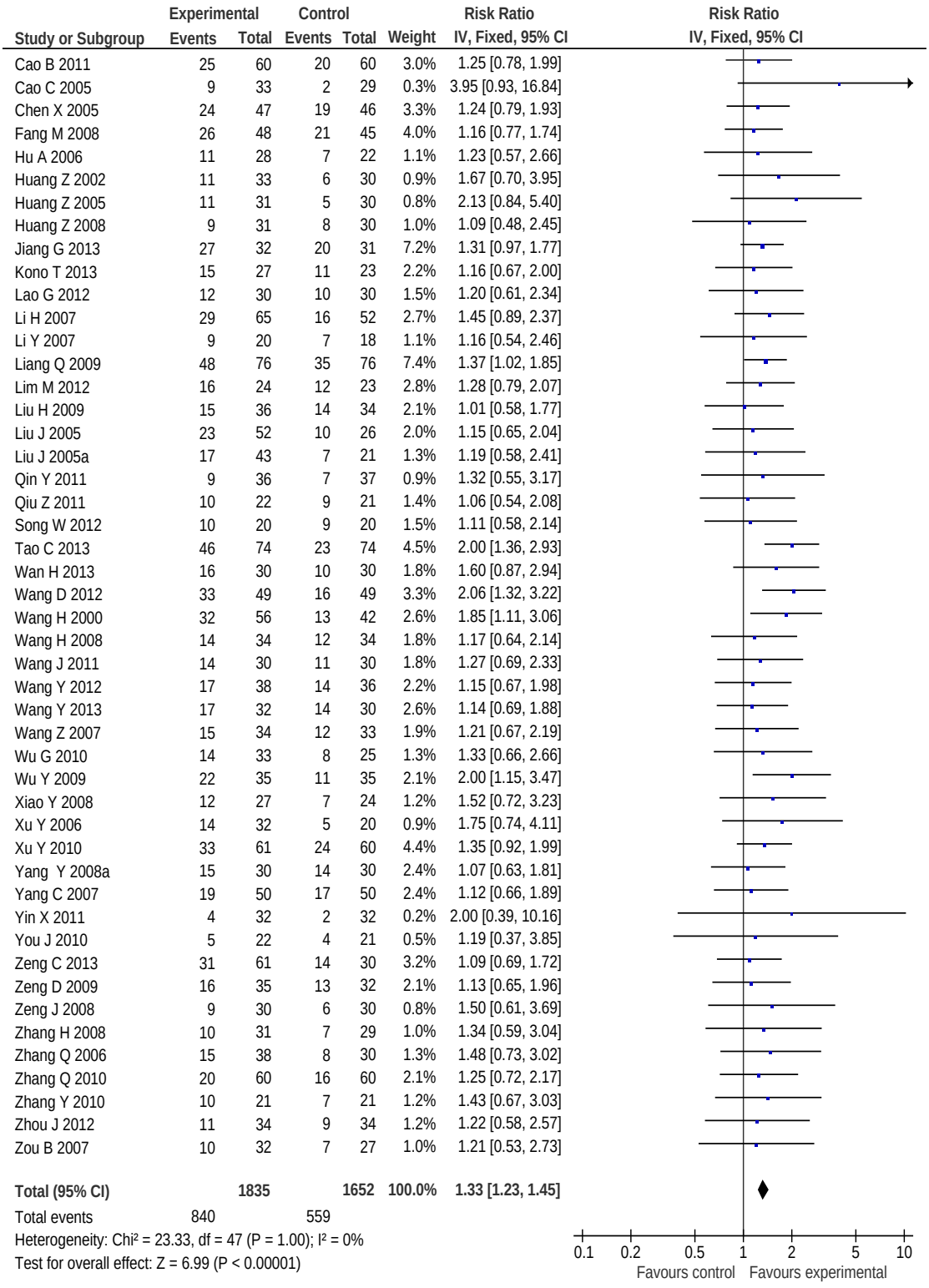


**Figure 4.3: Forest plot of risk ratio for tumour response rate (total group, n=48)**
control: chemotherapy alone; experimental: HM + chemotherapy

The funnel plot of tRR for the 48 studies showed some asymmetry, which suggests that more positive small studies were published than small negative studies. But there were only two of these small studies so this is unlikely to have affected the overall result (Figure 4.4).

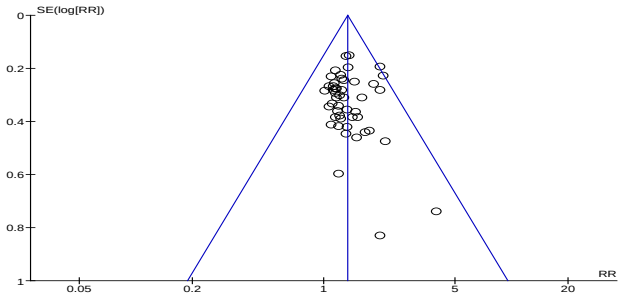


**Figure 4.4: Funnel plot of 48 studies that reported tumour response rate (total group)**

In the 47 studies, with 3,367 participants, that reported data for complete response rate (CR), nine studies reported zero events in both groups and therefore could not contribute to the meta-analysis result. In the remaining 38 studies, there was a significant increase in CR in favour of the combination therapy groups (RR 1.81, 95% CI [1.32, 2.47], $I^2$=0%) without heterogeneity. The relative improvement rate was 81% and there was an absolute improvement in CR of 2% in the combination therapy groups (RD 0.02, 95% CI [0.01, 0.03], $I^2$=0%) (Table 4.5; Figure 4.5). The funnel plot for all 47 studies was symmetric (Figure 4.6) indicating that the risk of publication bias was low.

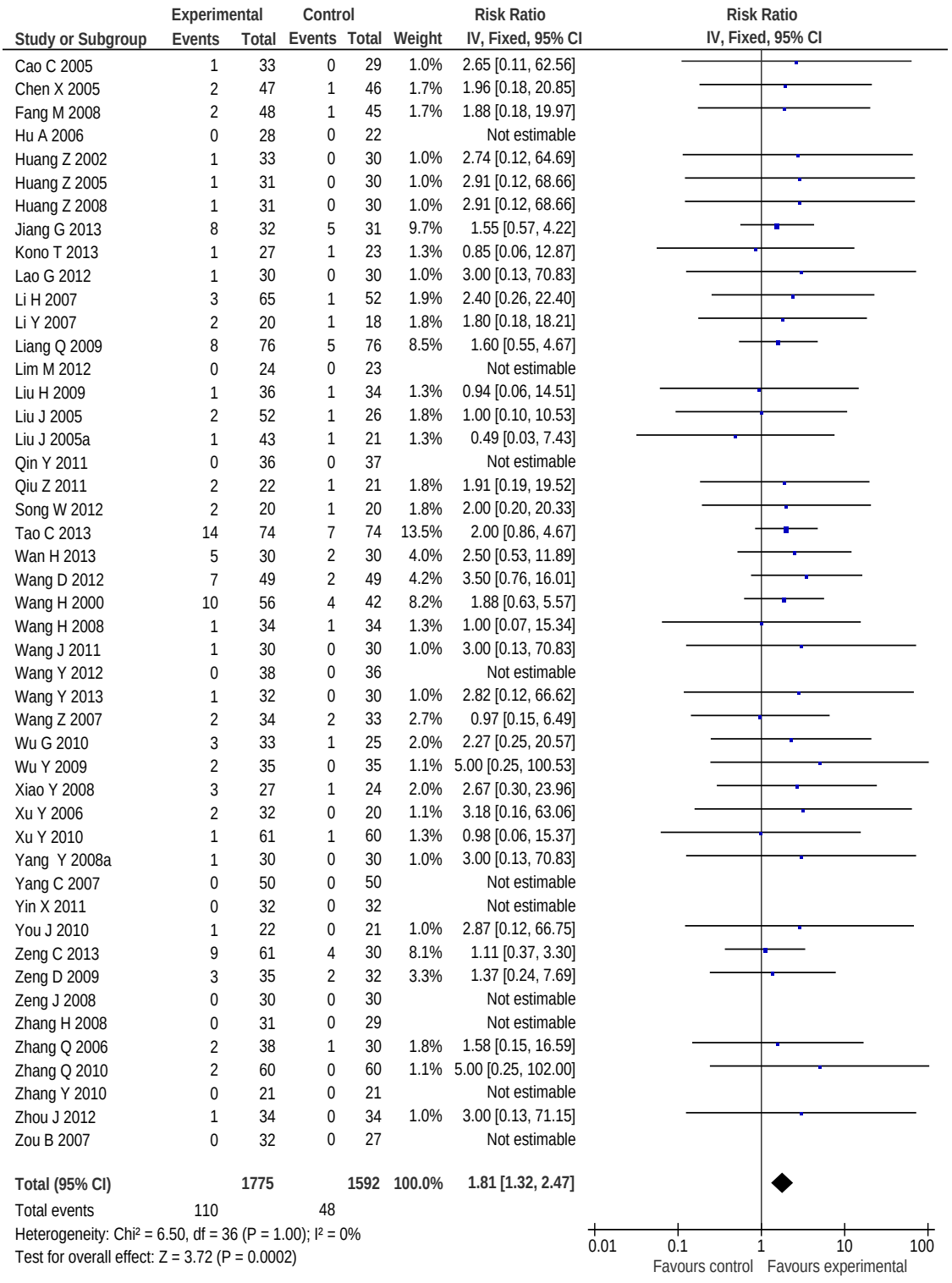


**Figure 4.5: Forest plot of risk ratio for complete response (total group, n=47)**
control: chemotherapy alone; experimental: HM + chemotherapy

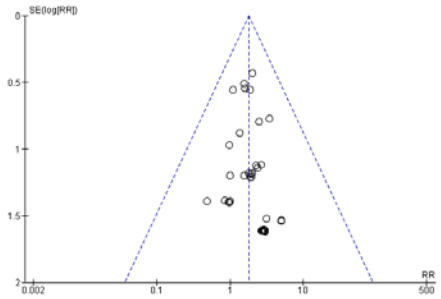


**Figure 4.6: Funnel plot of 47 studies that reported complete response (total group)**

*Sub-group analyses*

There was variation in the CMT regimens used in these studies. These CMT regimens can be classified as oxaliplatin regimens such as FOLFOX and non-oxaliplatin regimens, such as the 5-FU-based older generation CMTs. Evidence from large international trials have found that oxaliplatin regimens achieved higher tRR than those of non-oxaliplatin regimens (de Gramont et al., 2000).

Therefore, data was re-synthesized into two subsets: chemotherapy that employed oxaliplatin regimens, and non-oxaliplatin regimens. Among these studies, some provided separate data for participants who had been previously treated with chemotherapy and those who were previously untreated. Since the response to chemotherapy is known to be better in previously untreated participants, meta-analyses were conducted for these sub-groups where possible. In addition, when more than one study tested the same manufactured CHM intervention, data for these studies were pooled as sub-groups.

Oxaliplatin group

In the 42 studies (n=3,083) that used oxaliplatin regimens, one did not report CR (Cao et al., 2011), the test groups showed significantly improved tRR (RR 1.31, 95% CI [1.20, 1.42], $I^2$=0%) (Figure 4.7) and CR (RR 1.80, 95% CI [1.29, 2.51], $I^2$=0%) (Figure 4.8) compared to the control groups, without heterogeneity (Table 4.5).

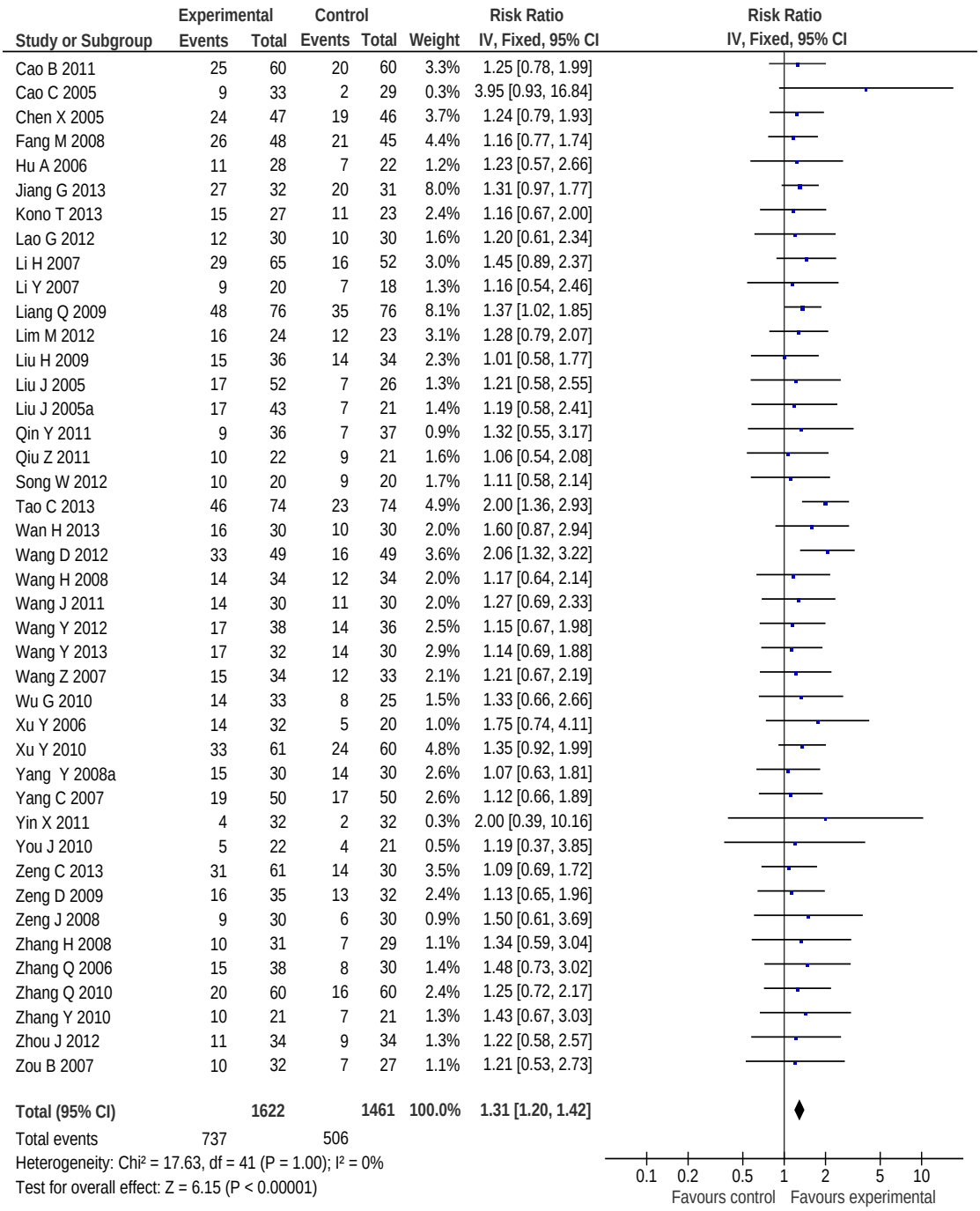


**Figure 4.7: Forest plot of risk ratio for tumour response rate (oxaliplatin group, n=42)**
control: chemotherapy alone; experimental: HM + chemotherapy

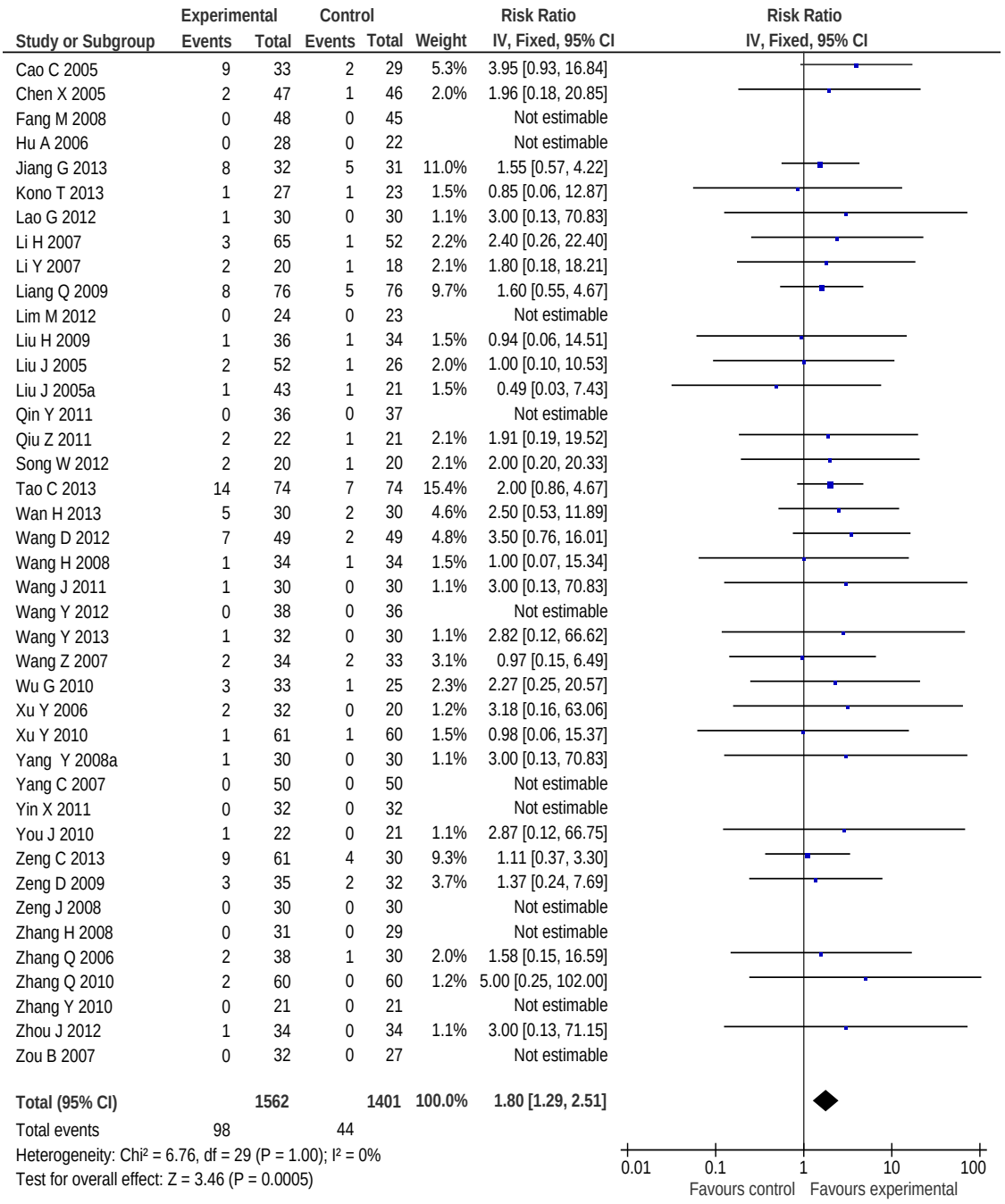


**Figure 4.8: Forest plot of risk ratio for complete response (oxaliplatin group, n=41)**
control: chemotherapy alone; experimental: HM + chemotherapy

The Funnel plot for tRR (Figure 4.9) was similar to that of the total group (Figure 4.6). The funnel plot was symmetric for CR (Figure 4.10).

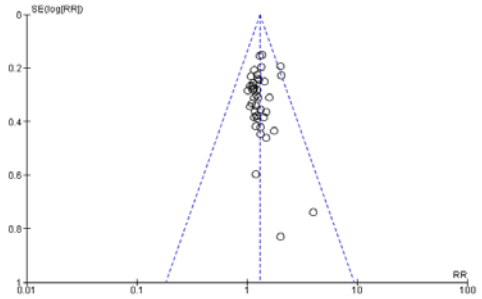


**Figure 4.9: Funnel plot of 42 studies that reported tumour response rate (oxaliplatin group)**

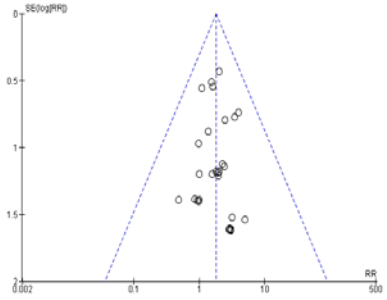


**Figure 4.10: Funnel plot of 41 studies that reported complete response (oxaliplatin group)**

Non-oxaliplatin group

The six studies (n=404) that employed a non-oxaliplatin regimen also showed significantly improved tRR (RR 1.73, 95% CI [1.31, 2.28], $I^2$=0%) and CR (RR 2.31, 95% CI [1.01, 5.29], $I^2$=0%) in favour of the integrated CHM and chemotherapy groups compared with the control groups without heterogeneity (Table 4.5; Figure 4.11 and 4.12).

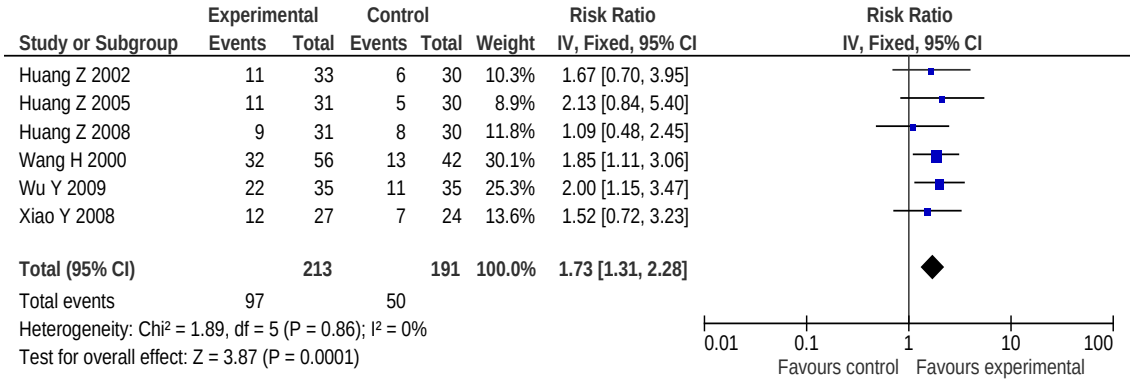


**Figure 4.11: Forest plot of risk ratio for tumour response rate (non-oxaliplatin group, n=6)**
control: chemotherapy alone; experimental: HM + chemotherapy

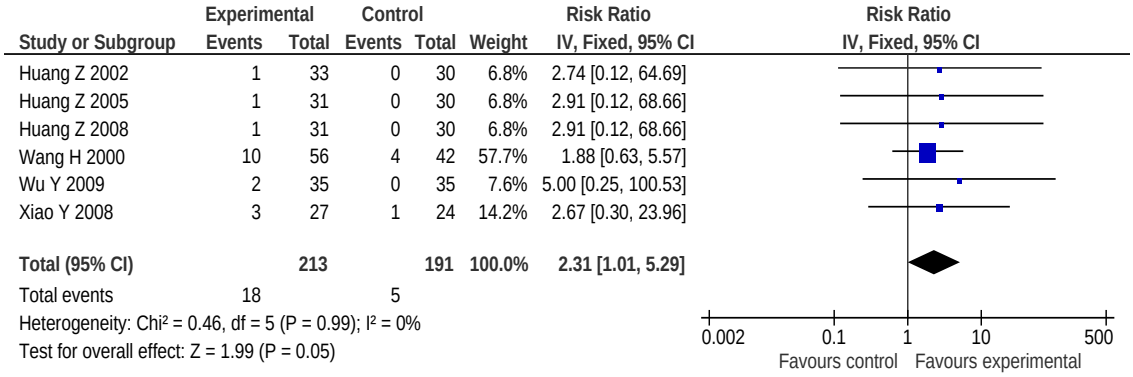


**Figure 4.12: Forest plot of risk ratio for complete response (non-oxaliplatin group, n=6)**
control: chemotherapy alone; experimental: HM + chemotherapy

Previously treated/previously untreated colorectal cancer patients

Previously treated ACRC patients have been found to be less responsive to current first-line settings of chemotherapy (Giantonio et al., 2007). Seven studies that reported tRR defined the participants as previously untreated. Three studies reported the tRR of previously treated participants who had ACRC.

The pooled tRR of the previously untreated category (seven RCTs) was significantly different between the treatment groups and the control groups (RR 1.25, 95% CI [1.01, 1.55], $I^2$=0%, n= 475) without heterogeneity in favour of the CHM plus chemotherapy groups (Figure 4.13). CR data was available for six of these studies of which one had zero events in both groups. The meta-analysis of the five studies showed that the difference between groups was not significant (RR 1.98, 95%CI [0.81, 4.87], p = 0.13, $I^2$ = 0%, n=281) (Figure 4.14).

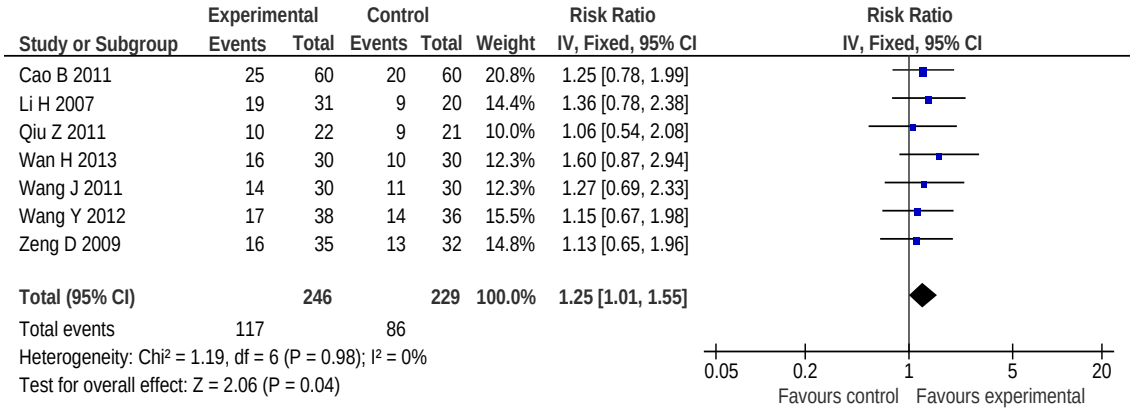


**Figure 4.13: Forest plot of risk ratio for tumour response rate (previously untreated colorectal cancer group, n=7)**
control: chemotherapy alone; experimental: HM + chemotherapy

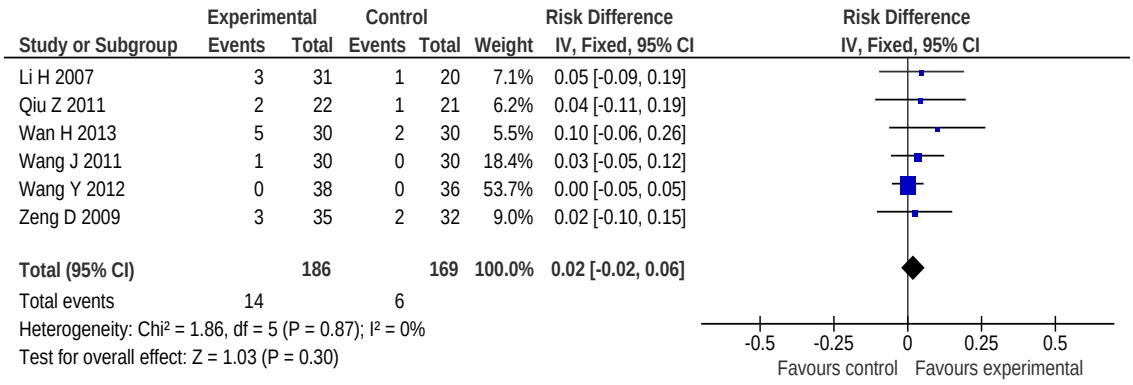


**Figure 4.14: Forest plot of risk ratio for complete response (previously untreated colorectal cancer group, n=6)**
control: chemotherapy alone; experimental: HM + chemotherapy

In the previously treated category, the tRR was not significantly different between the treatment groups (RR 1.65, 95% CI (0.94, 2.91), p=0.08, $I^2$=0%) (Figure 4.15), but the data were based on only three RCTs (n=188 particpants) and the result for the RD showed a significant effect (RD 0.14, 95% CI [0.02, 0.25], p=0.02, $I^2$=0%) (Table 4.5). There were insufficient data for meta-analysis of CR.

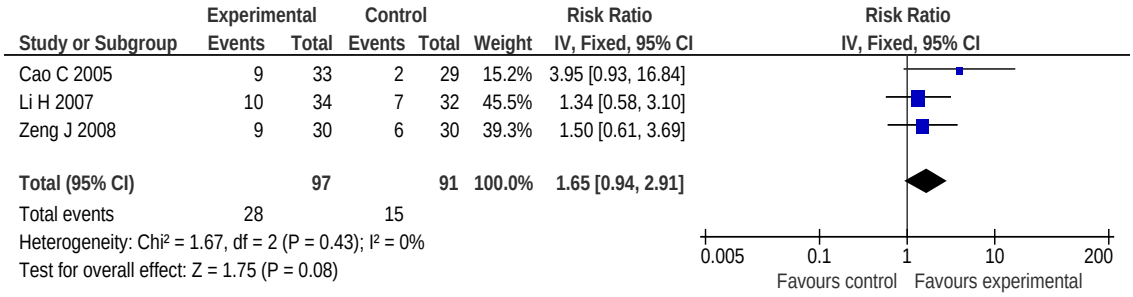


**Figure 4.15: Forest plot of risk ratio for tumour response rate (previously treated colorectal cancer group, n=3)**
control: chemotherapy alone; experimental: HM + chemotherapy

Manufactured CHM products

The tRR of *Aidi* injection, *Kang'ai* injection, and *Jianpi huoxue* decoction combined with an oxaliplatin regimen were reported in two studies each. The pooled tRR of the trials of these three HMs showed no differences between the treatment and control groups. For CR, there was no difference between groups for *Jianpi huoxue* decoction but there were insufficient data for meta-analysis of CR for the *Aidi* injection and *Kang'ai* injection groups (Table 4.5; Figure 4.16a, b, c, d).

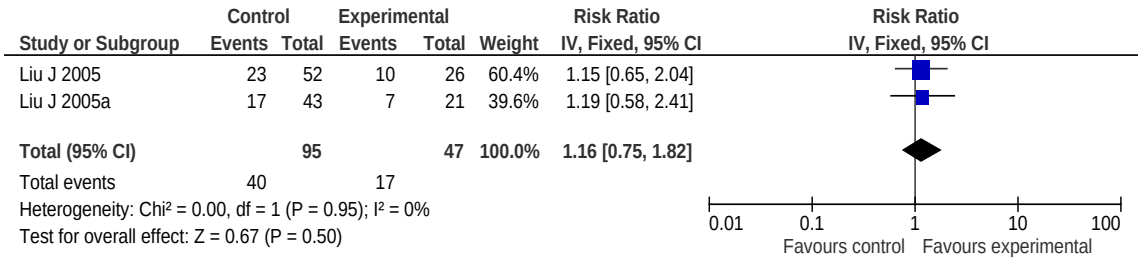


**Figure 4.16a: Forest plot of risk ratio for tumour response rate (Jianpi huoxue herbs, n=2)**

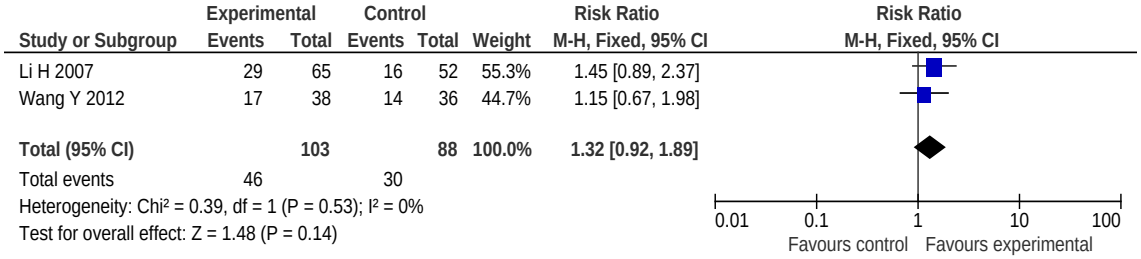


**Figure 4.16b: Forest plot of risk ratio for tumour response rate (AIDI injection, n=2)**

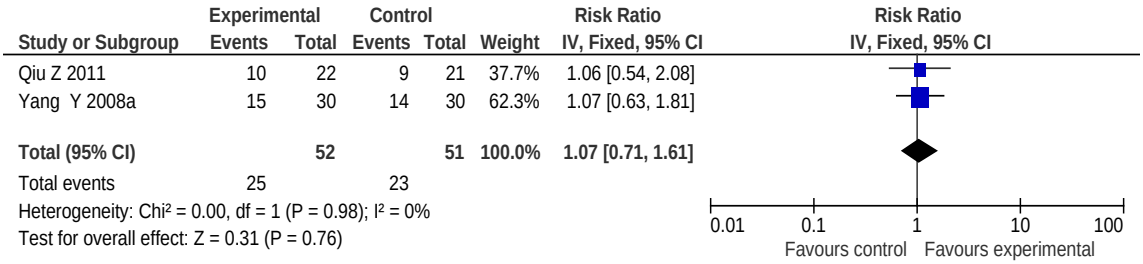


**Figure 4.16c: Forest plot of risk ratio for tumour response rate (Kang'ai injection, n=2)**

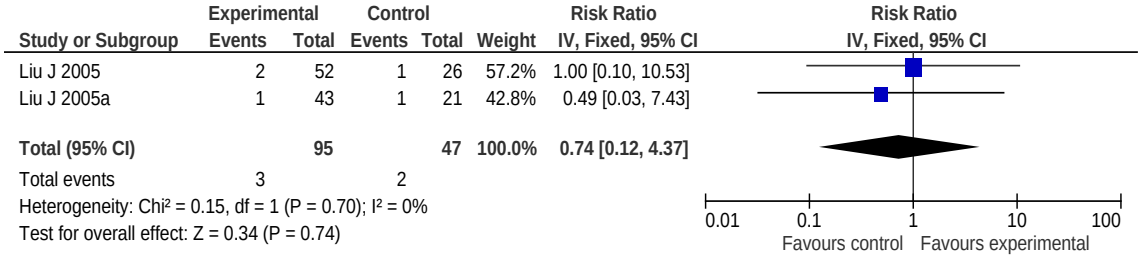


**Figure 4.16d: Forest plot of risk ratio for complete response (Jianpi huoxue herb, n=2)**

### *4.8.3.2 Discussion of tumour response rate results*

The meta-analysis found that chemotherapy, including oxaliplatin and non-oxaliplatin groups, combined with HMs significantly improved the tRR in CRC compared with the chemotherapy alone (Table 4.5). The review by Zhong et al. (2012) reported similar meta-analysis results.

In oxaliplatin and non-oxaliplatin groups, most of the available data were for oxaliplatin regimens, of which FOLFOX4 was the most common. There was a significant absolute increase of 11% in tRR in the combination therapy groups based on 42 RCTs with 3,070 participants with zero heterogeneity in the result. In the non-oxaliplatin chemotherapy sub-group, there were six RCTs (404 participants) in the pooled analysis and this also showed a significant increase in tRR in the combination therapy groups with an absolute difference of 4% between groups.

In addition, the meta-analysis found that the combination treatment significantly improved the CR in the total group by 2% (RD 0.02, 95% CI [0.01, 0.03], $I^2$=0%). In the sub-group of oxaliplatin regimens, the CR also showed a significant but small increase (1%) based on 41 RCTs, while the difference in the non-oxaliplatin regimens was much larger at 19% but this was based on only six studies. Overall incidence of CR was low, except in one study (Wang et al., 2000). When this study was excluded, the RD dropped to a marginal 4% (RD 0.04, 95% CI [-0.00, 0.08]) in the non-oxaliplatin group. The improvement of CR may be more meaningful than the tRR, in terms of translating to OS. For more discussion of the relationship between the tRR and OS, see section 4.8.3.6 (Discussion of results for survival and relationship with tumour response) below.

Objective tumour response is an important outcome in ACRC therapy and has been reported to have improved with advances in chemotherapy in the last two decades. Monofluorouracil therapy only had a 10% response rate for ACRC (Piedbois et al., 1994), but bimonthly high-dose leucovorin and fluorouracil bolus plus continuous infusion achieved 32.6% tumour response (de Gramont et al., 1997). Since irinotecan and oxaliplatin have became available in the last decade, several RCTs have demonstrated that 5-FU plus irinotecan or oxaliplatin elevated the tRR into the range 31% to54% (Hind et al., 2008). Despite the improved tRR with current first-line chemotherapy compared with the older generation chemotherapy, the CR improvement was still small (1.4% vs. 0.5%) (de Gramont et al., 2000).

The meta-analysis results in this review have shown the combination of CHM treatment and chemotherapy improved tRR with low heterogeneity. The funnel plot tests showed mild asymmetry indicating that problematic publication bias was unlikely. Animal studies have also demonstrated a better suppression rate of tumour growth when HM was combined with 5-FU, compared to chemotherapy alone. This has been reviewed in Chapter 2 of the literature review (section 2.9.6). However, in the clinical studies, most of the CHMs were tested in the context of multi-CHM formulas, so it is difficult to determine which individual HMs were more or less effective. Identification of the potent HMs or the optimal groups of HMs that are most frequently used and effective in improvement of tRR is explored in Chapter 6.

#### *4.8.3.3 Effect on carcinoembryonic antigen*

The carcinoembryonic antigen (CEA) results were treated as continuous data using mean difference (MD) with 95% interval. When the MD was negative, it was in favour of the combination groups. The CEA analysis, which pooled results from nine studies that included 651 participants who were at different stages of the disease, showed that the CHM interventions significantly reduced the CEA readings compared with the control groups (MD -3.97, 95% CI [-4.42, -3.52], $I^2$=93%), but with considerable heterogeneity (Table 4.6; Figure 4.17a).

**Table 4.6: Meta-analysis results of carcinoembryonic antigen for herbal medicine combined with systemic chemotherapy (9 studies)**

| Carcinoembryonic antigen (CEA) | No. studies (participants) | MD (95% CI, FE), $I^2$ |
|---|---|---|
| Total group CEA | 9 (651) | -3.97 [-4.42, -3.52], *p<0.00001, $I^2$=93% |
| Palliative setting CEA | 5 (387) | -5.32 [-5.84, -4.80], *p<0.00001, $I^2$=62% |
| Adjuvant setting CEA | 2 (133) | 0.07 [-1.00, 1.13], p=0.90, $I^2$=77% |

*statistically significant; MD: mean difference; FE: fixed effect; $I^2$: the proportion of heterogeneity.

Consequently, data were re-synthesized in two sub-groups: palliative therapy group (n=5) and adjuvant therapy group (n=2). Guo (1999) and Zheng (2011) included participants who had received either radical resection or palliative resection. Therefore, these studies were excluded from the sub-group analysis.

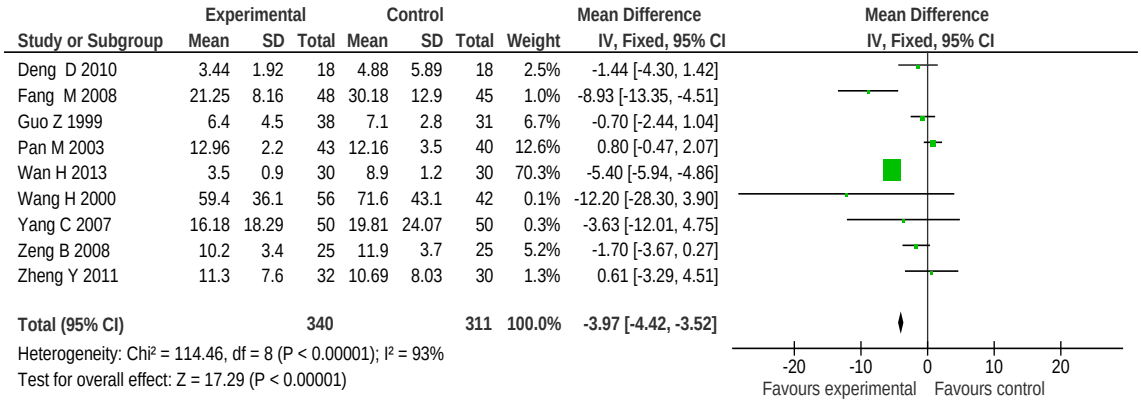


**Figure 4.17a: Forest plot of risk ratio for carcinoembryonic antigen (total group, n=9)**
control: chemotherapy alone; experimental: HM + chemotherapy

The pooled result for the palliative therapy group (5 studies) showed that the combination of HM significantly decreased the CEA level in serum (MD -5.32, 95% CI [-5.84, -4.80], $I^2$=62%), with reduced heterogeneity (Table 4.6; Figure 4.17b).

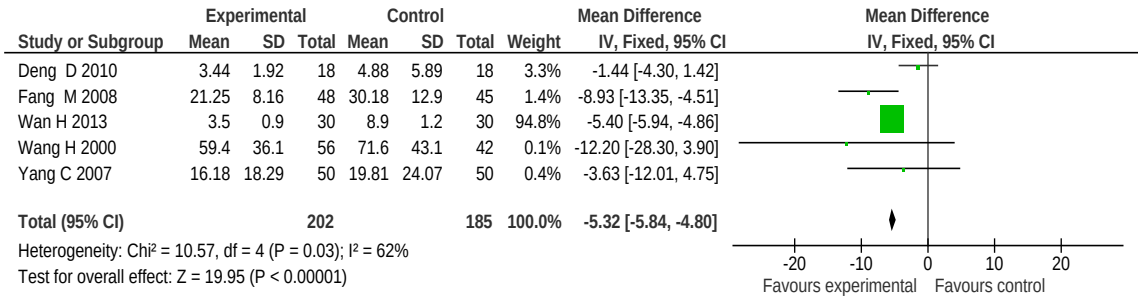


**Figure 4.17b: Forest plot of risk ratio for carcinoembryonic antigen (palliative setting, n=5)**

control: chemotherapy alone; experimental: HM + chemotherapy

Two studies were included in the adjuvant therapy group. The pooled result showed the CEA after treatment was not different between the two treatment groups, with reduced heterogeneity (Table 4.6; Figure 4.17c).

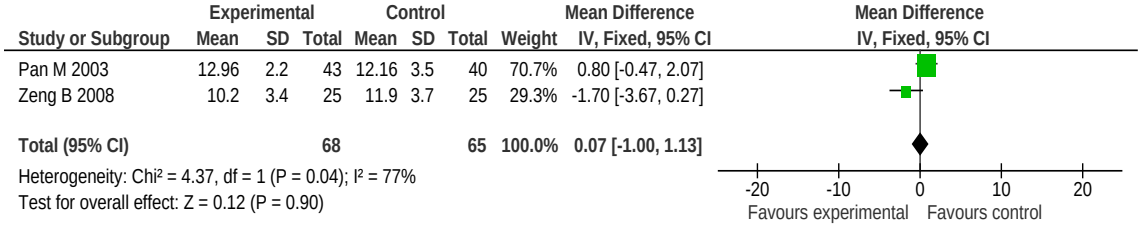


**Figure 4.17c: Forest plot of risk ratio for carcinoembryonic antigen (adjuvant setting, n=2)**
control: chemotherapy alone; experimental: HM + chemotherapy

#### *4.8.3.4 Discussion of carcinoembryonic antigen (CEA) results*

Carcinoembryonic antigen as a biomarker is not of value for screening detection of early CRC due to its low sensitivity and low specificity to asymptomatic early stage CRC, but it can assist in CRC diagnosis along with other diagnostic techniques. As a tool for assessing prognosis, for Dukes B or equivalent stage CRC patients, the pre-operational CEA test may provide independent prognostic information that could help in the management of the surgical procedure and inform the choice of future adjuvant therapies post-operation. When the CEA level fails to fall within six weeks post-operatively, this may suggest recurrence or metastatic CRC. CEA has up to 80% sensitivity for detecting recurrence and metastasis of the disease. CEA is also used for surveillance of post-operative patients. In palliative therapy, CEA can help monitor the outcome of treatment and an increasing CEA level is mostly associated with progressive disease (Duffy et al., 2003). In general, an ACRC patient with a positive CEA response (reduction) during chemotherapy was likely to have a better outcome than one without a positive CEA response (Wang et al., 2001). In the trial by de Gramont et al. (2000), the prognostic factors in the univariate analysis showed the CEA was correlated with the PFS (p=0.0015) and OS (p=0.0001), but not the tRR (p=0.5406).

Overall, the combination treatment significantly reduced the CEA readings in serum compared to chemotherapy alone in the palliative setting. This positive result is consistent with the pooled result for overall survival in which the combination treatment demonstrated greater prognosis compared to the CMT alone. However, data were only available for five studies and the results still showed substantial heterogeneity. In the adjuvant setting studies there were no clear differences between the two treatments groups at the end of treatment. However, CEA is more relevant to long-term outcomes such as DFS and OS in the adjuvant setting, so the meta-analysis results are difficult to interpret. There is a need for long-term follow-up to detect recurrence or metastasis for post-radical surgery patients. The heterogeneity may be due to the base-line differences in the studies, and differences in laboratory methods and disease severity.

#### *4.8.3.5 Effect on measures of survival and disease progression*

Thirty-two studies compared overall survival (OS), median OS (mOS), disease-free survival (DFS) and/or time to progression (TTP) between the combination therapy groups and CMT groups. Overall survival time at the end-points of one-year, two-years, three-years and five-years were pooled in sixteen, seven, nine, and four studies respectively. Thirteen studies also reported median survival time (mOS). Only one study reported death rate during follow-up (Yang et al., 2008). Six studies reported DFS in the adjuvant therapeutic setting for stages I, II and III CRC participants. Five studies reported the outcome of TTP in the palliative setting for ACRC participants.

Studies were grouped for each of these outcomes with sub-groups for adjuvant and palliative therapeutic settings. The data for OS and DFS at the end-points of one-year, two-years, three-years and five-years were treated as dichotomous data. The meta-analysis method was the same as that used for tRR.

**Table 4.7: Meta-analysis results of time to event (survival data) for herbal medicine combined with systemic chemotherapy (32 studies)**

| Outcomes | No. studies (participants) | RR (95% CI, FE), $I^2$; IR% | RD (95% CI, FE), $I^2$ |
|---|---|---|---|
| **One-year OS** | 16 (1265) | 1.06 [1.02-1.10], *p=0.005, $I^2$=52%; 6% | 0.07 [0.04, 0.10], *p<0.0001, $I^2$=47%. |
| One-year OS (palliative setting) | 9 (696) | 1.44 [1.25, 1.65], *p<0.00001, $I^2$=0%;44% | 0.21 [0.14, 0.29], *p<0.00001, $I^2$=0% |
| One-year OS (adjuvant setting) | 5 (346) | 1.01 [0.97, 1.06], p=0.63, $I^2$=0%;1% | 0.01 [-0.03, 0.05], p=0.58, $I^2$=0% |
| **Two-year OS** | 7 (494) | 1.13 [1.02, 1.25], *p=0.02, $I^2$=42%;13% | 0.11 [0.04, 0.18], *p=0.003, $I^2$=37% |
| Two-year OS (palliative setting) | 3 (231) | 1.50 [1.18, 1.90], *p=0.0009, $I^2$=0%;50% | 0.20 [0.09, 0.31], *p=0.0006, $I^2$=0% |
| Two-year OS (adjuvant setting) | 4 (263) | 1.06 [0.94, 1.19], p=0.34, $I^2$=15%;6% | 0.05 [-0.04, 0.14], p=0.30, $I^2$=26% |
| **Three-year OS** | 9 (740) | 1.27 [1.12, 1.42], *p<0.0001, $I^2$=0%;27% | 0.14 [0.08, 0.20], *p<0.00001, $I^2$=0% |
| Three-year OS (palliative setting) | 2 (171) | 2.01 [1.30, 3.10], *p=0.002, $I^2$=0%;101% | 0.15 [0.06, 0.25], *p=0.002, $I^2$=81% |
| Three-year OS (adjuvant setting) | 5 (346) | 1.18 [1.01, 1.37], *p=0.03, $I^2$=0%;18% | 0.11 [0.01, 0.21], *p=0.03, $I^2$=0% |
| **Five-year OS** | 4 (366) | 1.38 [1.11, 1.73], *p=0.004, $I^2$=0%;38% | 0.18 [0.08, 0.28], *p=0.0003, $I^2$=0% |
| Five-year OS (adjuvant setting) | 1 (83) | 1.34 [0.84, 2.14], p=0.23;34% | 0.13 [-0.08, 0.35], p=0.21 |
| **Disease-free survival (DFS)** | | | |
| One-year DFS | 3 (142) | 1.08 [0.99-1.18], p=0.09, $I^2$=36%; 8% | 0.08 [0.00, 0.16], *p=0.04, $I^2$=34% |
| Two-year DFS | 2 (153) | 1.23 [1.03, 1.47], *p=0.02, $I^2$=0%;23% | 0.16 [0.03, 0.29], *p=0.02, $I^2$=0% |
| Three-year DFS | 1 (70) | 1.18 [0.90, 1.55], p=0.24; 18% | 0.13 [-0.08, 0.33], p=0.22 |
| Five-year DFS | 1 (70) | 1.29 [0.84, 1.98], p=0.24; 29% | 0.13 [-0.08, 0.34], p=0.23 |

*statistically significant; RR: risk ratio; IR: improvement rate; RD: risk difference; FE: fixed effect; $I^2$: proportion of heterogeneity.

One-year overall survival

The 16 studies that reported one-year OS included 1,265 participants at different stages of CRC. The pooled result showed a significantly improved one-year OS with 6% relative improvement rate (RR 1.06, 95% CI [1.02, 1.10], $I^2$=52%) when CHMs were combined with CMT interventions, with substantial heterogeneity (Table 4.7; Figure 4.18a).

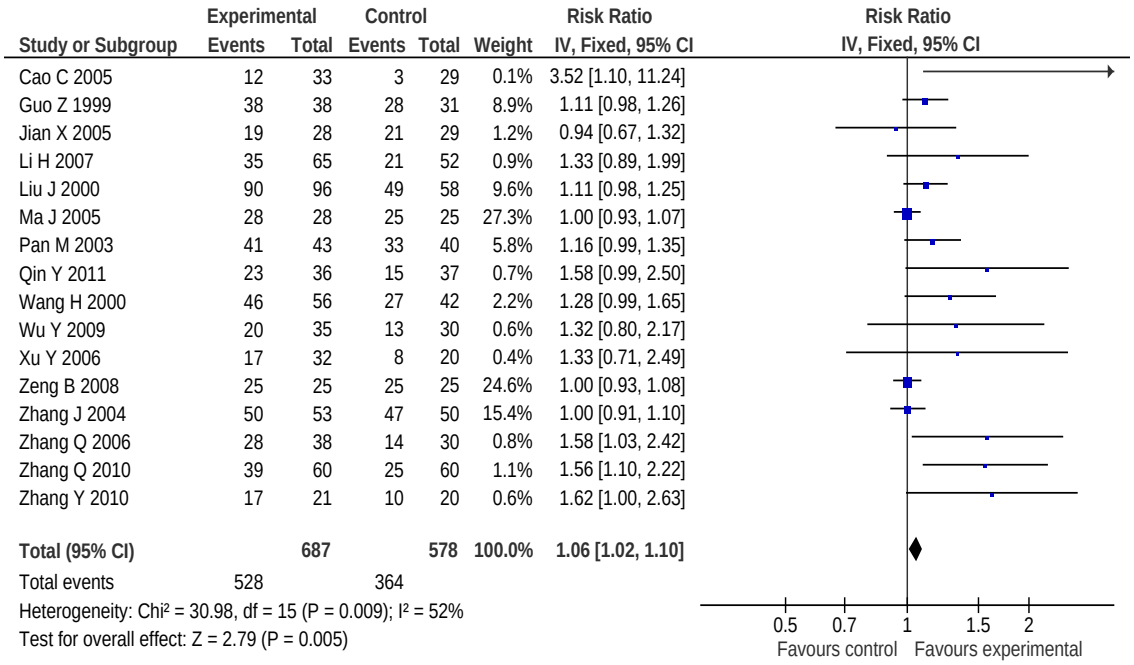


**Figure 4.18a: Forest plot of risk ratio for one-year overall survival (total group, n=16)**
control: chemotherapy alone; experimental: HM + chemotherapy

Sensitivity analysis showed that the tumour burden was substantially different between participants who have chemotherapy in the palliative setting compared with the adjuvant setting. Within a palliative setting, the participants' tumours are usually at an advanced stage and unable to be removed by radical resection. Participants in an adjuvant setting are usually in the early stage of the disease and the tumours have been radically removed. Therefore, the outcome of overall survival was analysed separately for these two groups and data were re-synthesized in two sub-groups: palliative setting; and adjuvant setting.

Nine studies were in the palliative settings for advanced CRC. The result showed that one-year OS was significantly improved in the combination groups compared with the control groups that used chemotherapy. Translated to a percentage, there was a 44% (RR 1.44, 95% CI [1.25, 1.65], $I^2$=0%) greater chance of survival for one year when chemotherapy was combined with CHMs than for chemotherapy alone, with no heterogeneity. In absolute terms, 21% more participants survived for one year in the combination groups than in the control groups (RD 0.21, 95% CI [0.14, 0.29], p<0.00001, $I^2$=0%) (Table 4.7; Figure 4.18b).

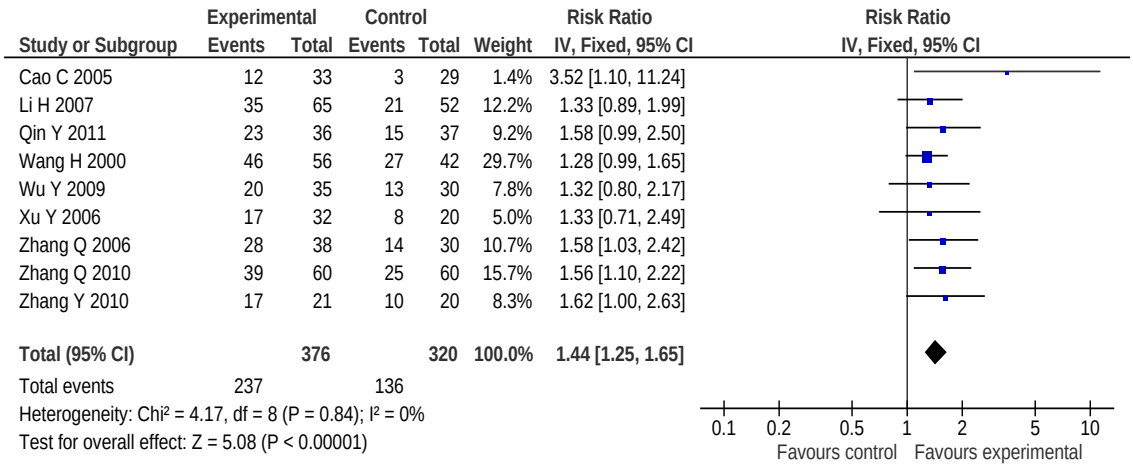


**Figure 4.18b: Forest plot of risk ratio for one-year overall survival (palliative setting, n=9)**
control: chemotherapy alone; experimental: HM + chemotherapy

Five studies were in the adjuvant settings for stage II or III CRC following resection. There was no statistically significant difference between the two treatment groups for one-year OS (Table 4.7; Figure 4.18c).

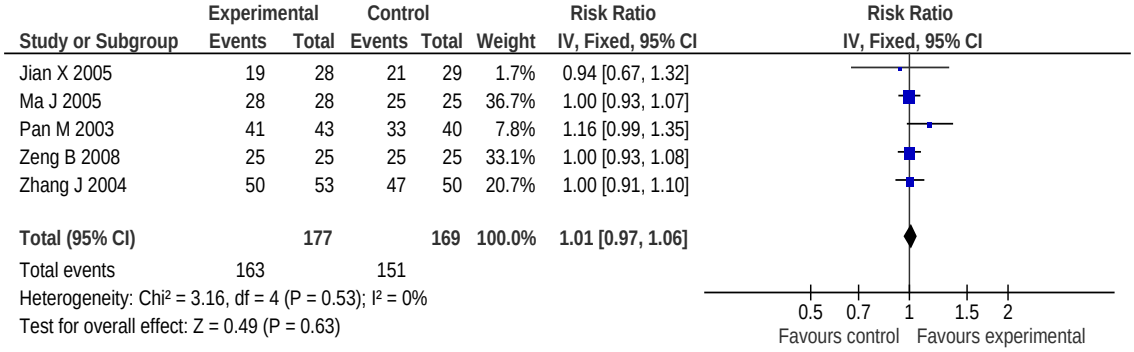


**Figure 4.18c: Forest plot of risk ratio of one-year overall survival (adjuvant setting, n=5)**
control: chemotherapy alone; experimental: HM + chemotherapy

The funnel plot of the 16 studies that reported one-year OS was asymmetric (Figure 4.19.). There were more studies located in the positive zone, which indicates the possibility of reporting bias.

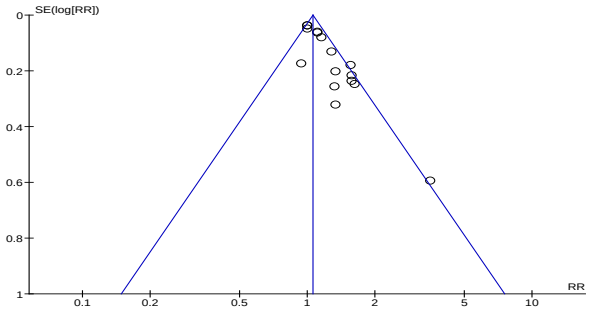


**Figure 4.19: Funnel plot of 16 studies that reported one-year overall survival**

Two-year overall survival

Seven studies reported two-year OS. Four hundred and ninety-six participants at different stages of the disease were involved. The result showed significantly improved two-year OS when CHMs were combined with the CMT interventions (RR 1.13, 95% CI [1.02, 1.25], $I^2$=42%), with moderate heterogeneity (Table 4.7; Figure 4.20a).

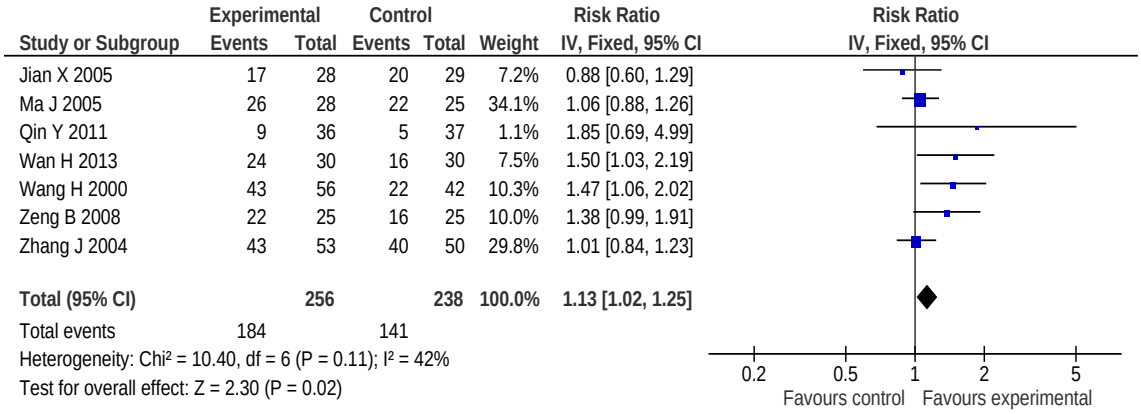


**Figure 4.20a: Forest plot of risk ratio for two-year overall survival (total group, n=7)**
control: chemotherapy alone; experimental: HM + chemotherapy

Sensitivity analysis was undertaken. Data were re-synthesized into two sub-groups: palliative setting and adjuvant setting. Three studies (n=231) were included in the palliative setting for the ACRC group. The result showed significantly improved two-year OS in the combination groups compared with the control groups (RR 1.50, 95% CI [1.18, 1.90], $I^2$=0%). The risk difference was similar to the one-year OS in the same setting (RD 0.20, 95% CI [0.09, 0.31], $I^2$=0%) (Table 4.7; Figure 4.20b).

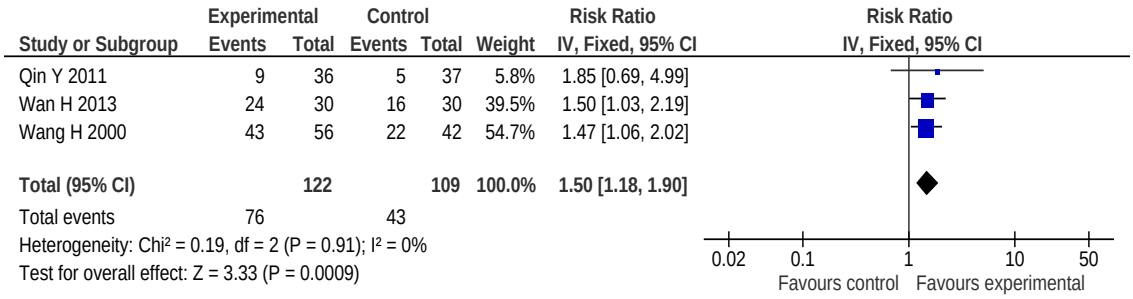


**Figure 4.20b: Forest plot of risk ratio for two-year overall survival (palliative setting, n=3)**
control: chemotherapy alone; experimental: HM + chemotherapy

Four studies were in adjuvant settings for stage II or III after radical surgery. There was no statistically significant difference between the two treatment groups for two-year OS (Table 4.7; Figure 4.20c), with no important heterogeneity.

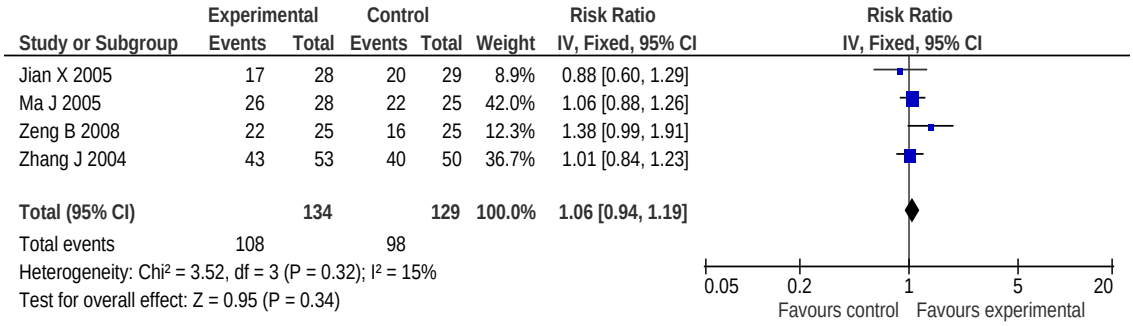


**Figure 4.20c: Forest plot of risk ratio for two-year overall survival (adjuvant setting, n=4)**
control: chemotherapy alone; experimental: HM + chemotherapy

Three-year overall survival

Three-year OS was reported by nine studies involving 763 participants at different stages of the disease. The result showed significantly improved three-year OS when CHMs were combined with the CMTs, with zero percent heterogeneity (RR 1.27, 95% CI [1.12, 1.42], $I^2$=0%), compared to CMT alone (Table 4.7; Figure 4.21a).

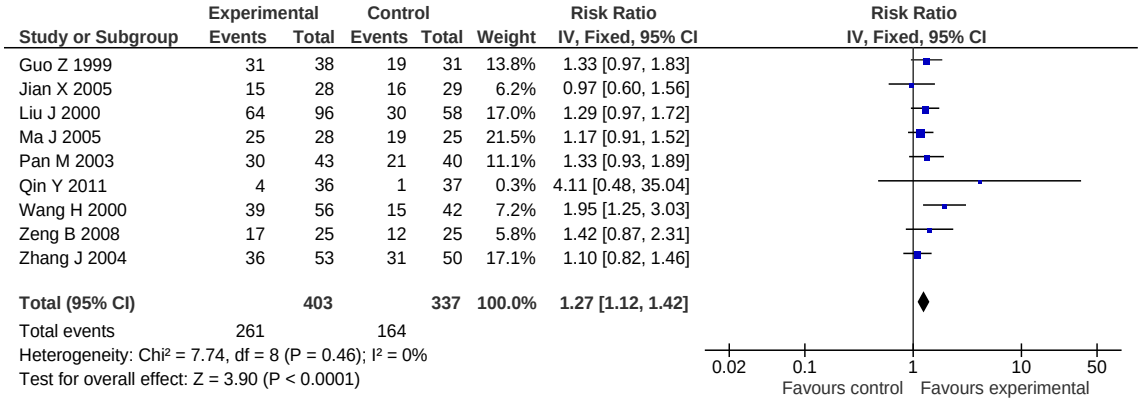


**Figure 4.21a: Forest plot of risk ratio for three-year overall survival (total group, n=9)**
control: chemotherapy alone; experimental: HM + chemotherapy

The studies were divided into two groups: palliative setting and adjuvant setting. Two studies were of palliative settings for ACRC. The statistical result was significantly in favour of the combination group (n=171 participants) (RR 2.01, 95% CI [1.30, 3.10], $I^2$=0%) (Table 4.7; Figure 4.21b).

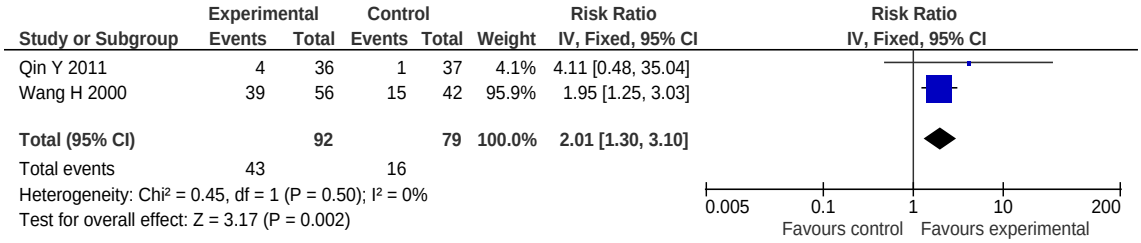


**Figure 4.21b: Forest plot of risk ratio for three-year overall survival (palliative setting, n=2)**
control: chemotherapy alone; experimental: HM + chemotherapy

Five studies (n=346 participants) were of adjuvant settings for stage II or III after radical surgery. The combination groups had significantly improved three-year OS compared with the CMT control groups without heterogeneity (RR1.18, 95% CI [1.01, 1.37], $I^2$=0%). The absolute three-year survival rate of the combination groups was 11% higher than the control groups (RD 0.11, 95% CI [0.01, 0.21], p=0.03, $I^2$=0%) (Table 4.7; Figure 4.21c).

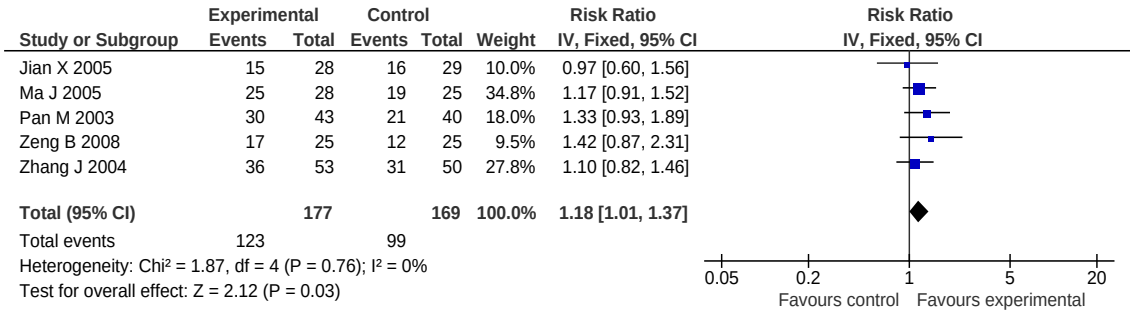


**Figure 4.21c: Forest plot of risk ratio for three-year overall survival (adjuvant setting, n=5)**
control: chemotherapy alone; experimental: HM + chemotherapy

Five-year overall survival

Five-year OS was reported by four studies involving 366 participants at different stages of the disease. The result showed significantly improved five-year OS when CHMs were combined with the CMT interventions (RR 1.38, 95% CI [1.11, 1.73], $I^2$=0%) (Table 4.7; Figure 4.22).

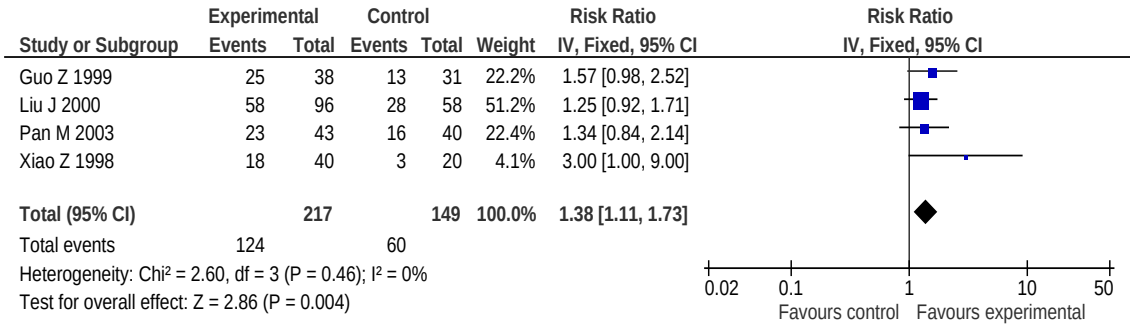


**Figure 4.22: Forest plot of risk ratio for five-year overall survival (total group, n=4)**
control: chemotherapy alone; experimental: HM + chemotherapy

Only one study reported the five-year OS in the adjuvant setting for stage II or III after radical surgery. There was no statistically significant difference between the two groups (Table 4.7). No data were separately reported for five-year OS in the palliative setting.

Median overall survival

Twelve studies that were in the palliative setting reported median overall survival time (OS). Participants in these studies had stages III or IV. Four studies reported separate median OS data for stage IV, whereas the others reported median OS data for stages III and IV combined (Table 4.8).

The median OS is the time at which 50% of the patients are alive. The median OS is commonly reported for survival outcome in medical studies. The hazard ratio (HR) and its variance, which express the chance of the event happening at a particular time point, are considered the most appropriate statistical analyses of the OS data (time to event) (Micheal et al., 2005). However, 12 studies presented the OS as aggregate data for the groups without values for the variance. This made it impossible to use the log hazard ratio and its variance for analysis of the pooled effect of the additional CHM treatments on median OS. The data are presented as a histogram in Figure 4.23. The mean values and standard deviations (SD) were calculated using the formula in Excel. The mean was 15.84 months for the test groups and 11.83 months for the control groups; the SD was 5.27 months for test groups and 4.47 months for control groups. The estimated mean difference significantly favoured the test groups (MD 4.01, 95% CI [0.25, 7.77], p=0.04).

**Table 4.8: The characteristics of 12 studies that reported median overall survival data without variance**

| Study (first author, year) | Participant (n) T/C | Stage (n) T/C | Intervention: T/C | Median OS (m) T/C |
|---|---|---|---|---|
| Cao B 2011 | 60/60 | IV (all) | Yiqi zhuyu decoction + FOLFOX4 vs. FOLFOX4 + placebo | 21.0/18.0 |
| Cao C 2005 | 33/29 | IV (all) | Shenmai injection + FOLFOX vs. FOLFOX | 11.07/5.77 |
| Cazacu M 2003 | 47/37 | III: 18/16<br>IV: 29/21 | Mistletoe Viscum album + 5-FU/LV vs. 5-FU/LV | III: 24.9/18.0<br>IV: 16.6/7.0 |
| Ding X 2010 | 30/30 | Ad (all) | Co-kushen injection + FOLFOX vs. FOLFOX | 19.8/18.6 |
| Li H 2007 | 65/52 | III: 27/19<br>IV: 38/33 | Aidi injection + FOLFOX4 vs. FOLFOX4 | 12.5/10.8 (all stages) |
| Liang Q 2009 | 76/76 | III: 45/46<br>IV: 31/30 | Shenqi Fuzheng injection + FOLFOX vs. FOLFOX | 7.75/7.0 (all stages) |
| Lui W 2011 | 16/16 | IV (all) | Yierkang capsule+FOLFOX vs. FOLFOX | 12.0/9.0 |
| Qin Y 2011 | 36/37 | Ad (all) | Fuzhongguben tang+FOLFOX vs. FOLFOX | 15.6/11.2 |
| Wang H 2000 | 56/42 | III: 25/17<br>IV: 31/25 | Mutouhui Glycoside Pill + 5-FU/LV vs. 5-FU/LV | 23.6/15.7 |
| Wang Z 2007 | 34/33 | IV (all) | Delisheng injection + FOLFOX vs. FOLFOX | 10.6/9.5 |
| Yang Y 2008 | 18/19 | Ad (all) | Quxie capsule + FOLFOX vs. FOLFOX | 17.0/13.0 |
| Zhang Y 2010 | 21/20 | Ad (all) | Jianpi Jiedu decoction + FOLFOX4 vs. FOLFOX4 | 13.6/10.2 |

Ad: advanced stage; m: month; 5-FU: 5-Fluorouracil; LV: Leucovorin; Ox.: Oxaliplatin; T/C: Test group /Control group; FOLFOX: Ox. + 5-FU + LV.

Only one study in this review reported the hazard ratio (HR) result for median OS and median PFS. Cao B et al. (2011) studied *Yiqizhuyu* decoction (YZD) combined with FOLFOX4 in patients with metastatic colorectal cancer (MCRC). One hundred and twenty participants were randomly allocated

to the combination group or control group (FOLFOX4 + placebo for the CHM) in the ratio of 1:1. All participants were treated until disease progression or for 48 weeks. The median OS was 21.0 months for the test group and 18.0 months for the control group. There was a significant difference between the two treatment groups that favoured the test group (HR: 0.65, 95% CI [0.43, 0.99], p=0.043). The median PFS were 9.0 months for the test group and 8.0 months in the control group. There was no significant difference between the two groups (HR: 0.78, 95% CI [0.53, 1.15], p=0.215).

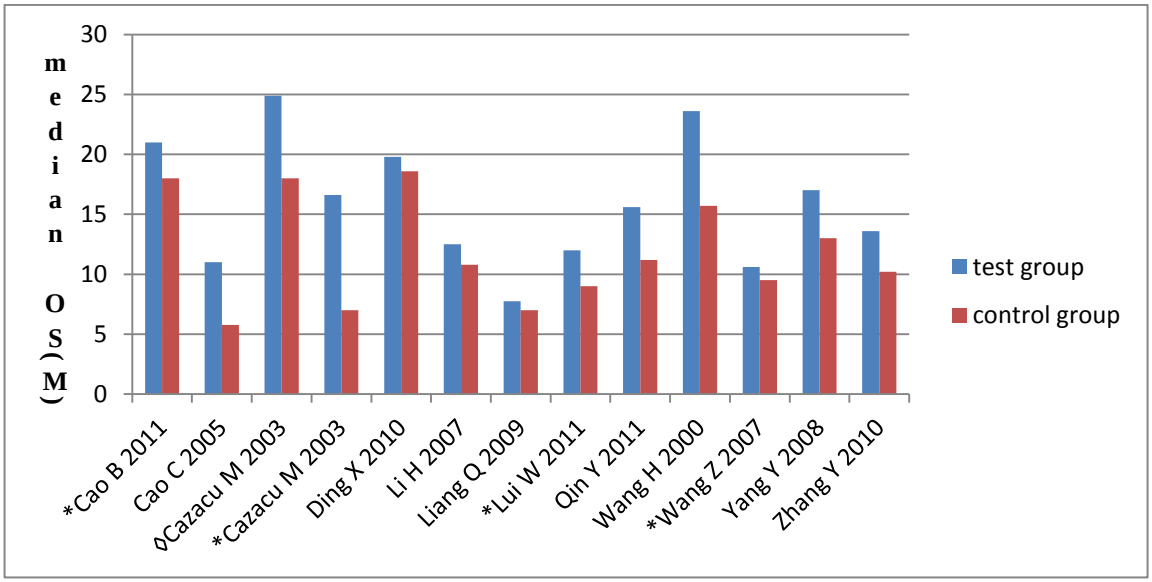


**Figure 4.23: Comparison of median overall survival between test and control group in palliative setting (data without variance)**

* data for stage IV; ◊ data for stage III; m: month; test group: HM + chemotherapy; control group: chemotherapy alone

The disease-free survival (DFS) and time to progression (TTP)

The DFS, TTP and/or PFS were reported in 10 studies. DFS is usually used as an outcome for patients who are post radical surgery to remove their tumour, whereas TTP and PFS is used as an outcome for patients who are at such an advanced stage that the tumour is unable to be totally removed (see Chapter 3). Both PFS and TTP in a clinical study have often been used as surrogate end points (Beauchemin et al., 2014).

In one study, combined participants with post radical surgery and palliative surgery (Guo, 1999) reported a disease recurrence rate of 21.05 % for the test group compared with 48.34 % for the control group. Another study, Zhang et al. (2013), reported the disease progression rate instead of DFS. For the test group, this was 18.57% (6/32), compared with 34.38% (11/32) in the control group. The authors did not provide information on the censor time, so this study was not pooled with the other three studies. Therefore, DFS data at the end-points of one-year, two-years, three-years, and five-years were pooled for the remaining three studies as follows:

*One-year disease-free survival*: In three studies, 142 participants with stage II or III were included. The result showed no significant improvement in the one-year DFS when CHMs were added to the CMT interventions, with no important heterogeneity. However, the absolute improvement rate was 8% which showed a significant improvement (RD 0.08, 95% CI [0.00, 0.16]) (Table 4.7; Figure 4.24a).

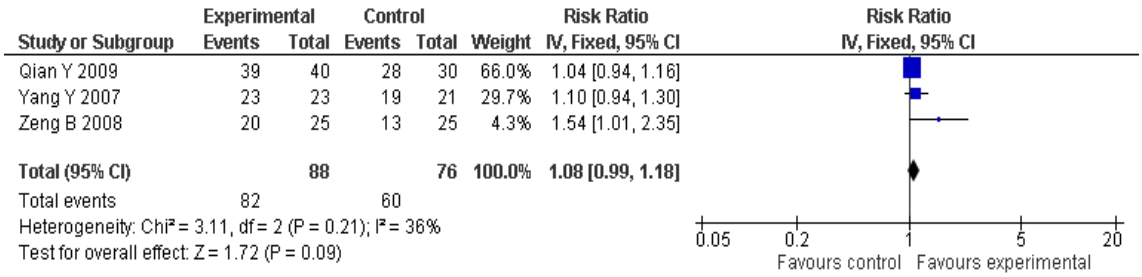


**Figure 4.24a: Forest plot of risk ratio for one-year disease-free survival (n=3)**
control: chemotherapy alone; experimental: HM + chemotherapy

*Two year DFS*: Two studies involving stage I, II and III CRC participants (n=153) reported two-year DFS data. The result showed a statistically significant improvement when CHMs were added to the CMT intervention (Table 4.7; Figure 4.24b).

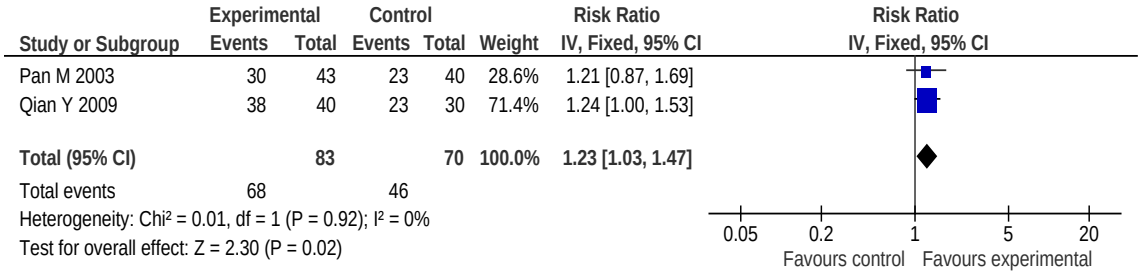


**Figure 4.24b: Forest plot of risk ratio for two-year disease-free survival (n=2)**
control: chemotherapy alone; experimental: HM + chemotherapy

*Three year disease-free survival*: Qian et al. (2009) reported three-year DFS. The pooled result showed no statistically significant improvement when the CHM was added to the CMT intervention (Table 4.7). Qian et al. (2009) also reported that the addition of CHM significantly prolonged the mean recurrence time (in months) compared to the chemotherapy alone (MD 12.80, 95% CI [8.13, 17.47], p<0.00001).

*Five year disease-free survival*: Pan et al. (2003) reported the five-year DFS for 83 stage I, II or III CRC participants. The result showed an increased five-year DFS when CHMs were included in the intervention, but it was not statistically significant (Table 4.7).

*Time to progression and progression free survival (TTP/PFS)*: Since these outcomes are very similar, they were included in the same pool where possible. Five studies provided data for TTP or PFS which compared the median TTP/PFS between the HM plus FOLFOX regimen and the FOLFOX regimen

alone groups in the palliative setting. The data did not provide the variance. Therefore, the data were treated as in the median OS section and are presented in Table 4.9 and Figure 4.25.

The estimated mean values and standard deviations (SD) were calculated using the formula in Microsoft Excel. The mean was 10.42 months for the test groups and 8.46 for the control groups; the SD was 3.43 months for test groups and 1.20 months for control groups. The mean difference was not significantly different (MD 1.96, 95% CI [-1.23, 5.15], p=0.23) between groups. Notably, the TTP reported by Xu et al. (2010) were considerably higher than the other studies. A likely reason is this study enrolled participants with various stages of CRC including some in Stage I and II whereas the other studies were of stage III or IV.

**Table 4.9: Five studies reported median time to progression and progression free survival (data without variance)**

| **Study (first author, year)** | Cao B 2011 | Ding X 2010 | Lao G 2012 | Xu Y 2010 | Zhang Y 2010 |
|---|---|---|---|---|---|
| **T gp; mTTP (m)** | 9.0 | 9.2 | 9.3 | 16.5 | 8.1 |
| **C gp; mTTP (m)** | 8.0 | 8.7 | 8.5 | 10.2 | 6.9 |

C gp: Control group (chemotherapy alone); T gp: test group (HM+chemotherapy); mTTP: median TTP; m: month.

In addition, Cao et al. (2011) reported there was no significant difference in PFS between the two groups (HR 0.78, 95% CI [0.53, 1.15], p=0.215). Yang et al. (2008) (n=37) found that the mean TTP was 17.76 ± 5.62 months for the test group and was significantly higher than the mean of 12.68 ± 9.26 months for the control group (MD 5.05, 95% CI [0.17, 9.99], p=0.04).

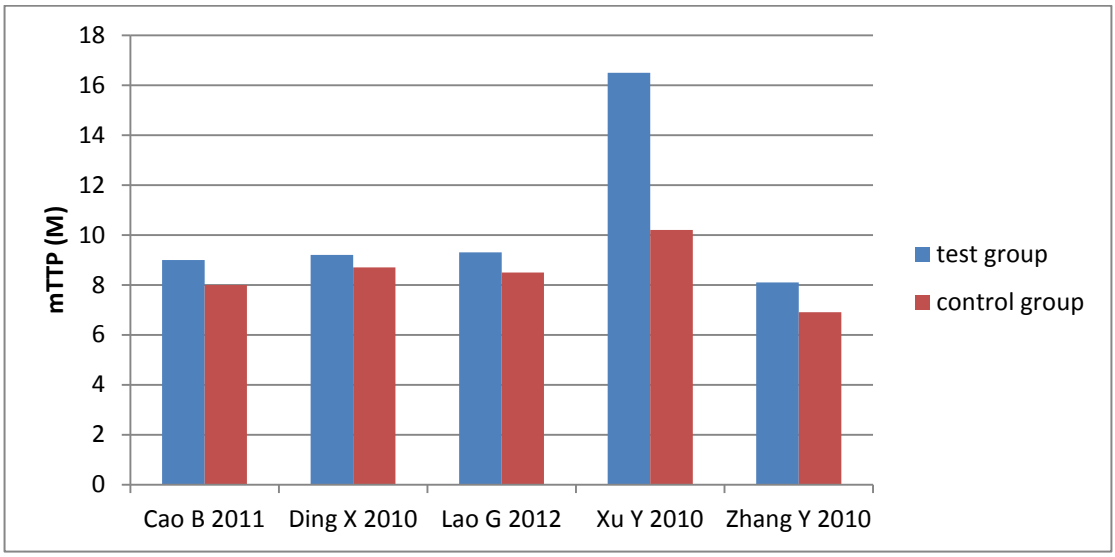


**Figure 4.25: Comparison of median time to progression between test group and control group (data without variance)**

test group: HM + chemotherapy; control group: chemotherapy alone; mTTP: median TTP; m: month.

#### *4.8.3.6 Discussion of results for survival and relationship with tumour response*

The meta-analysis results showed that in palliative settings, the combination of CHM plus CMT treatment statistically significantly elevated the one-year, two-year, and three-year OS. The estimated difference in median OS was also significantly higher in the test groups. However, the funnel plot test showed an asymmetric distribution in the one-year OS, so the possibility of reporting bias cannot be ruled out. In the adjuvant setting, a statistical advantage of additional HM treatment appeared at three-year OS, one-year DFS and two-year DFS. Most of the CRC patients in the adjuvant setting die from relapse of the disease. Up to 80% of these relapses occur during the first three years following radical resection of the tumour. The median time from relapse to death is 12 months (Sargent et al., 2005). Therefore, treatment in the adjuvant setting aims to prevent disease relapse. There were too few studies to assess the possibility of publication bias for the adjuvant setting. Overall, the results suggest combination treatment may prolong OS in the adjuvant setting and in the palliative setting.

The combination of CHM with CMT treatment may have enhanced the anti-cancer effect of the CMT, in terms of improved tRR and CEA in the palliative setting, and this could have translated into improvements in one year, two-year, and three-year OS. The relation between tumour response and survival in first-line chemotherapy is an interesting issue in ACRC treatment. In the last two decades, palliative chemotherapy has shown great advances in the treatment of ACRC in terms of tRR. In contrast, the improvement in OS is relatively modest and is not always significantly different between test group and control group in ACRC trials (Buyse et al., 2000).

In the last two decades, the tRR increased from approximately 10% in first-line monofluorouracil therapy (Piedbois et al., 1994) to 45%-56% in first-line oxaliplatin-based or irinotecan-based combination regimens (Hind et al., 2008). In addition, the median OS has also been elevated from 9.1 months (5-FU monotherapy) to 20 months using the same settings. These results show that the improvements in chemotherapy have produced considerably greater gains in tRR than improvements in median OS.

Thus, the question has been raised as to whether a therapy that significantly improves tRR in ACRC treatment will necessarily translate into significant benefits in OS. The evidence from RCTs has been contradictory. The Advanced Colorectal Cancer Meta-Analysis Project conducted a meta-analysis that included nine RCTs that compared 5-FU with 5-FU plus intravenous LV for the treatment of ACRC. The 5-FU plus LV regimens, administered either weekly or monthly, showed a highly significant benefit over single-agent 5-FU in tRR (23% vs. 11%; OR 0.45, $p<0.0000001$) but no significant difference in OS (OR 0.97, $p=0.57$) (Doroshow et al., 1992). Also, improvements in response rates in the FOLFOX4 regimen did not ultimately translate into extended OS when compared to 5-FU/LV (median OS 16.2 vs. 14.7 months, $p =0.12$) in a RCT (de Gramont et al., 2000).

In contrast, the Advanced Colorectal Cancer Meta-Analysis Project conducted another meta-analysis that was based on individual data of 1,178 patients included in eight RCTs comparing 5FU alone with 5FU/MTX. The 5FU/MTX group generated almost twice the tRR of the 5FU alone control group (19% vs. 10%). This difference was highly significant (OR 0.51, 95% CI [0.37, 0.70], $p < 0.0001$). There was a small improvement in median OS for the 5FU/MTX groups (10.7 months vs. 9.1 months) which was significantly different (OR 0.87, 95% CI [0.77, 0.98], $p = 0.024$) (Piedbois et al., 1994).

Buyse et al. (2000) also conducted a meta-analysis to test the relationship between tumour response to first-line 5-FU-based chemotherapy and survival in ACRC. The comparisons were between bolus fluoropyrimidines as a control treatment versus fluoropyrimidine modifications. The data comprised individual data from 3,791 patients enrolled in 25 RCTs of first-line treatment. These analyses demonstrated that the experimental fluoropyrimidine modification groups achieved higher rates of improved tRR (n= 454 / 2031, 22.4%) than the bolus fluoropyrimidine groups (n= 209 /1760, 11.9%) and there was a significant difference between groups (OR 0.48, 95% CI [0.40, 0.57], $p<0.0001$). However, the benefits to OS for the experimental fluoropyrimidine modifications treatments (n= 223/2031, 11%) were considerably less obvious than the controls (n=180/1760, 10.2%) although the difference remained significant (HR 0.90, 95% CI [0.84, 0.97], $p=0.003$). This meta-analysis found that while there was a relationship between tumour response and survival time, it was relatively small with the survival hazard reduction being only an eighth of the response odds reduction (Buyse et al., 2000).

Investigators have proposed a number of hypotheses to explain this relatively small benefit to survival in ACRC. First, in the previous studies, the tRR were considerably higher than the CR. The clinically effective response, which is measured as tRR, is a combination of CR and PR (partial response). The duration of survival is related to the degree of tumour response. A meta-analysis by Graf et al. (1994) found the CRC median survival time decreased according to the degree of response as follows: 21 months for CR, 15 months for PR, 12 months for SD, and 4 months for PD (logrank $X^2$ (3) = 166, $p<0.001$) (Graf et al., 1994). However, there is generally a low CR in ACRC chemotherapy regardless of regimen. According to De Gramont et al. (2000), none of the first-line chemotherapy trials they reviewed reported high CRs in ACRC. Buyse et al. (2000) found that most of the trials had less than 5% CR, but they found a higher survival rate in the CR cohort.

A similar phenomenon was seen in a RCT which used three chemotherapy regimens in ACRC patients and evaluated the impact of CR on OS. The trial enrolled 1,508 patients who were randomised to three treatment arms: IFL (irinotecan + FU/LV); FOLFOX4 (oxaliplatin + FU/LV); and IROX (irinotecan + oxaliplatin). Among the 4% (62/1508) of the participants who achieved CR from first-line treatments: 43 were from the FOLFOX4, 11 were from the IROX, and 8 were from the IFL (Dy et al., 2007). The median OS was 44.3 months for participants who achieved CR with CMT, and 17.1 months for

participants without CR. This relationship between higher CR and higher OS was consistent for both 5-FU-based regimens and oxaliplatin-based regimens. The trial also found that the factors associated with the achievement of CR were FOLFOX4 treatment and patients with low tumour burden.

The overall tRRs in ACRC trials have been low and were less than 50% in most of the phase 3 trials of up-to-date first-line settings (Hind et al., 2008). Although the tRR has doubled over the last two decades, an expectation of a high yield in OS is not realistic. The results of the various RCTs suggest that complete remission (CR) may be a more clinically meaningful measurement in predicting overall survival (OS) than tumour response rate (tRR).

Second, unplanned second-line therapies tend to confound the outcomes of tRR and OS in RCTs. In ACRC trials, most of the patients whose disease progresses or fails to respond to first-line therapies receive second-line therapies. This approach has been observed to increase tRR and prolong OS. For example, in the de Gramont et al. (2000) study (see above) the total OS of 16.2 months for FOLFOX4 included patients with and without second-line treatment, whereas for those who did not receive second-line treatment the median OS was 14.8 months. Also, when the control treatment fails to produce a response, patients in the control group often cross over to receive the experimental treatment. Consequently, the outcomes for OS could be overestimated in these patients. Thus, investigators have suggested that OS might no longer be an appropriate primary measure of the efficacy of first-line therapy in advanced CRC (de Gramont et al., 2000). Instead, the PFS is a more appropriate primary measure of efficacy for ACRC studies because it can measure the anti-tumour activity of first-line chemotherapy (Louvet et al., 2001).

Third, the sample size of the study could affect the statistical power of the studies. In ACRC trials, most of the experimental therapies improve the outcome of OS by two to three months compared with the control therapies (Hind et al., 2008). Such a marginal difference may be undetectable in a trial in which the sample size is not large enough. A similar effect was evident in the review by Piedbois et al. (1994) that included eight relatively small RCTs. The results of the individual studies showed that only three of eight trials showed an improvement in tRR for 5-FU/MTX compared with 5-FU alone, and only one of these three trials reported an improvement in survival. However, the meta-analysis that gathered data from 866 individual patients confirmed there were significant improvements in both tRR and OS for 5-FU/MTX therapy (Piedbois et al., 1994). When the effect size is modest, a single trial may not be able to answer the question but a meta-analysis of a number of trials can enhance the power and find a statistically significant effect.

Overall, the advanced chemotherapies significantly increase tRR and this translates into an increase in OS for patients with ACRC, but the effect on OS is small and is not always predictable.

In this review, the meta-analysis results showed the addition of the CHMs to the interventions improved the tRR (T/C: 45.78% vs. 33.84%) (Figure 4.3). More importantly, the CR rate of the combination groups was double that of the CMT alone groups (T/C: 7.57% vs. 3.74%) (Figure 4.5). These benefits were also present in the one-year, two-year, and three-year OS rates. The OS for test groups compared with control groups were: 63.03% vs. 42.50% (one-year OS in palliative setting) (Figure 4.18b); 62.30% vs. 39.45% (two-year OS in palliative setting) (Figure 4.20b); and 46.74% vs. 20.25% (three-year OS in palliative setting) (Figure 4.21b). These meta-analysis results are consistent with the trends in the outcomes of international studies regarding the relationship between tRR and OS.

#### *4.8.3.7 Effect on Quality of life (QoL)*

Sixty-two studies reported quality of life (QoL) outcomes. Fifty-five studies used Karnofsky performance scale (KPS) as a measurement of QoL. Multi-dimensional assessments of QoL were reported in 6 studies.

Karnofsky performance scale

KPS is a single-dimension scale that provides a global measure of a patient's physical function. The reliability and validity of the KPS have been investigated and verified (Granda-Cameron et al., 2008).

**Table 4.10: Meta-analysis results of quality of life for herbal medicine combined with systemic chemotherapy (n=40)**

| Outcomes | No. studies (participants) | RR (95% CI, FE), $I^2$; IR% | MD (95% CI, FE), $I^2$ | RD (95% CI, FE), $I^2$ |
|---|---|---|---|---|
| ***KPS*** | | | | |
| Total group (dichotomous data) | 40 (2973) | 1.86 [1.68, 2.06], *p<0.00001, $I^2$=0%; 86% | NA | 0.22 [0.19, 0.25], *p<0.00001, $I^2$=21% |
| Total group (continuous data) | 13 (827) | NA | 7.17 [6.20, 8.14], *p<0.00001, $I^2$=80% | NA |
| ***Body weight*** | | | | |
| Total group | 7 (650) | 1.95 [1.45, 2.62], *p<0.0001, $I^2$=0%;95% | NA | 0.15 [0.09, 0.21], *p<0.00001, $I^2$=0% |

*statistically significant; RR: risk ratio; IR: improvement rate; MD: mean difference; RD: risk difference; FE: fixed effect; $I^2$: proportion of heterogeneity.

Two approaches to reporting KPS scores were used in the included studies:

1. Forty studies defined a KPS score gain ≥10 points as 'improved', KPS score reduction ≥10 points as 'worse', and a change of less than 10 points as 'stable'. For meta-analysis, the numbers of patients who were judged as 'improved' in each group were pooled as

dichotomous data. RR was used to compare the two intervention groups at the end of treatment.

2. Thirteen studies reported the mean KPS score for each intervention group before treatment and at the end of treatment. These data were pooled as continuous data, and analysed as the MD between the two treatment groups at the end of treatment. There was no baseline imbalance before treatment.

The pooled data of the 'improved' events for KPS for 40 studies (n=2,973) showed significantly improved KPS scores when CHMs were added to CMT interventions (RR 1.86, 95% CI [1.68, 2.06], $I^2$= 0%) (Table 4.10; Figure. 4.26). However, the funnel plot was asymmetric (Figure 4.27) with more small studies located in the positive zone, which indicated the possibility of publication bias.

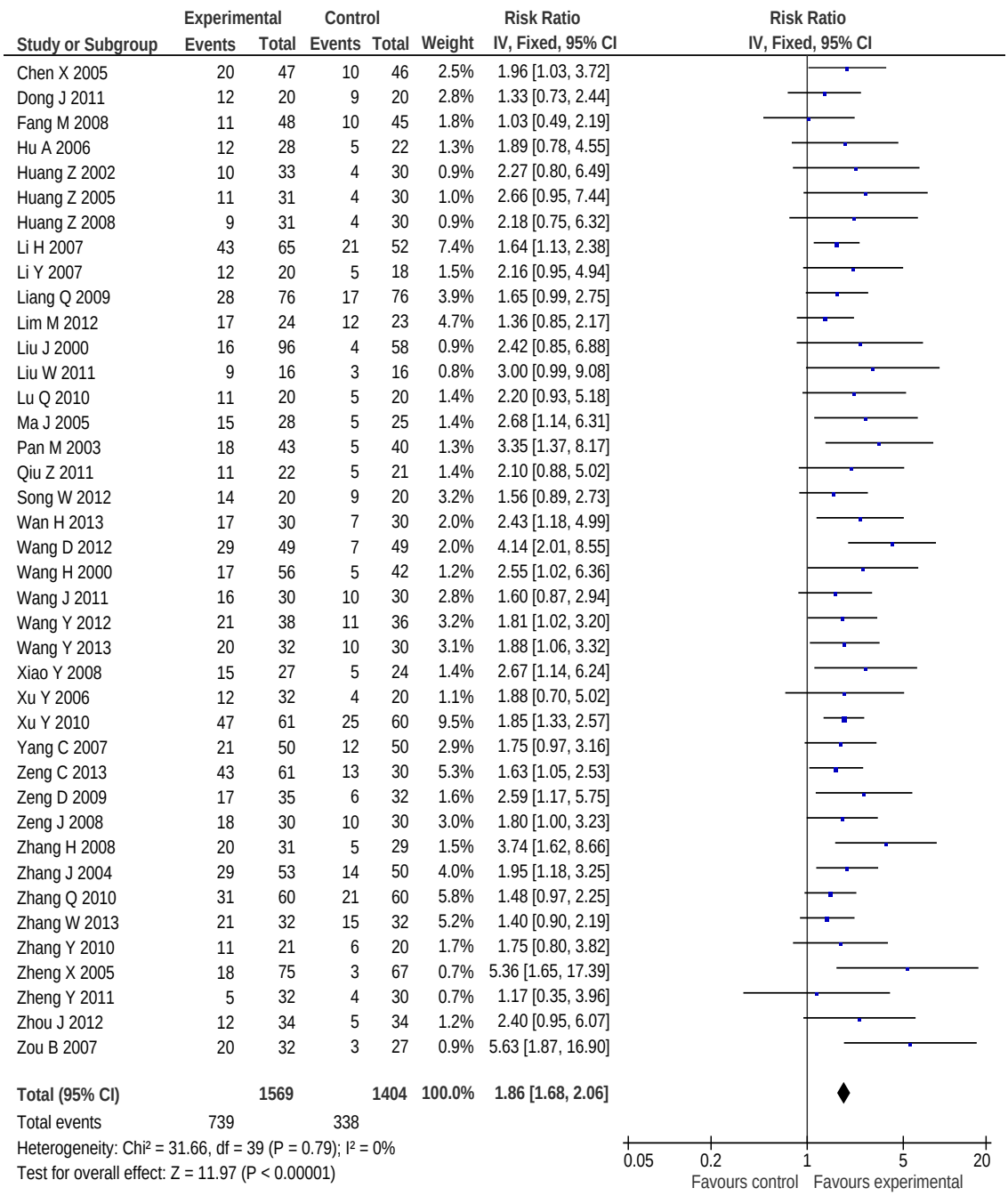


**Figure 4.26: Forest plot of risk ratio for Karnofsky performance scale score gain ≥10 points (n=40)**

control: chemotherapy alone; experimental: HM + chemotherapy

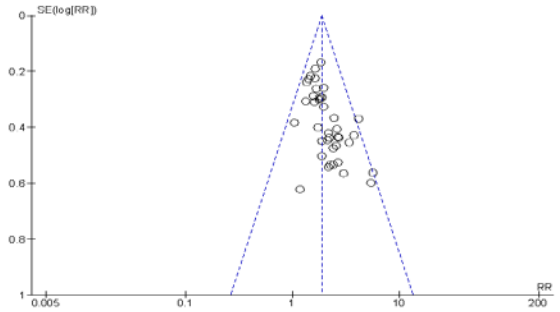


**Figure 4.27: Funnel plot of 40 studies that reported Karnofsky performance scale score gain ≥10 points**

Thirteen studies reported the mean scores of KPS for each treatment group. Eleven of these studies reported that the mean scores of KPS were increased in the combination therapy groups, while two studies reported that the mean scores were slightly reduced (Wu et al., 2010; Yang et al., 2005). Conversely, in the CMT control groups, only two studies reported the mean scores were slightly increased (Lao et al., 2012; Mao & Huang 2005), while the other 11 studies reported the mean scores were reduced after treatment. This suggests that CMT was generally associated with reduced KPS.

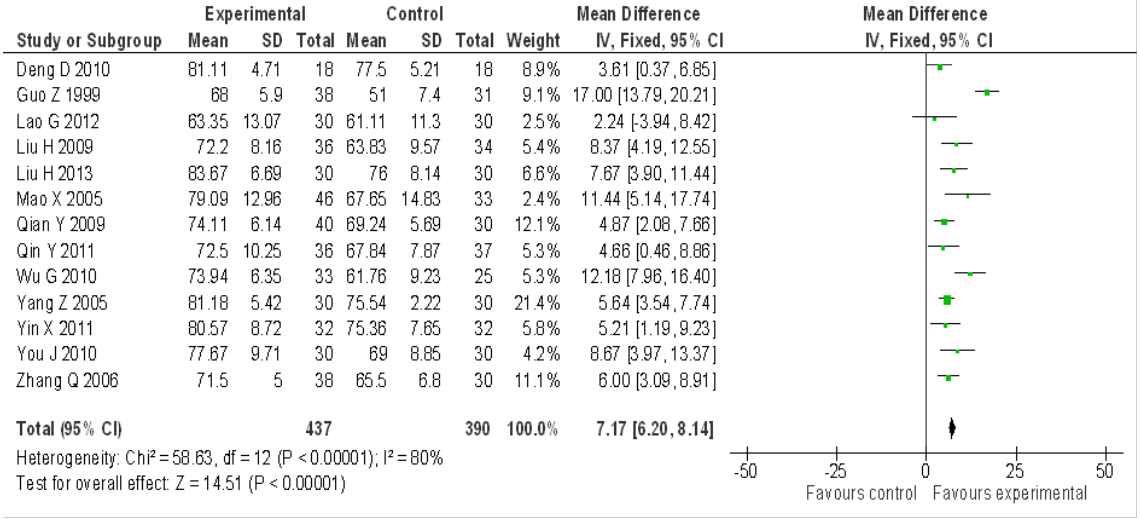


**Figure 4.28: Forest plot of mean difference for 13 studies that reported Karnofsky performance scale as mean ± SD scores**

control: chemotherapy alone; experimental: HM + chemotherapy; SD: standard deviation.

Analysis of the pooled data for the 13 studies that reported mean KPS scores for each treatment group after the intervention were higher in the combination therapy groups (MD 7.17, 95% CI [6.20, 8.14], $p<0.00001$, $I^2=80\%$) with substantial heterogeneity (Table 4.10; Figure 4.28). The funnel plot showed asymmetric scatter, with two studies (Guo, 1999; Wu et al., 2010) outlying the 95% confidence zone (Figure 4.29). A sensitivity analysis was therefore conducted by excluding two small studies located in the bottom of the funnel plot (Lao et al., 2012; Mao & Huang, 2005), and the two outlier studies (Guo, 1999; Wu et al., 2010) were excluded. The result showed a reduction in the magnitude of the difference but the KPS remained significantly improved in the combination treatment groups

compared with the CMT alone groups (9 studies, n= 561 participants, MD 5.76, 95% CI [4.68, 6.84], $I^2$= 0%) (Figure 4.30).

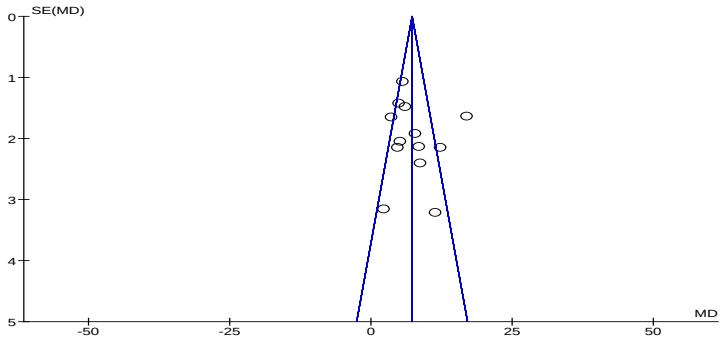


**Figure 4.29: Funnel plot of 13 studies of Karnofsky performance scale reported as mean ± SD scores**

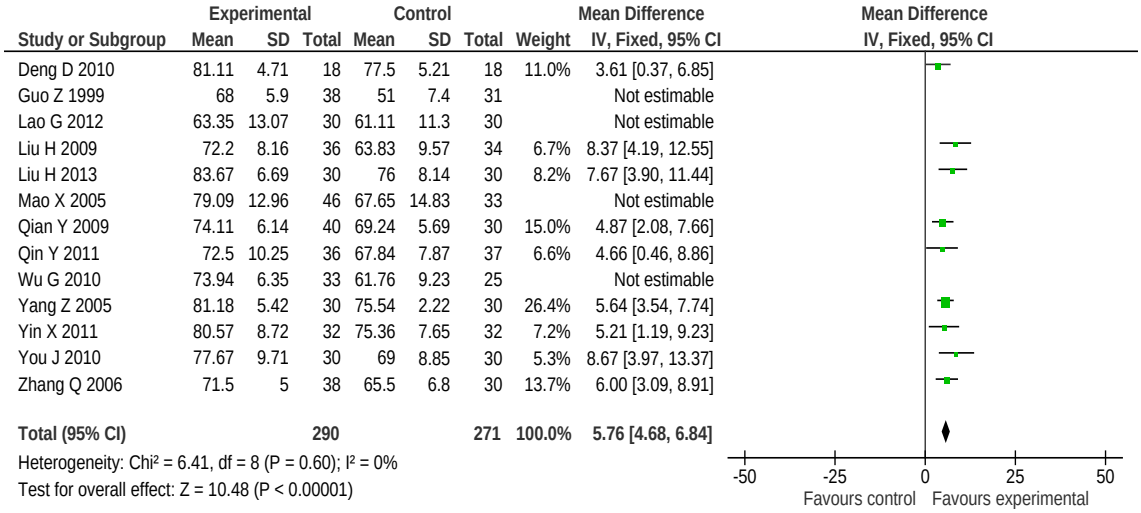


**Figure 4.30: Forest plot of mean difference for 9 studies that reported Karnofsky performance scale as mean ± SD scores (outlier removal)**

control: chemotherapy alone; experimental: HM + chemotherapy; SD: standard deviation

Multi-dimensional quality of life assessments

Multi-dimensional assessments of QoL were reported in the following six studies:

- Zhang et al. (2007) used the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30) (Aaronson et al., 1993); the Self-reported Anxiety Scale (SAS); and the Depression Scale (SDS);
- Yang (2008a) used Su Ying's QoL Questionnaire; and the KPS;
- Jian et al. (2005) and Zheng (2011) used the Chinese 1990 version of the standard criteria for assessment of QoL of tumour patients (The Ministry of Health PRC, 1991);
- Zhang et al. (2010) used a combination of: a visual analogue scale for pain; KPS; and body weight gain; and

- Jiang et al. (2013) used the Functional Assessment of Cancer Therapy-Colorectal (FACT-C) (Ward et al., 1999).

Since all the scales were different, there were few opportunities for data pooling. Therefore, most results were assessed for each study separately.

Zhang et al. (2007) assessed changes in negative emotion (anxiety and depression) and QoL using the Chinese version of the European Organisation for Research and Treatment of Cancer (EORTC QLQ-C30) Questionnaire, Self-reported Anxiety Scales (SAS) and Depression Scales (SDS) on day 1 prior to the chemotherapy (B1), day 2 during chemotherapy (T2), day 7 (T7) and day 14 (T14) after chemotherapy. The results showed the negative emotion and global QoL of patients in both arms deteriorated at the T2 measurement point, but improved gradually at T7 to reach a peak at the T14 measurement point. At T14, the improvements were greater in the combination therapy group compared with the CMT control ($p<0.05$) for the physical, role, and emotional sub-scales and the global QoL, but not for the cognitive or social sub-scales. For the symptom scales, significant benefits were found at T14 for relief of fatigue, insomnia, constipation, and diarrhoea but not for nausea and vomiting, pain or appetite.

In Yang (2008a), QoL was measured by Su Ying's QoL Questionnaire (12 items) which uses a score from 5 to 1 according to increase in severity, the more severe the symptoms the lower the score. The total scores for the two treatment groups were balanced before treatment (MD -0.17, 95% CI [-1.69, 1.35], p=0.83). When the two groups were compared after treatment, the result was significantly in favour of the combination therapy group (MD 4.10, 95% CI [2.36, 5.84], $p<0.00001$).

Jian et al. (2005) and Zheng et al. (2011) used the 1990 version of the Chinese standard criteria for assessment of QoL that uses a scoring system that ranges from 60 to 0 according to increase in severity, the more severe the symptoms the lower the score (The Ministry of Health PRC, 1991). Patients who recorded a score more than 40 (which referred to as 'satisfactory') after treatment were pooled as dichotomous data. The difference between the two treatment groups was significantly in favour of the combination therapy groups (RR 1.35, 95% CI [1.06, 1.71], p=0.02, $I^2$=47%) with no important heterogeneity (p=0.17).

Zhang et al. (2010) assessed QoL using a combination of KPS, a visual analogue scale for pain, and body weight. However, the criteria used in assessment were not described in detail. The authors reported that 18 out of 21 patients (85.7%) were judged as receiving 'clinical benefit' in the combination therapy group, compared with 11 out of 20 patients (55.0%) in the control group. The difference between the two groups was significantly in favour of the combination therapy group (RR 1.56, 95% CI [1.01, 2.40], p=0.04).

Jiang et al. (2013) used the FACT-C for assessment of QoL. The FACT-C questionnaire includes social/family, physical, emotional, and functional sub-scales, and a subscale of specific items for colorectal cancer (36 items in total). The scoring system ranges from 0 to 4 for each item, and the more severe the symptoms the lower the score (Ward et al., 1999). When the two groups were compared after treatment, the result was significantly in favour of the combination therapy group (MD 50.1, 95% CI [34.44, 65.76], p<0.00001).

Body weight

Assessment of body weight was reported in 7 studies. A body weight gain of ≥1.0 kg was defined as 'improved'; a loss ≥1.0 kg was defined as 'worse'; and a change between 'improved' and 'worse' was classified as 'stable'. The 'improved' events for body weight were pooled.

The pooled analysis for body weight gain in these seven studies (n=690) showed there was a significant difference in the incidence of 'improved' in favour of the combination treatment groups (Table 4.10, Figure 4.31).

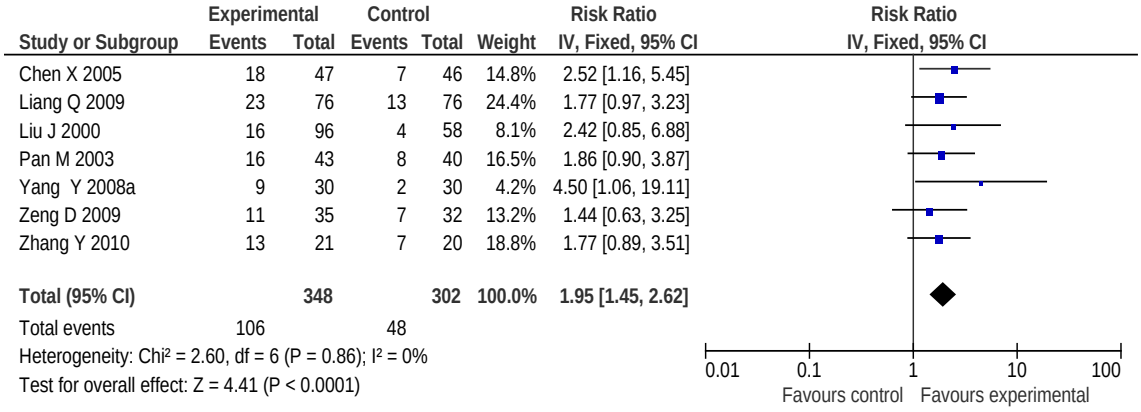


**Figure 4.31: Forest plot of risk ratio for body weight gain ≥ 1.0 Kg (seven studies)**
control: chemotherapy alone; experimental: HM + chemotherapy

### *4.8.3.8 Discussion of results for quality of life and body weight*

The results of the above meta-analyses showed that the combination treatments significantly elevated the QoL of CRC patients based on internationally well-recognised measurements such as the KPS, EORTC QLQ- C30 and FACT-C. These results were consistent with another review of CHM use in CRC (Zhong et al., 2012).

As an outcome measure, QoL is as important as survival for assessing palliative chemotherapy (Seymour et al., 1997). QoL in the healthcare perspective refers to an individual's well-being which includes emotional, social, and physical dimensions. KPS is a single-dimension scale that provides a global measurement of a patient's physical function. Its reliability and validity have been investigated

and verified, and it is used as a surrogate measure for a global evaluation of a patient's health status (Granda-Cameron et al., 2008). A high KPS score is associated with low symptom distress (Hwang et al., 2003). KPS can be rated by healthcare personnel or by patients themselves and it is extensively used in clinical trials on cancer patients (Yates et al., 1980).

A multi-dimensional QoL scale, the EORTC QLQ- C30 (Chinese version) was used in one of the included studies (Zhang et al., 2007). The QLQ-C30 comprises nine multi-item scales: five functional scales (physical, role, cognitive, emotional, and social); three symptom scales (fatigue, pain, and nausea and vomiting); and a global health and QoL scale. A multicultural clinical study found that the EORTC QLQ-C30 was a reliable and valid measure of the QoL for cancer patients (Aaronson et al., 1993). Studies have also found that there is high correlation between EORTC QLQ-C30 and KPS in terms of physical functioning (Pearson's correlation $r$=0.62, $p$<0.05) (Guzelant et al., 2004) and in global health/ QoL scores (Pearson's correlation $r$ = 0.39, $p$< 0.01) (Teunissen et al., 2004).

Since only one of the included studies used EORTC QLQ- C30 (Chinese version), meta-analysis was not possible. The study reported a significant improvement in physical functioning in favour of the group taking a CHM formula containing *Astragalus membranaceus* after 14 days of treatment with significant benefits for the relief of fatigue, insomnia, constipation, and diarrhoea. Significant benefits also were found for the role sub-scale, emotional sub-scale and global QoL (Zhang et al. 2007).

These findings suggest that the improvements in QoL reported for integrated HM treatment in ACRC patients may not be limited to physical functioning. There may be beneficial effects on broader QoL domains, including emotional state, which are not captured by KPS scores. The extent to which these effects are related to improvements in fatigue and other symptoms such as insomnia cannot be determined, but these results indicate that broader-based QoL scales should be used in future trials to further investigate such effects.

It should be noted that the included studies did not provide detailed information on how the KPS assessments were implemented and whether the lack of blinding was considered. Therefore, the possibility of detection bias cannot be ruled out. The funnel plot test also suggested the possibility of reporting bias in this set of data. Since the QoL was not the primary outcome in most of the included studies, negative results for KPS are likely to have not been reported.

Weight loss is a significant issue in ACRC patients with most tending to lose weight. However, most studies did not include this as a separate measure. To a certain extent, weight loss is captured in KPS, so this may account for the few studies that provided a separate measure. On the other hand, since all studies favoured the combination therapy groups there may have been selective outcome reporting of this aspect.

### 4.8.4 **Effects on adverse events (AE) associated with chemotherapy**

In total, 47 studies reported data on the alleviation of CMT-related adverse events (AEs). The WHO toxicity criteria grades 0 to 4 (Miller et al., 1981) and the National Cancer Institute Common Toxicity Criteria (NCI-CTC) (National Cancer Institute, 1999) were used to assess the events. These systems use similar grading criteria, so pooling of data was undertaken where possible (Hind et al., 2008). The outcomes for nausea and vomiting, diarrhoea, myelosuppression (neutropenia, thrombocytopenia, and anaemia), neurotoxicity, alopecia, liver impairment, kidney impairment, and stomatitis were pooled. Neutropenia, nausea and vomiting, and diarrhoea were the most commonly reported adverse events. Analyses were based on all grades of the AEs. When the Risk Ratio (RR) is less than 1 (IV, Fixed, 95% CI) it favours the test groups. Negative numbers for RD favour the test groups.

Three studies (Deng et al., 2010; Fang et al., 2008; Zheng et al., 2011) used recombinant human granulocyte colony-stimulating factor (rhG-CSF) to stimulate granulopoiesis in some of the participants. The rhG-CSF can influence monocytes, lymphocytes and the hemostatic system (Anderlini, 2009). Therefore, these three studies were not included for the analysis of neutropenia, thrombocytopenia and anaemia. The sub-groups for oxaliplatin regimes and non-oxaliplatin regimes were analysed separately.

Table 4.11 shows the overall meta-analysis results for the ten main categories of AE associated with chemotherapy. The results are given for the total pools followed by sub-groups based in the category of chemotherapy used. The most commonly reported outcomes were: neutropenia (38 RCTs); nausea and vomiting (35 RCTs); neurotoxicity (26 RCTs); and diarrhoea (22 RCTs). More detailed results for each of the ten categories are presented in the following sections. These include Funnel plots when there were ten or more RCTs in the pooled result for the particular AE, and forest plots for the total results and the sub-groups of oxaliplatin and non-oxaliplatin regimes.

**Table 4.11: Meta-analysis results of chemotherapy induced adverse events (56 studies)**

| **Outcomes/ groups** | **No. studies (participants)** | **RR (95% CI, FE), $I^2$; IR%** | **RD (95% CI, FE), $I^2$** |
|---|---|---|---|
| **Neutropenia** | | | |
| Total group | 38 (2,764) | 0.57 [0.52, 0.62], *p<0.00001, $I^2$=15%; -43% | -0.24 [-0.27, -0.21], *p<0.00001, $I^2$=35% |
| Oxaliplatin group | 29 (1,974) | 0.61 [0.55, 0.67], *p<0.00001, $I^2$=0%; -39% | -0.26 [-0.30, -0.22], *p<0.00001, $I^2$=0% |
| Non-oxaliplatin group | 8 (728) | 0.35 [0.28, 0.46], *p<0.00001, $I^2$=0%; -65% | -0.23 [-0.28, -0.17], *p<0.00001, $I^2$=77% |
| **Thrombocytopenia** | | | |
| Total group | 18 (1,254) | 0.64 [0.53, 0.780], *p<0.00001, $I^2$=0%; -36% | -0.10 (-0.14, -0.06), *p<0.00001, $I^2$=27% |
| Oxaliplatin group | 17 (1,185) | 0.66 [0.54, 0.80], *p<0.00001, $I^2$=0%; -34%% | -0.09 [-0.13, -0.05], *p<0.0001, $I^2$=25% |
| Non-oxaliplatin group | 1 (69) | 0.49 [0.25, 0.96], *p=0.04; -51% | -0.25 [-0.47, -0.03], |

| Outcomes/ groups | No. studies (participants) | RR (95% CI, FE), $I^2$; IR% | RD (95% CI, FE), $I^2$ |
|---|---|---|---|
| | | | *p=0.03. |
| **Anaemia** | | | |
| Total group (oxaliplatin group) | 15 (1,083) | 0.65 [0.54, 0.79], *p<0.0001, $I^2$=0%; -35% | -0.13 [-0.18, -0.08], *p<0.00001, $I^2$=14% |
| **Nausea & vomiting** | | | |
| Total group | 35 [2,407] | 0.61 [0.55, 0.66], *p<0.00001, $I^2$=40%; -39% | -0.26 [-0.30, -0.23], *p<0.00001, $I^2$=48% |
| Oxaliplatin group | 27 [1,843] | 0.65 [0.59, 0.71], *p<0.00001, $I^2$=28%; -35%. | -0.24 [-0.28, -0.19], *p<0.00001, $I^2$=50% |
| Non-oxaliplatin group | 8 (564) | 0.36 [0.28, 0.47], *p<0.00001, $I^2$=0%; -64% | -0.34 [-0.41, -0.28], *p<0.00001, $I^2$=27% |
| **Diarrhoea** | | | |
| Total group | 22(1483) | 0.60 [0.51, 0.71], *p<0.00001, $I^2$=13%; -40% | -0.13 [-0.16, -0.09], *p<0.00001, $I^2$=56% |
| Oxaliplatin group | 18 (1236) | 0.65 [0.55, 0.76], *p<0.00001, $I^2$=0%; -35% | -0.10 [-0.15, -0.06], *p<0.00001, $I^2$=33% |
| Non-oxaliplatin group | 4 (247) | 0.22 [0.12, 0.40], *p<0.00001, $I^2$=0%; -80% | -0.20 [-0.27, -0.12], *p<0.00001, $I^2$=83% |
| **Neurotoxicity** | | | |
| Total group (oxaliplatin group) | 26 (1,803) | 0.77 [0.70, 0.84], *p<0.00001, $I^2$=25%; -23%. | -0.13 [-0.18, -0.09], *p<0.00001, $I^2$=39%. |
| **Alopecia** | | | |
| Total group | 9 (637) | 0.53 [0.40, 0.71], *p<0.0001, $I^2$=13%; -47% | -0.12 [-0.17, -0.06], *p<0.0001, $I^2$=65% |
| Oxaliplatin group | 5 (383) | 0.82 [0.50, 1.330], p=0.41, $I^2$=0%. | -0.03 [-0.10, 0.04], p=0.36, $I^2$=0% |
| Non-oxaliplatin group | 4(254) | 0.41 [0.29, 0.60], *p<0.00001, $I^2$=0%; -59%. | -0.32 [-0.43, -0.21], *p<0.00001, $I^2$=0% |
| **Liver impairment** | | | |
| Total group | 19(1307) | 0.76 [0.63, 0.93], *p=0.007, $I^2$=34%; -24% | -0.07 [-0.11, -0.04], *p<0.0001, $I^2$=27% |
| Oxaliplatin group | 14(852) | 0.85 [0.69, 1.05], p=0.12, $I^2$=18%; -15% | -0.06 [-0.11, -0.01], *p=0.01, $I^2$=16% |
| Non-oxaliplatin group | 5 (455) | 0.37 [0.22, 0.65], *p=0.0005, $I^2$=4%; -63% | -0.09 [-0.15, -0.04], *p=0.0006, $I^2$=52% |
| **Kidney impairment** | | | |
| Total group | 6 (421) | 0.80 [0.51, 1.26], p=0.33, $I^2$=0%; -20% | -0.02 [-0.06, 0.03], p=0.48, $I^2$=0% |
| Oxaliplatin group | 5 (359) | 0.88 [0.55, 1.40], p=0.58, $I^2$=0%; -12% | -0.01 [-0.05, 0.04], p=0.79, $I^2$=0% |
| Non-oxaliplatin group | 1 (62) | 0.31 [0.07, 1.43], p=0.13; -69% | -0.14 [-0.30, 0.03], p=0.10 |
| **Stomatitis** | | | |
| Total group (oxaliplatin group) | 10 (801) | 0.76 [0.61, 0.94], *p=0.01, $I^2$=17%; -24% | -0.07 [-0.13, -0.02], *p=0.009, $I^2$=42% |

*statistically significant; RR: risk ratio; IR: improvement rate; RD: risk difference; FE: fixed effect; $I^2$: proportion of heterogeneity

#### *4.8.4.1 Neutropenia*

In the 38 studies that reported assessable data for neutropenia, there was a statistically significant reduction in neutropenia when CHMs were added to the intervention for all grades of neutropenia (RR 0.57, 95% CI [0.52, 0.62], $I^2$=15%). The incidence of neutropenia in the test groups was 43% lower than in the control groups (Table 4.11; Figure 4.32). The funnel plot was asymmetric with the smaller studies showing a positive shift to the left side of the 95% confidence region (Figure 4.33). Therefore, reporting bias due to selective reporting or publication bias cannot be ruled out.

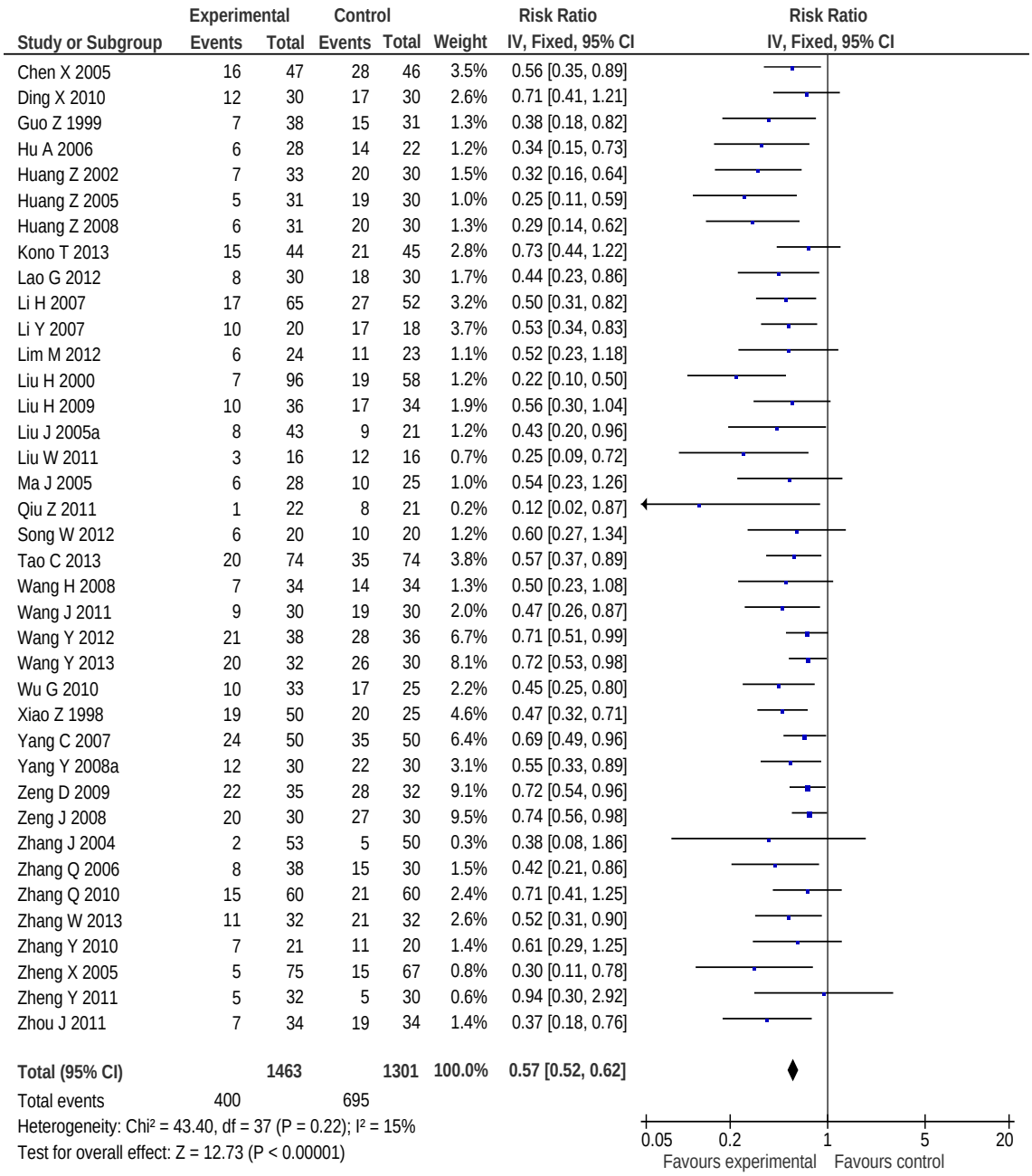


**Figure 4.32: Forest plot of risk ratio for neutropenia (total group, n=38)**
control: chemotherapy alone; experimental: HM + chemotherapy

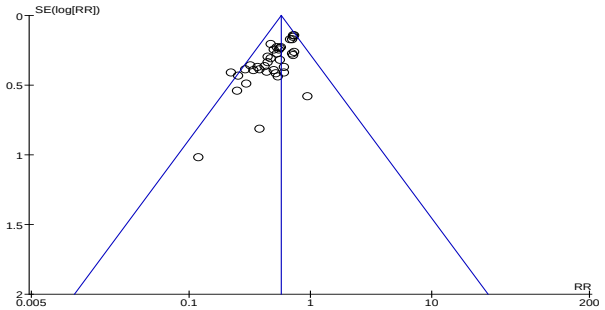


**Figure 4.33: Funnel plot of 38 studies that reported neutropenia (total group)**

Since the studies employed different types of chemotherapy regimens, these were evaluated separately. Evidence from international studies has shown that patients treated with oxalipaltin regimens have a higher incidence of neutropenia than patients treated with the older generation of 5-FU regimens (de Gramont et al., 2000). Therefore, studies were divided into two sub-groups: oxalipaltin group (n=29 studies) and non-oxalipaltin group (n=9 studies). The result showed the combination treatment significantly reduced neutropenia in both the oxalipaltin group (RR 0.61, 95% CI [0.55, 0.67], $I^2$=0%) (Figure 4.34) and the non-oxalipaltin group (RR 0.35, 95% CI [0.28, 0.46], $I^2$=0%) (Figure 4.35) compared to the CMT alone (Table 4.11). The funnel plot for the oxaliplatin group was asymmetric and similar to the total group of neutropenia (Figure 4.36).

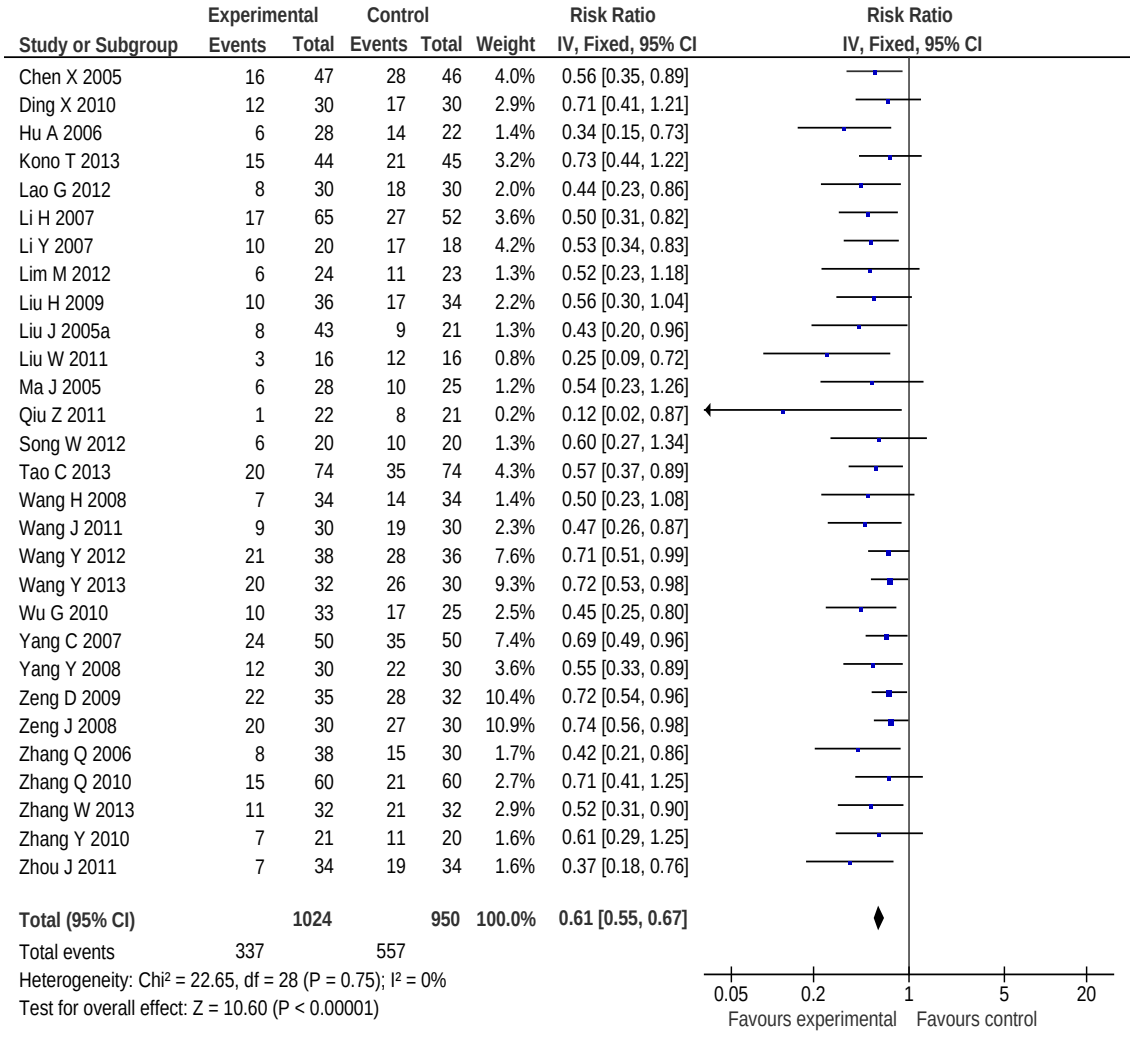


**Figure 4.34: Forest plot of risk ratio for neutropenia (oxaliplatin group, n=29)**
control: chemotherapy alone; experimental: HM + chemotherapy

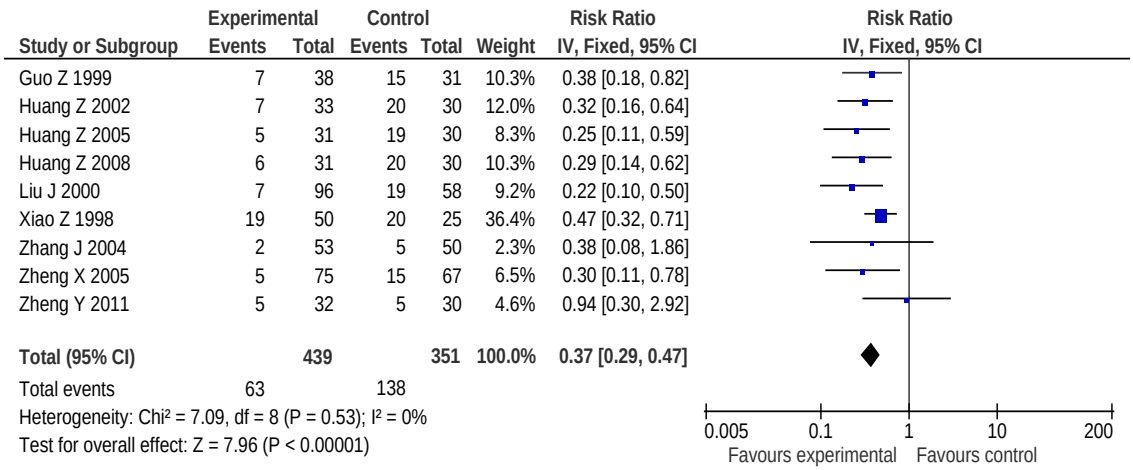


**Figure 4.35: Forest plot of risk ratio for neutropenia (non-oxaliplatin group, n=9)**
control: chemotherapy alone; experimental: HM + chemotherapy

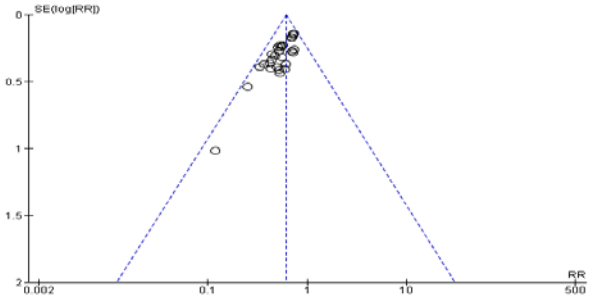


**Figure 4.36: Funnel plot of 29 studies that reported neutropenia (oxaliplatin group)**

### *4.8.4.2 Thrombocytopenia*

There were 18 studies that reported assessable data for thrombocytopenia. There was a statistically significant reduction in thrombocytopenia (all grades) (RR 0.64, 95% CI [0.53, 0.780], $I^2$=0%) when CHMs were added to the CMT intervention. In comparison with the CMT alone group, there was a 36% reduction in incidence of thrombocytopenia in the combination group (Table 4.11, Figure 4.37a). Only one study (Guo, 1999) did not use an oxaliplatin regimen. Analysis of the 17 studies that used oxaliplatin showed the combination treatment significantly reduced thrombocytopenia (RR 0.66, 95% CI [0.54, 0.80], $I^2$=0%) (Table 4.18; Figure 4.37b). The funnel plot was somewhat asymmetric with small studies showing a left shift (Figure 4.38).

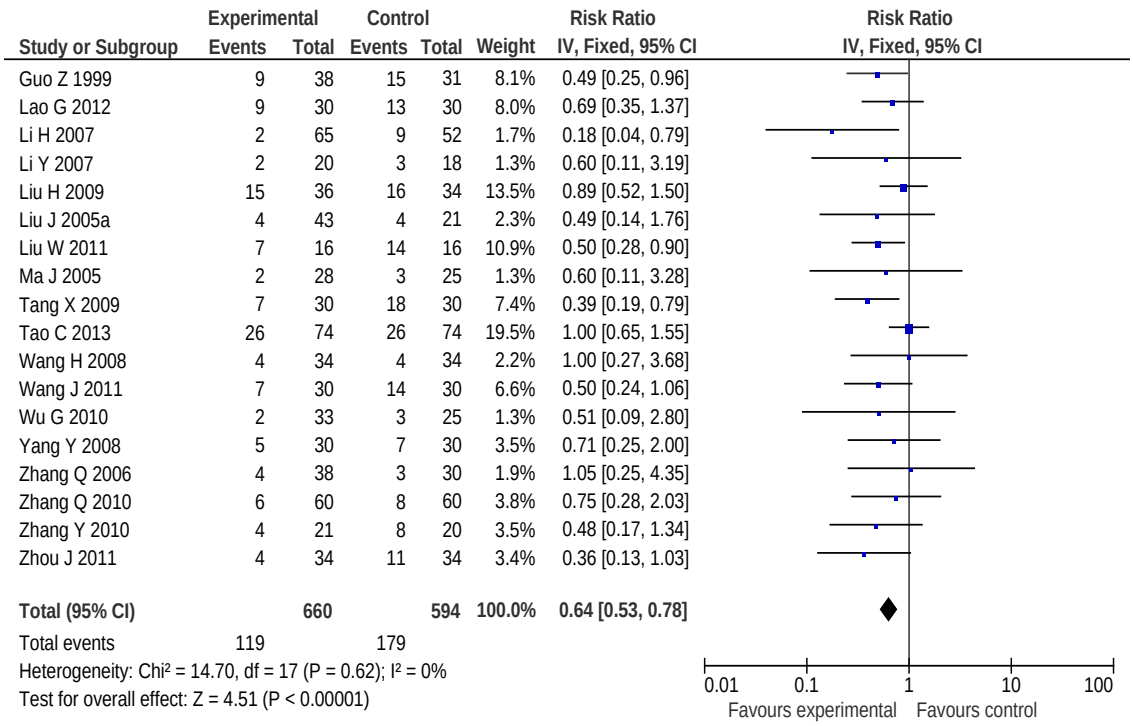


**Figure 4.37a: Forest plot of risk ratio for thrombocytopenia (total group, n=18)**
control: chemotherapy alone; experimental: HM + chemotherapy

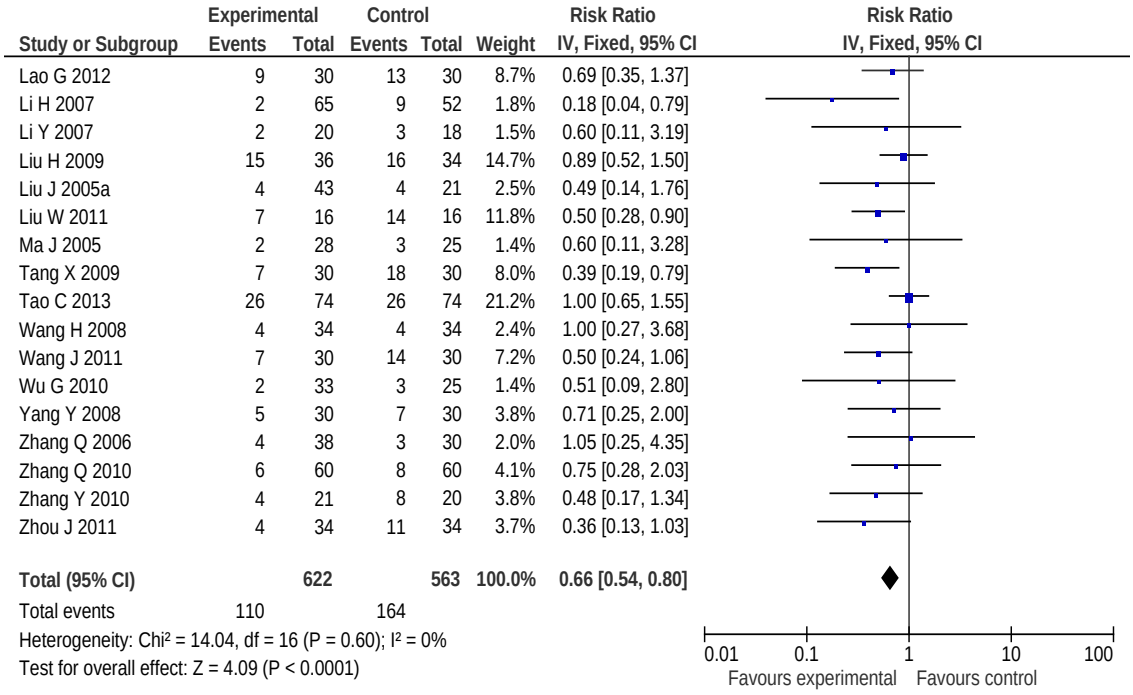


**Figure 4.37b: Forest plot of risk ratio for thrombocytopenia (oxaliplatin group, n=17)**
control: chemotherapy alone; experimental: HM + chemotherapy

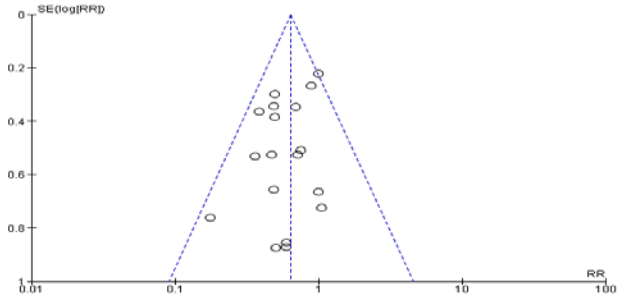


**Figure 4.38: Funnel plot of 18 studies that reported thrombocytopenia (total group)**

### *4.8.4.3 Anaemia*

Fifteen studies reported assessable data for anaemia. All studies were in the oxaliplatin group. There was a statistically significant reduction in anaemia (all grades) when additional CHMs were included in the intervention (RR 0.65, 95% CI [0.54, 0.79], $I^2$=0%) (Table 4.11; Figure 4.39). There was a 35% reduction in anaemia incidence in the combination therapy group compared with the CMT alone group. The funnel plot was roughly symmetric (Figure 4.40).

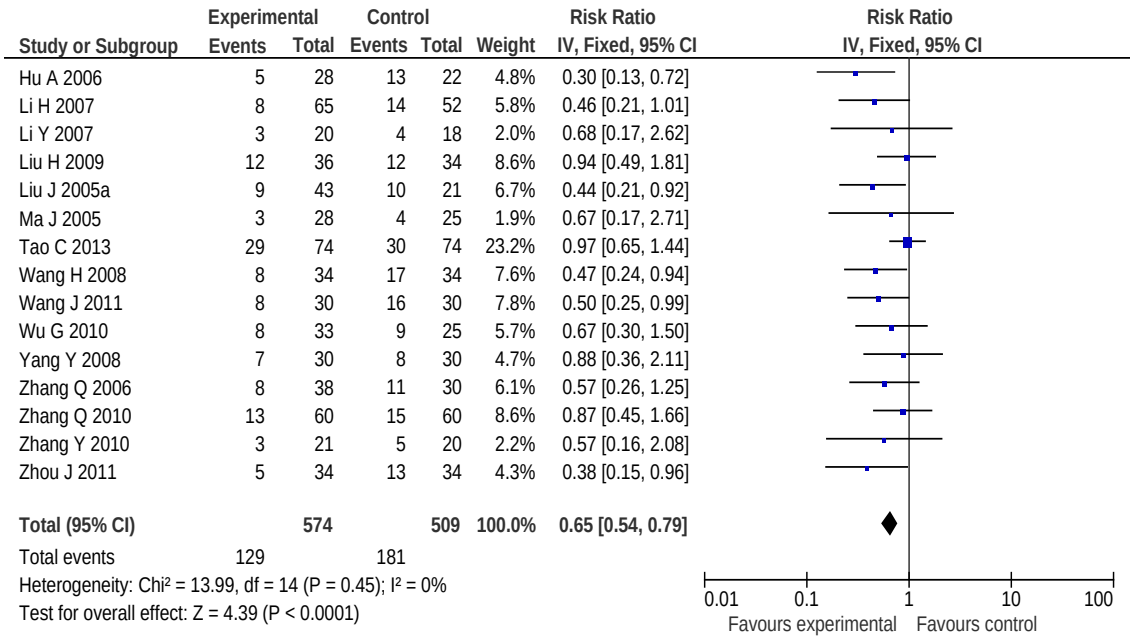


**Figure 4.39: Forest plot of risk ratio for anaemia in comparison with two treatments (n=15)**
control: chemotherapy alone; experimental: HM + chemotherapy

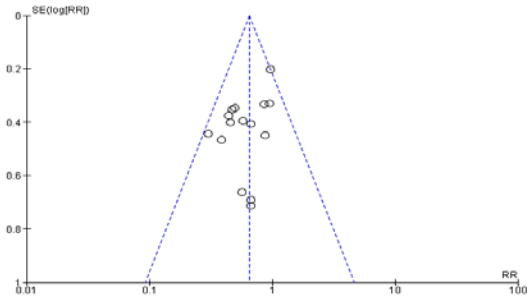


**Figure 4.40: Funnel plot of 15 studies that reported anaemia**

#### *4.8.4.4 Nausea and vomiting*

Thirty-five studies reported assessable data for nausea and/or vomiting. There was a statistically significant reduction in nausea and/or vomiting events (all grades) when additional HMs were included in the intervention with moderate heterogeneity (RR 0.61, 95% CI [0.55, 0.66], $I^2$=40%). The improvement rate was 39% (Table 4.11; Figure 4.41). The funnel plot was asymmetric due to two studies on the bottom left side of the positive zone which may indicate reporting bias (Figure 4.42). However, the removal of these two studies did not affect the overall result (RR 0.61 [0.56, 0.67], $I^2$=36%).

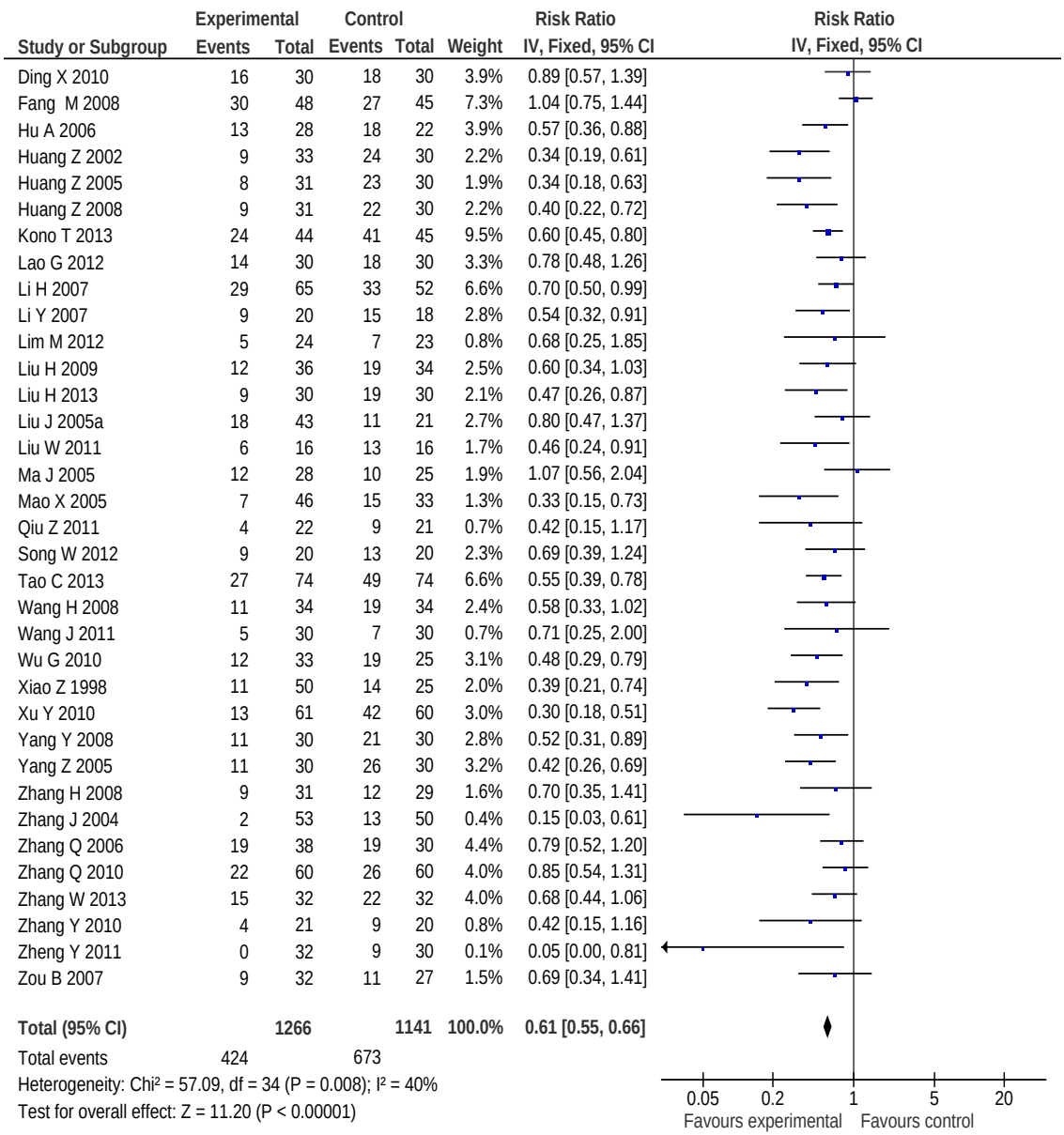


**Figure 4.41: Forest plot of risk ratio for nausea and vomiting (total group, n=35)**
control: chemotherapy alone; experimental: HM + chemotherapy

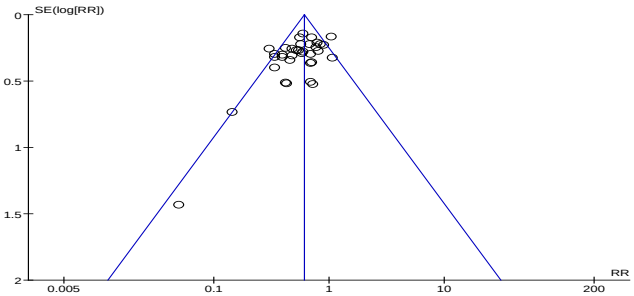


**Figure 4.42: Funnel plot of 35 studies that reported nausea and vomiting (total group)**

The studies were divided into two groups: oxaliplatin group (n=27 studies) and non-oxaliplatin group (n=8 studies). The result showed that the combination of CHM treatment with oxaliplatin-based CMT

significantly reduced nausea and vomiting (RR 0.65, 95% CI [0.59, 0.71], $I^2$=28%), with no important heterogeneity (Table 4.11; Figure 4.43a). The non-oxaliplatin group also showed significantly reduced nausea and vomiting (RR 0.36, 95% CI [0.28, 0.47], $I^2$=0%) when CHMs were combined with CMT (Table 4.11; Figure 4.43b). The funnel plot for the oxaliplatin group was somewhat asymmetric due to a single small study which was unlikely to affect the overall result (Figure 4.44).

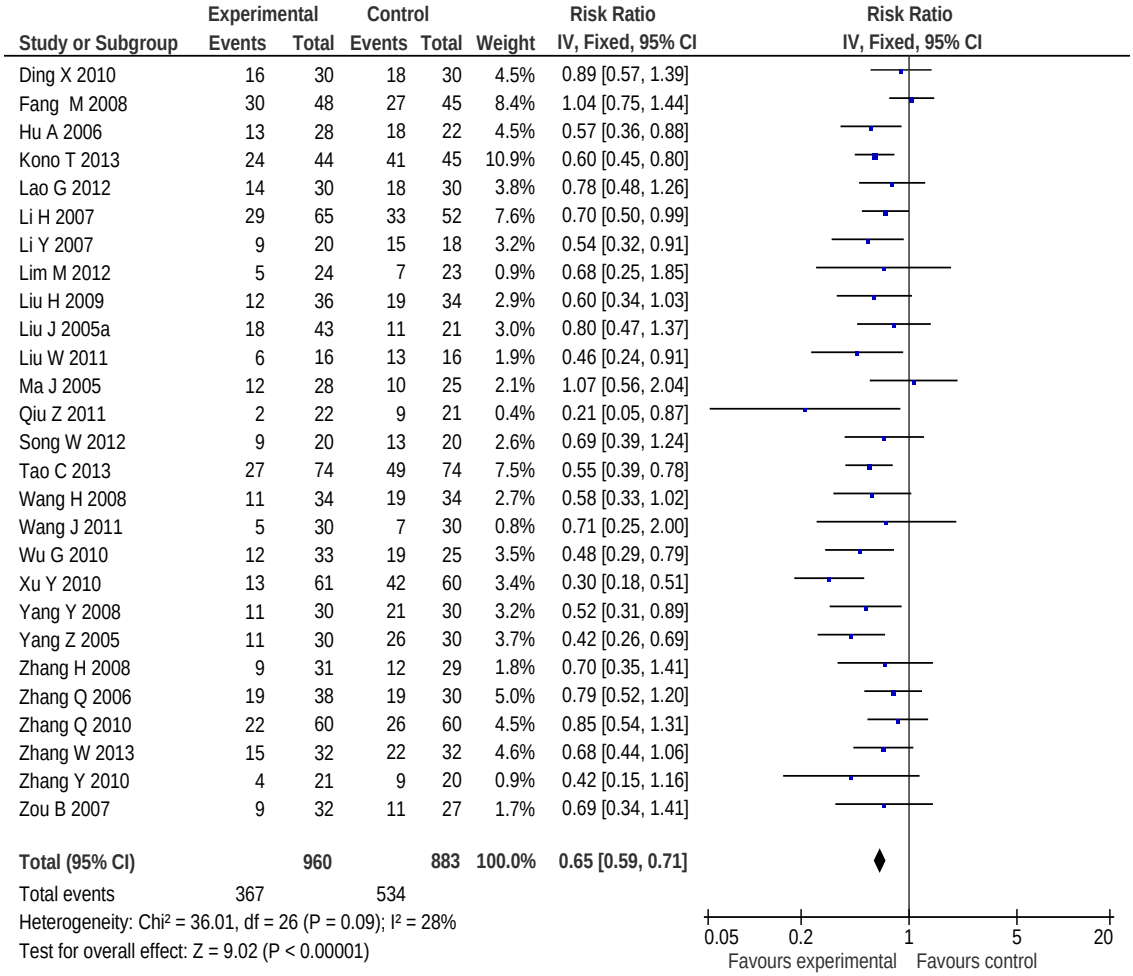


**Figure 4.43a: Forest plot of risk ratio for nausea and vomiting (oxaliplatin group, n=27)**
control: chemotherapy alone; experimental: HM + chemotherapy

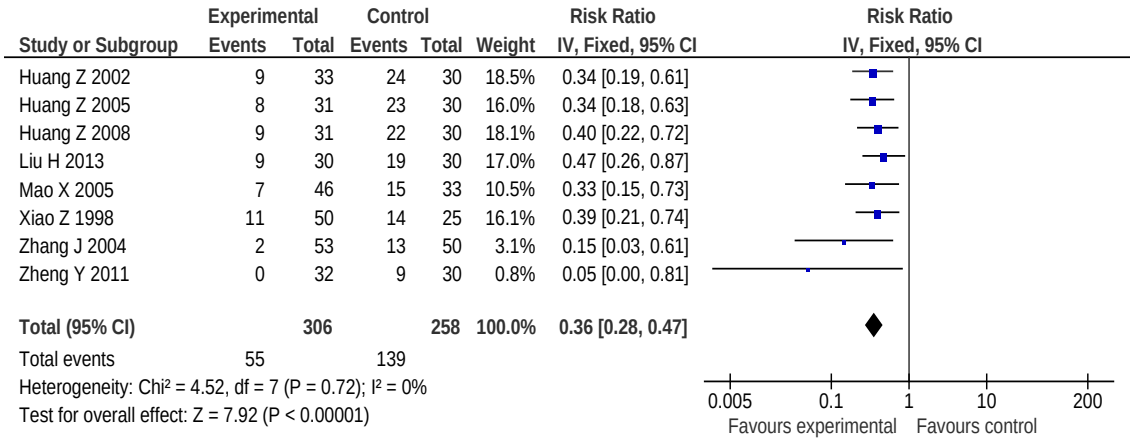


**Figure 4.43b: Forest plot of risk ratio for nausea and vomiting (non-oxaliplatin group, n=8)**
control: chemotherapy alone; experimental: HM + chemotherapy

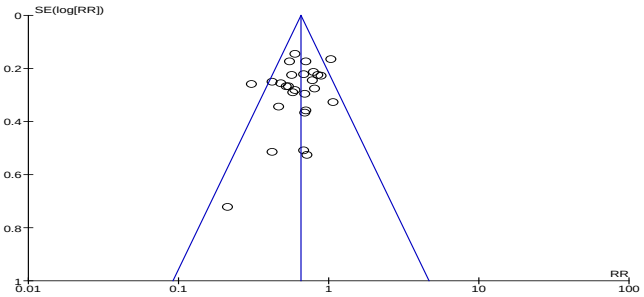


**Figure 4.44: Funnel plot of 27 studies that reported nausea and vomiting (oxaliplatin group)**

### *4.8.4.5 Diarrhoea*

Twenty-two studies reported assessable data for diarrhoea. All grades of diarrhoea events were statistically significantly reduced when CHMs were added to the CMT interventions (Table 4.11; Figure 4.45), with no important heterogeneity (RR 0.60, 95% CI [0.51, 0.71], $I^2$=13%). The reduction of diarrhoea was 40% more for CHMs integrated with CMT compared with CMT alone. The funnel plot was slightly asymmetric (Figure 4.46).

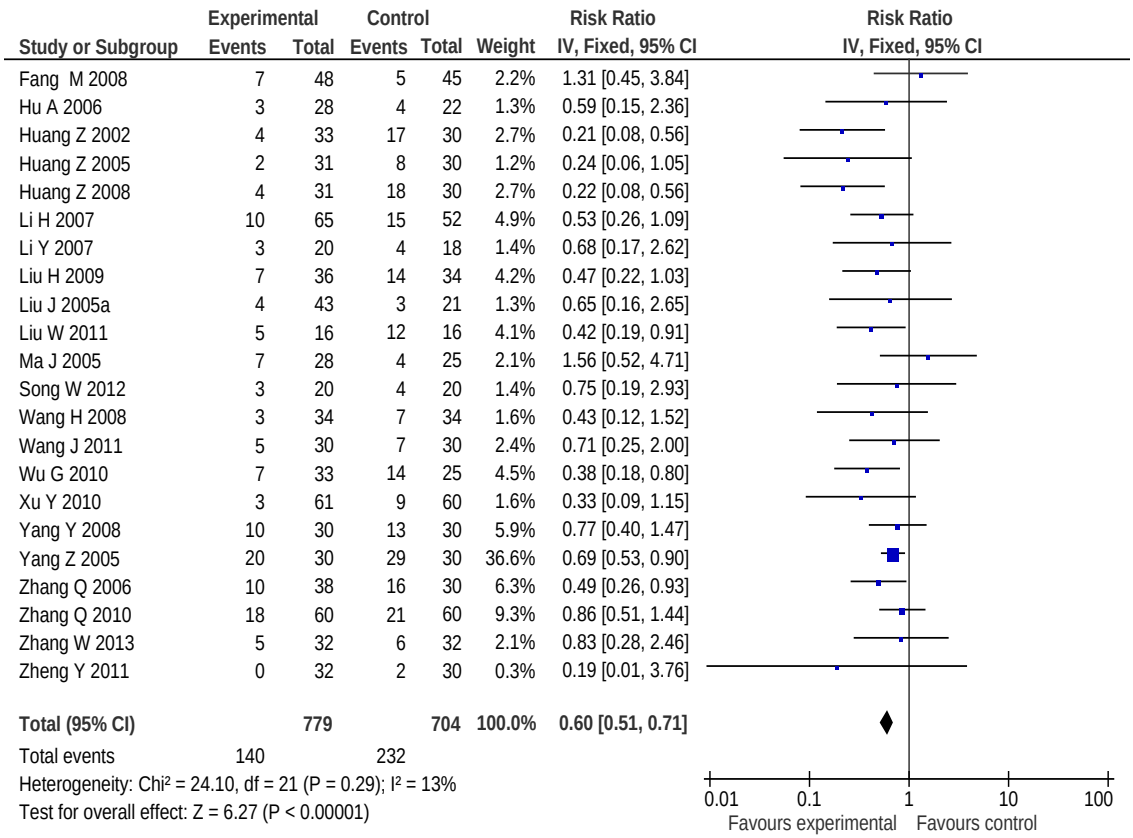


**Figure 4.45: Forest plot of risk ratio for diarrhoea in comparison with two treatments (total group, n=22)**

control: chemotherapy alone; experimental: HM + chemotherapy

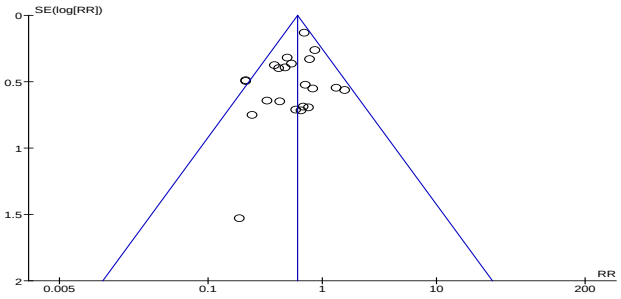


**Figure 4.46: Funnel plot of 22 studies that reported diarrhoea (total group)**

The oxaliplatin group (n=18) and non-oxaliplatin group (n=4) were analysed separately. The results showed that the combination treatments significantly reduced diarrhoea events in both the oxaliplatin group (RR 0.65, 95% CI [0.55, 0.76], $I^2$=0%) (Table 4.11; Figure 4.47a) and the non-oxaliplatin group (RR 0.22, 95% CI [0.12, 0.40], $I^2$=0%), (Table 4.11; Figure 4.47b). The funnel plot for the oxaliplatin group was roughly symmetric (Figure 4.48).

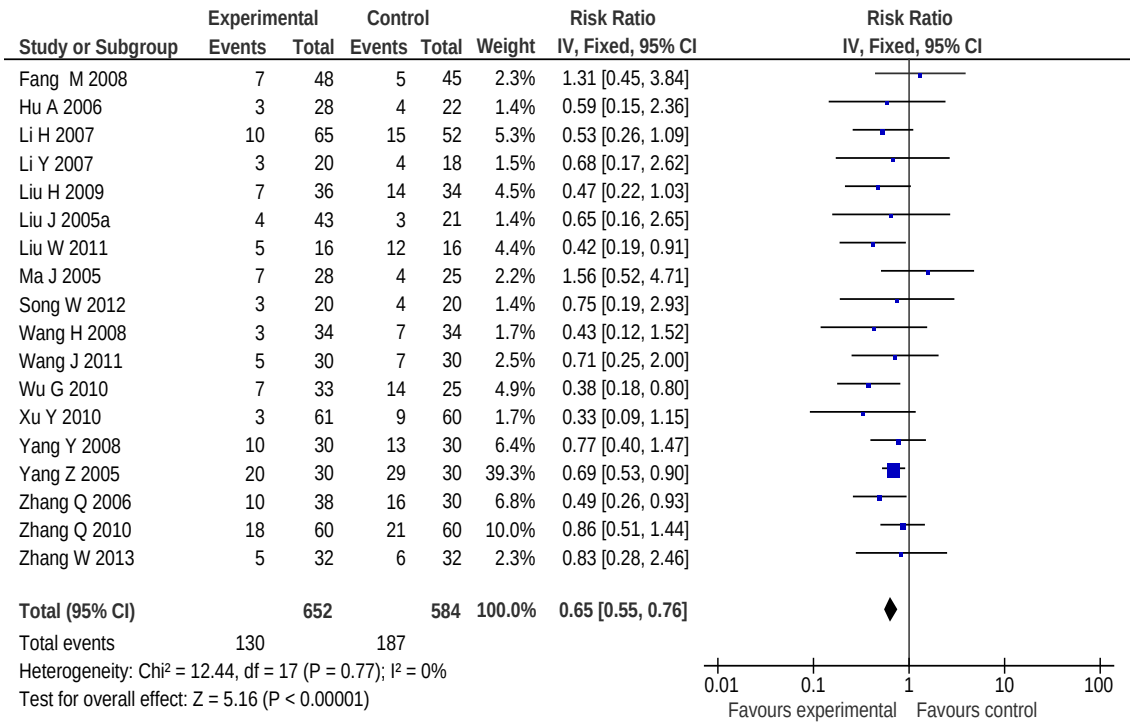


**Figure 4.47a: Forest plot of risk ratio for diarrhoea (oxaliplatin group, n=18)**
control: chemotherapy alone; experimental: HM + chemotherapy

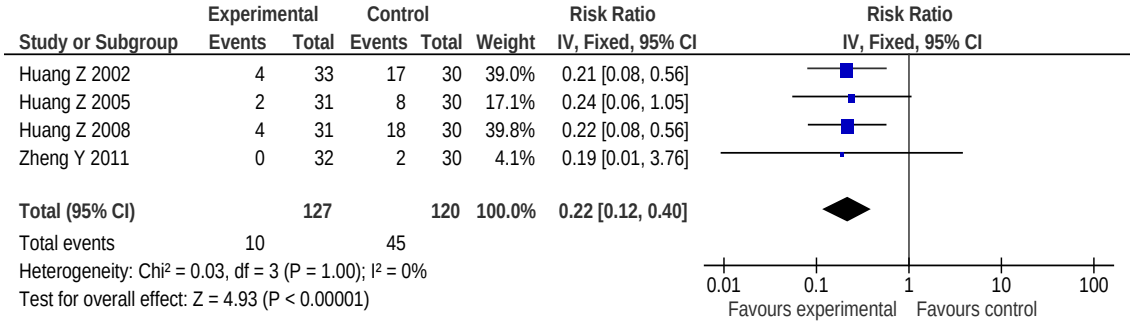


**Figure 4.47b: Forest plot of risk ratio for diarrhoea (non-oxaliplatin group, n=4)**
control: chemotherapy alone; experimental: HM + chemotherapy

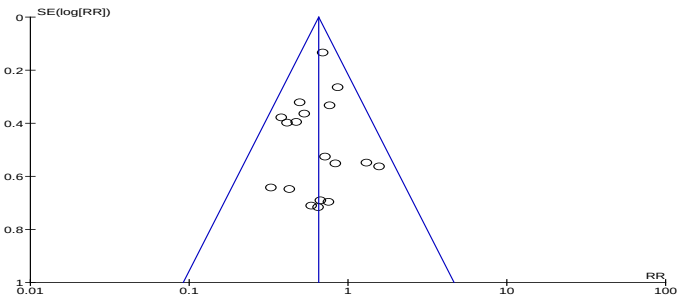


**Figure 4.48: Funnel plot of 18 studies that reported diarrhoea (oxaliplatin group)**

### *4.8.4.6 Neurotoxicity*

Neurotoxicity events were only reported for oxaliplatin regimen groups, with 26 studies reporting assessable data. There was a statistically significant reduction in neurotoxicity events (all grades) (RR 0.77, 95% CI [0.70, 0.84], $I^2$=25%) when CHMs were added to the CMT intervention (Table 4.11; Figure 4.49), and there was no important heterogeneity. The funnel plot was roughly symmetric (Figure 4.50).

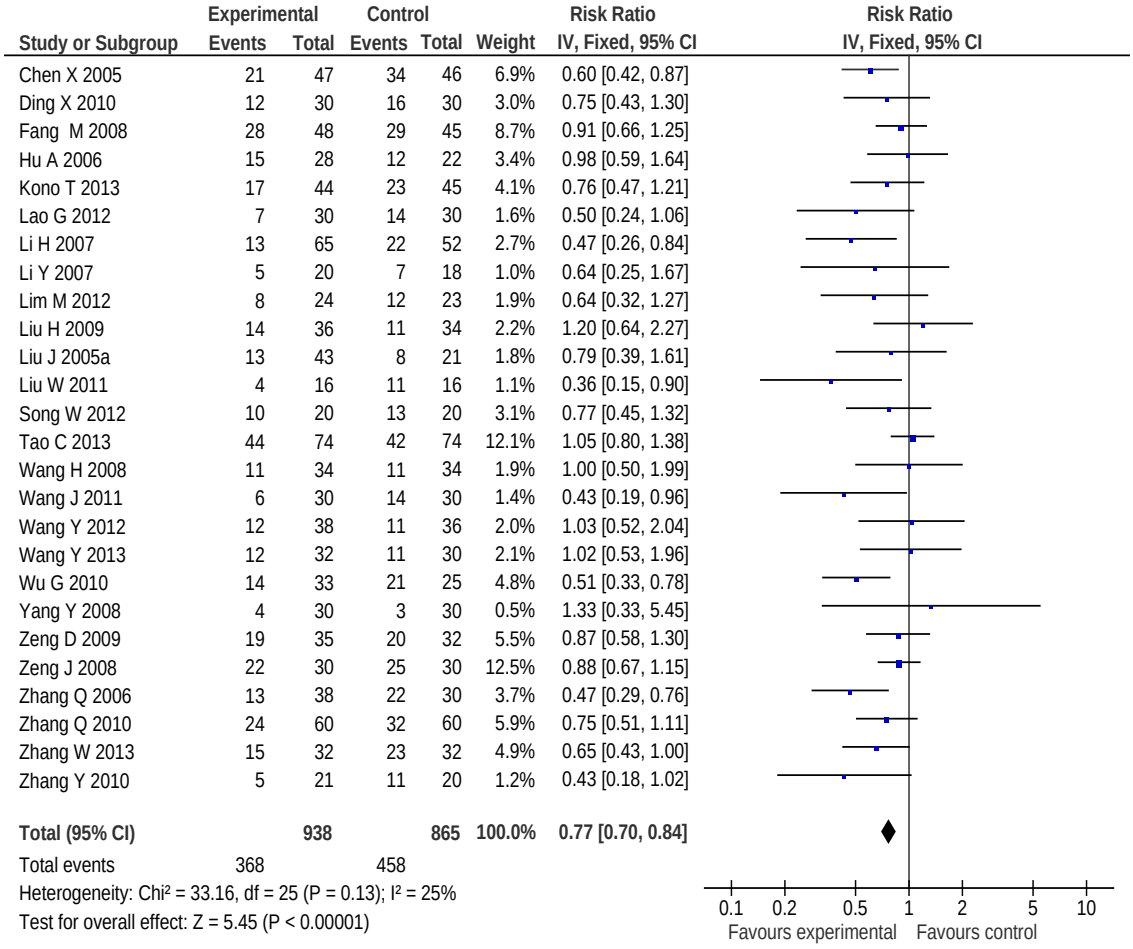


**Figure 4.49: Forest plot of risk ratio for neurotoxicity (n=26)**
control: chemotherapy alone; experimental: HM + chemotherapy

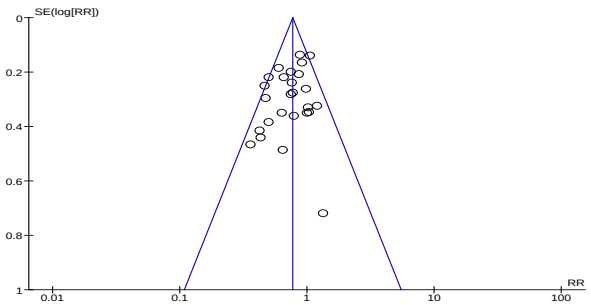


**Figure 4.50: Funnel plot of 26 studies that reported neurotoxicity**

### *4.8.4.7 Alopecia*

Nine studies reported assessable data for alopecia events. There was a statistically significant reduction in alopecia (all grades) when additional CHMs were combined with the CMT intervention, and there was no important heterogeneity (RR 0.53, 95% CI [0.40, 0.71], $I^2$=13%) (Table 4.11; Figure 4.51a).

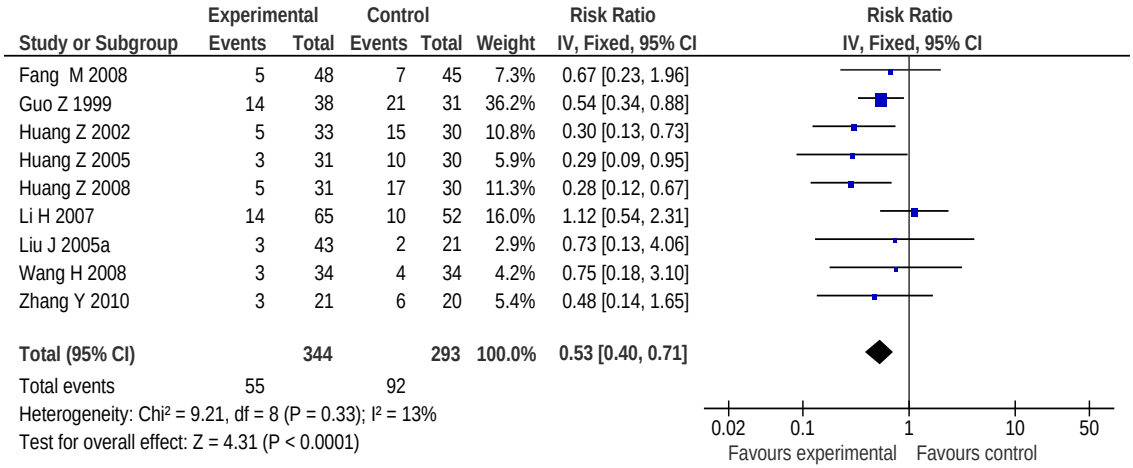


**Figure 4.51a: Forest plot of risk ratio for alopecia (total group, n=9)**
control: chemotherapy alone; experimental: HM + chemotherapy

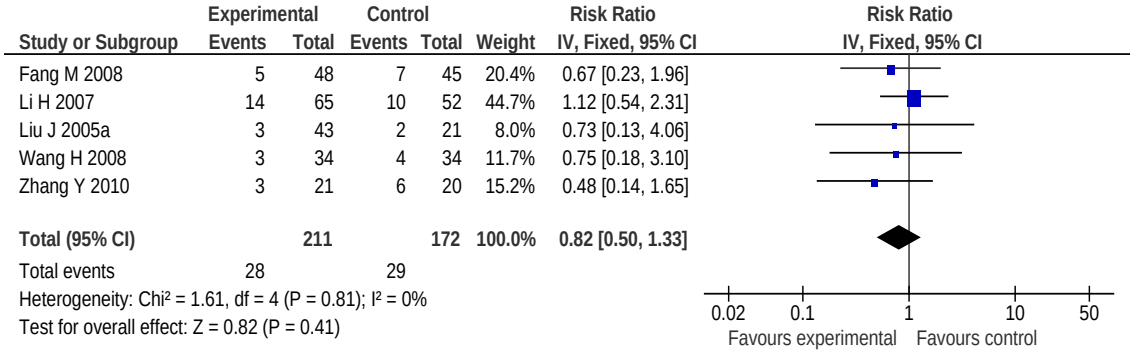


**Figure 4.51b: Forest plot of risk ratio for alopecia (oxaliplatin group, n=5)**
control: chemotherapy alone; experimental: HM + chemotherapy

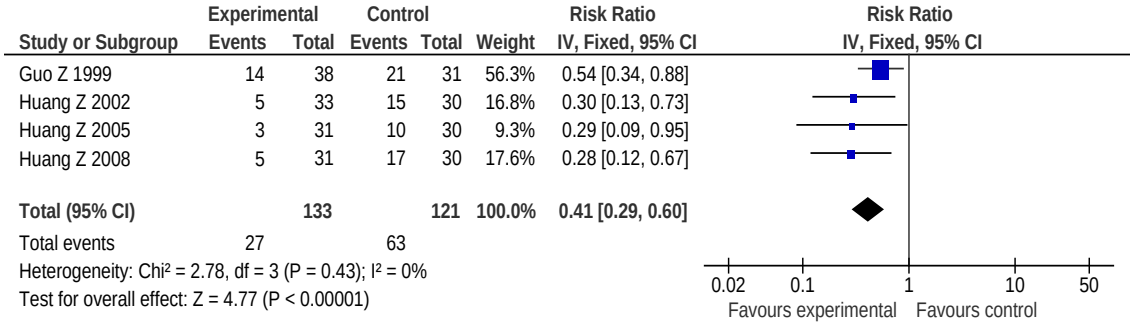


**Figure 4.51c: Forest plot of risk ratio for alopecia (non-oxaliplatin group, n=4)**
control: chemotherapy alone; experimental: HM + chemotherapy

The oxaliplatin group (5 studies) and non-oxaliplatin group (4 studies) were analysed separately. The pooled results showed there was no significant difference between the two treatment groups for alopecia events in the oxaliplatin group (RR 0.82, 95% CI [0.50, 1.33], $I^2$=0%) (Table 4.11; Figure 4.51b). However, there was a significant reduction in alopecia events in the combination therapy groups for the non-oxaliplatin group (RR 0.41, 95% CI [0.29, 0.60], $I^2$=0%) (Table 4.11; Figure 4.51c).

#### *4.8.4.8 Liver impairment*

Nineteen studies reported assessable data for liver impairment. There was a statistically significant reduction in all grades of liver impairment events (i.e. elevated transaminases) when CHMs were combined with the CMTs (Table 4.11; Figure 4.52), and there was no important heterogeneity (RR 0.76, 95% CI [0.63, 0.93], p=0.007, $I^2$=34%). The Funnel plot was asymmetric (Figure 4.53).

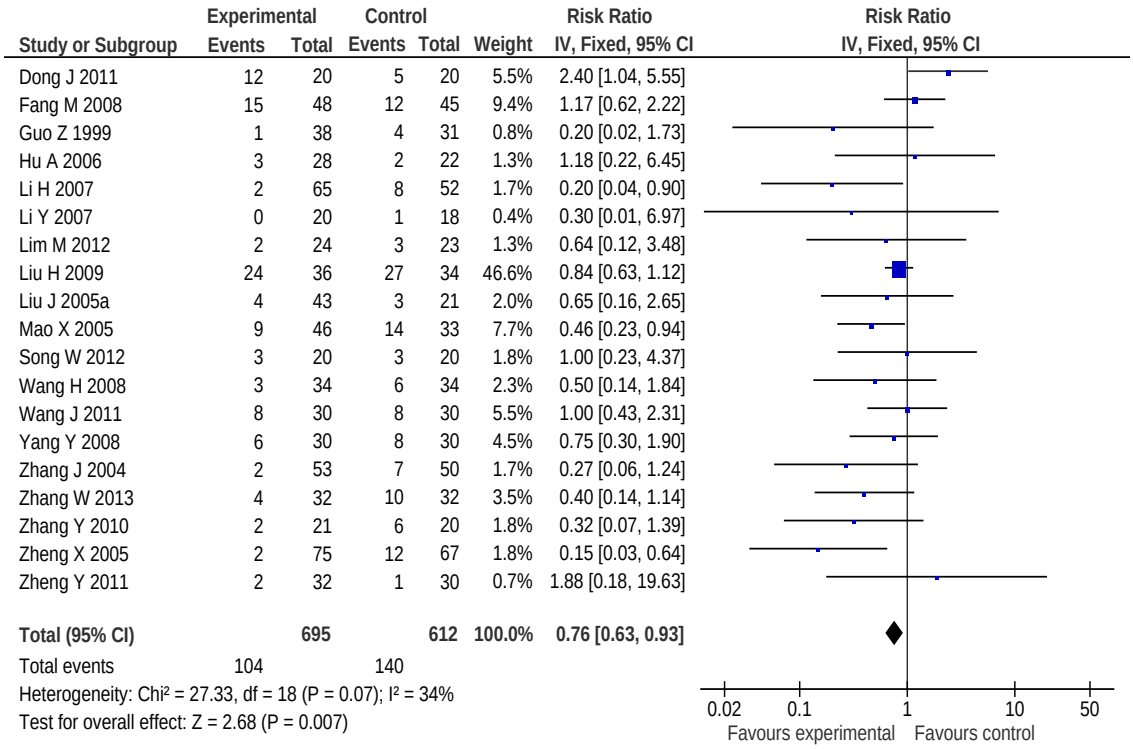


**Figure 4.52: Forest plot of risk ratio for liver impairment (total group, n=19)**
control: chemotherapy alone; experimental: HM + chemotherapy

The oxaliplatin group (n=14) and non-oxaliplatin group (n=5) were analysed separately. In the oxaliplatin sub-group, the result showed the combination treatment did not significantly reduce liver impairment compared to the CMT alone control groups (RR 0.85, 95% CI [0.69, 1.05], $I^2$=18%) (Table 4.11; Figure 4.54a).

Oxaliplatin undergoes little metabolism in the the liver and is not excreted hepatically (Cassidy & Misset, 2002). Therefore, it is reasonable to expect that the two treatments groups would show no

significant difference in hepatic damage. However, in the non-oxaliplatin sub-group, the pooled result was significantly in favour of the combination treatments (Table 4.11; Figure 4.54b). The funnel plot was asymmetric for the oxaliplatin group suggesting that studies that found no difference between groups may not have reported this outcome (Figure 4.55).

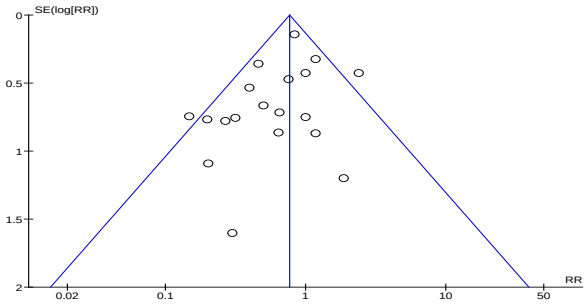


**Figure 4.53: Funnel plot of 19 studies that reported liver impairment (total group)**

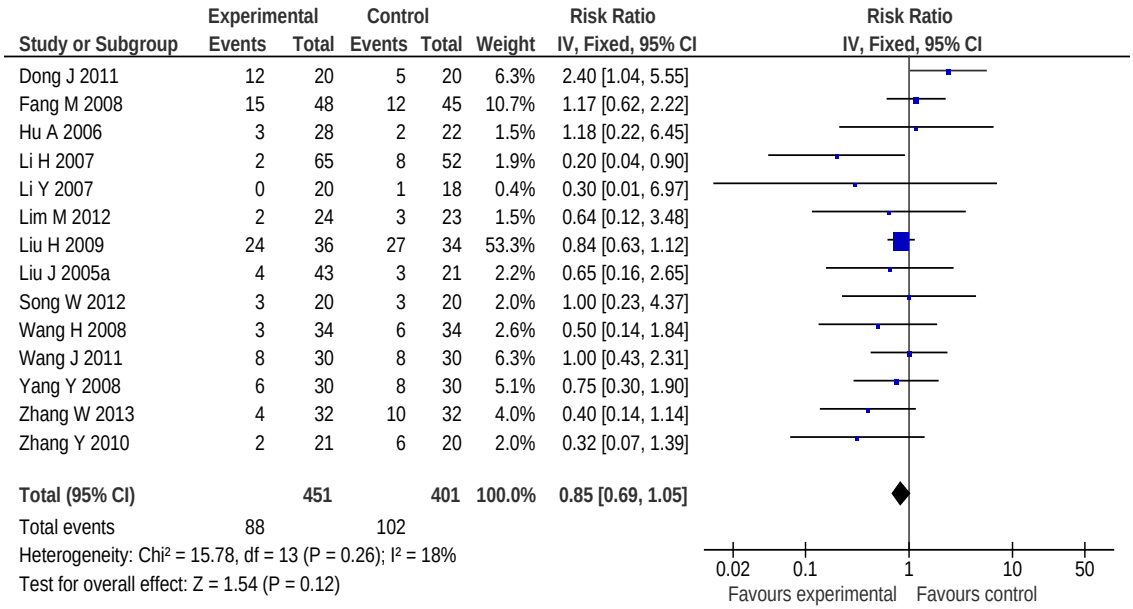


**Figure 4.54a: Forest plot of risk ratio for liver impairment (oxaliplatin group, n=14)**
control: chemotherapy alone; experimental: HM + chemotherapy

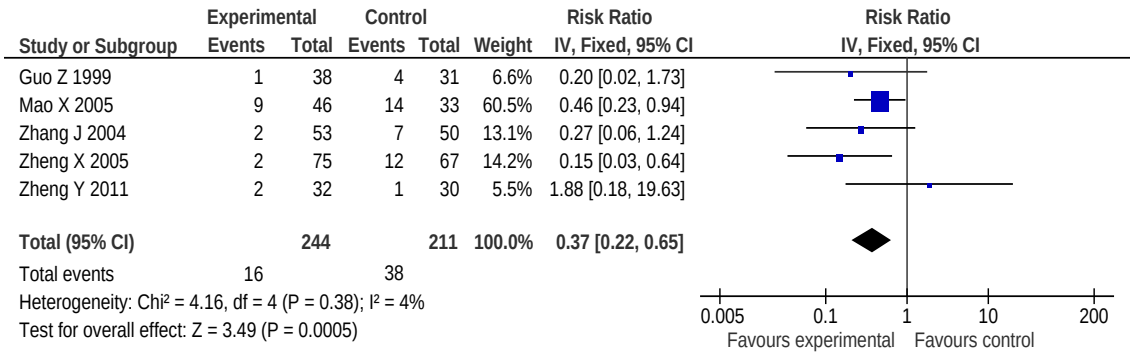


**Figure 4.54b: Forest plot of risk ratio for liver impairment (non-oxaliplatin group, n=5)**
control: chemotherapy alone; experimental: HM + chemotherapy

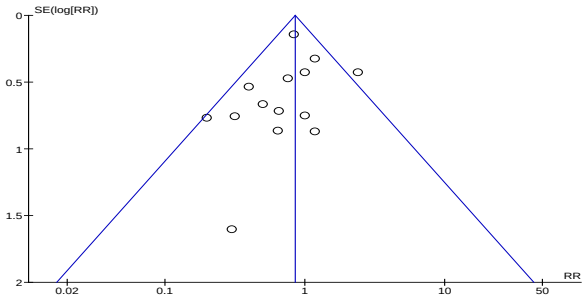


**Figure 4.55: Funnel plot of 14 studies that reported liver impairment (oxaliplatin group)**

### *4.8.4.9 Kidney impairment*

Assessable data were available in 6 studies for kidney impairment events. The pooled results showed there was no difference between the two treatment groups for kidney impairment adverse events (RR 0.80, 95% CI [(0.51, 1.26]), p=0.33, $I^2$=0%) (Table 4.11; Figure 4.56a).

The oxaliplatin group (n=5) and non-oxaliplatin group (n=1) were analysed separately. In the oxaliplatin group, the result also showed there was no difference between the two treatment groups for kidney impairment adverse events (RR 0.88, 95% CI [0.55, 1.40], p=0.58, $I^2$=0%) (Table 4.18; Figure 4.54b). The result for kidney impairment may be due to the mildness of kidney toxicity events in both treatment groups. This finding is consistent with other international studies that have shown that there are fewer kidney toxicity events when using third generation platinum drugs such as oxaliplatin (Cassidy & Misset, 2002).

Zheng et al. (2011) was the only study that used non-oxaliplatin chemotherapy and reported kidney impairment events. The analysis showed there was no difference between the two treatment groups for kidney impairment (RR 0.31, 95% CI [(0.07, 1.44]), p=0.13) (Table 4.18).

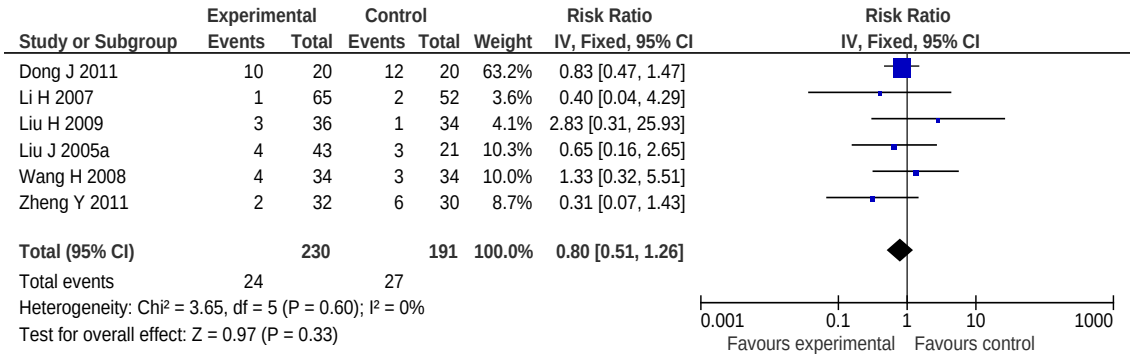


**Figure 4.56a: Forest plot of risk ratio for kidney impairment (total group, n=6)**
control: chemotherapy alone; experimental: HM + chemotherapy

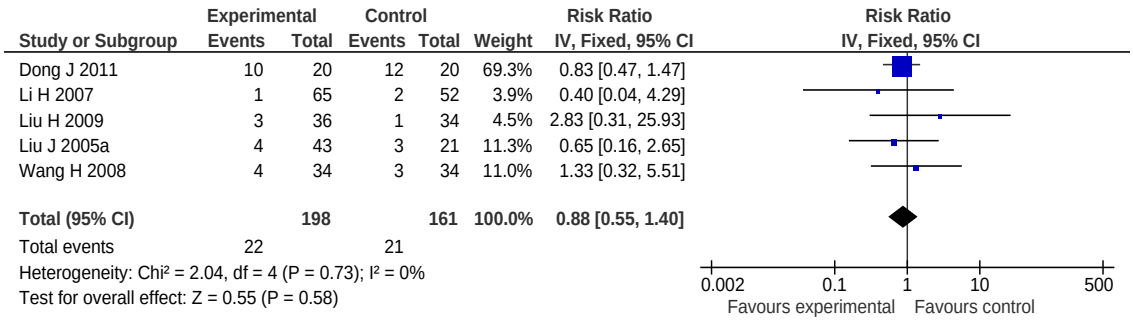


**Figure 4.56b: Forest plot of risk ratio for kidney impairment (oxaliplatin group, n=5)**
control: chemotherapy alone; experimental: HM + chemotherapy

### *4.8.4.10 Stomatitis*

Stomatitis events were only reported for the oxaliplatin regimens group (n=10 studies). There was a significant difference between the groups for the stomatitis events in favour of the combination therapy groups (RR 0.76, 95% CI [0.61, 0.94], $I^2$=17%) (Table 4.11; Figure 4.57). The funnel plot was symmetric for stomatitis (Figure 4.58).

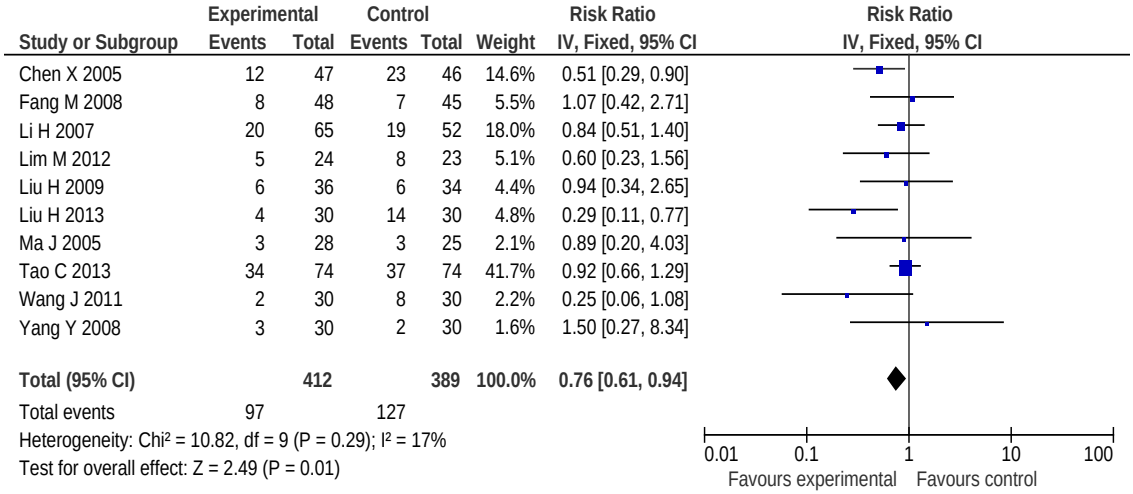


**Figure 4.57: Forest plot of risk ratio for stomatitis (n=10)**
control: chemotherapy alone; experimental: HM + chemotherapy

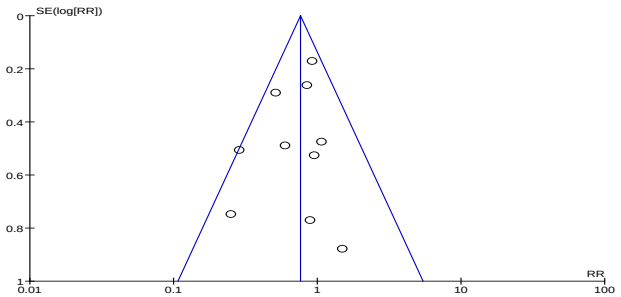


**Figure 4.58: Funnel plot of 10 studies that reported stomatitis**

#### *4.8.4.11 Discussion of adverse events associated with chemotherapy*

The addition of CHMs to the CMT regimens significantly reduced the incidence of hematologic toxicity (neutropenia, thrombocytopenia, and anaemia events), and gastrointestinal toxicity (nausea and vomiting, diarrhoea) induced by the CMTs. The sub-group meta-analysis based on the type of CMT reduced the heterogeneity of the results for neutropenia, nausea and vomiting, and diarrhoea events. One of the reasons for this may be associated with differences in the hematologic toxicity and gastrointestinal toxicity between the oxaliplatin regimens and non-oxaliplatin regimens.

Dose-dependant neurotoxicity is particularly associated with oxaliplatin regimens (Cassidy & Misset, 2002). These toxicity events appear to have been alleviated when the CHMs were added to the oxaliplatin-based CMT regimens. Two previous CRC systematic reviews did not analyse these adverse events (Zhong et al., 2012; Guo et al., 2012). Kono et al. (2011) reported a retrospective study that enrolled 90 patients who received oxaliplatin regimen treatment for ACRC. The study compared adjuvant oxaliplatin-based CMT combined with the CHM formula GJG (group A), which is a combination of 10 HMs, with three other groups: calcium gluconate and magnesium sulfate + oxaliplatin regimen (group B); GJG + calcium gluconate and magnesium sulfate + oxaliplatin regimen (group C); and oxaliplatin regimen alone (group D). The authors found that the patients in group A had a 50% neurotoxicity incidence, whereas the other groups had higher neurotoxicity incidences: group B, 100%; group C, 78.9%; and group D, 91.7%, when the cumulative dose of oxaliplatin exceeded 500 mg /$m^2$. Pre-clinical studies have suggested that GJG increases the level of dynorphin and nitric oxide. Dynorphin alleviates the symptoms of numbness or pallesthesia via the opiate system, and the nitric oxide increases the blood supply to nerve tissues (Kono et al., 2011). The same authors conducted a well-designed phase II, randomised, double-blind, placebo-controlled study with the same herbal formula in the test arm, and a placebo in the control arm. The results showed that the incidence of grade 2 or greater oxaliplatin-induced peripheral neurotoxicity (OPN) up until the 8th cycle of chemotherapy was 39% in the test arm versus 51% in the control arm (RR 0.76, 95% CI [0.47, 1.21]). The incidence of grade 3 OPN was 7% and 13% in the test arm and control arm respectively (RR 0.51, 95% CI [0.14-1.92]). No concerns regarding toxicity emerged with the GJG treatment. The authors concluded that the GJG intervention delayed the onset of grade 2 or greater OPN without impairing FOLFOX efficacy (Kono et al., 2013).

It is interesting to note that the RD analysis method generated more substantial heterogeneity ($I^2$>50% or large $Chi^2$ with $p<0.1$) than using the RR method for dichotomous data (Table 4.11). Empirical evidence had found that RD analysis was more likely to generate heterogeneity compared with OR or RR, since RD more directly correlates to the control group event rate (Engels et al., 2000). There is evidence that increasing the control group event rate is associated with higher heterogeneity in dichotomous data. In general, analysis using RR and OR produce more consistent results across

varying baseline risks than analysis using RD, and it appears that RR is more reliable for providing a consistent prediction clinically (Deeks et al., 2002).

In a number of meta-analysis pools, asymmetric funnel plots indicated the possibility of publication bias or selective reporting within the group of studies for a particular outcome. Since the AEs induced by CMT were not the primary outcome in the majority of the included studies, a non-significant result may be not have been reported by the authors. The lack of a clinical trial protocol for the majority of the studies meant it was not possible to determine whether these assessments had been conducted and not reported or not conducted at all. It is also possible that small, pilot scale studies that did not show significant results were not published.

### 4.8.5 **Effects on immunoregulation**

Twenty-six studies investigated immune system activity during treatment. The percentages of T cell subsets (CD3+, CD4+, CD8+); the ratio of CD4+ to CD8+; CD4+CD25+ regulatory T (Treg) cells; and NK cell activity (%) in serum were reported. These immune parameters provided measurements of the status of the person's cell-mediated immunity (CMI) which plays a critical role in defending the host against cancer and in reducing metastasis (Swann & Smyth, 2007). The MD results are presented for both the fixed effect model and random effect model. A positive result indicated increased T cell subsets (CD3+ and CD4+), increased ratio CD4+ to CD8+, or increased NK cell activity in serum, which are considered favourable. For CD8+ cells, and CD4+CD25+ regulatory T cells, decreases are favourable.

#### *4.8.5.1 CD3+ cells*

Fourteen studies reported assessable data for percentage of CD3+ cells. The addition of CHM interventions to the CMTs increased the mean percentage of CD3+ cells compared to before treatment. This general result was reported in all studies except one study in which the percentage of CD3+ decreased. In the control groups, five studies showed no change in the percentage of CD3+ cells after treatment, whereas the rest of the studies showed decreases.

The pooled result at the end of treatment showed the CD3+ cells were significantly higher when CHMs were included in the intervention (MD 6.38, 95% CI [5.61,7.15], $p<0.00001$, $I^2=93\%$) compared with CMT alone, and there was important heterogeneity (Table 4.12; Figure 4.59). The results of the two analysis models were similar. The funnel plot was asymmetric (Figure 4.60).

The cell-mediated immunity is influenced by stage, tumour burden, the treatments (Ji et al., 2010). Since the individual data for the participants was not available, it is difficult to determine the causes of the heterogeneity. A sensitivity analysis was therefore undertaken to investigate the sources of the

heterogeneity. Four outlying studies (Song & Zhang, 2012; You et al., 2010; Zeng et al., 2008; Zhang & Song 2013) that are scattered outside the 95% confidence interval zone of the funnel plot and one small study (Dong et al., 2011) that is isolated at the bottom of the funnel plot (Figure 4.60) were excluded from analysis.

**Table 4.12: Meta-analyses results for immunity regulation of herbal medicine in combination with systemic chemotherapy (26 studies)**

| Outcomes | No. studies (participants) | MD (95% CI, FE), $I^2$ | MD (95% CI, RE), $I^2$ |
|---|---|---|---|
| **CD3+ (%)** | | | |
| Total group | 14 (820) | 6.38 [5.61, 7.15], *p<0.00001, $I^2$=93% | 6.94 [3.72, 10.15], *p<0.0001, $I^2$=93% |
| Excluding outliers | 9 (566) | 4.97 [4.06, 5.88], *p<0.00001, $I^2$=41% | 4.21 [2.79, 5.62], *p<0.00001, $I^2$=41% |
| **CD4+ (%)** | | | |
| Total group | 15 (932) | 7.27 [6.73, 7.82], *p<0.00001, $I^2$=97% | 6.93 [3.43, 10.42], *p<0.00001, $I^2$=97% |
| Excluding outliers | 9 (559) | 4.82 [4.11, 5.53], *p<0.00001, $I^2$=39% | 4.60 [3.53, 5.67], *p<0.0001, $I^2$=39% |
| **CD8+ (%)** | | | |
| Total group | 14 (872) | 1.68 [1.07, 2.28], *p<0.00001, $I^2$=99% | 0.65 [-4.55, 5.85], p=0.81, $I^2$=99% |
| Excluding outliers | 9 (520) | -1.39 [-2.30, -0.48], *p=0.003, $I^2$=37% | -1.35 [-2.58, -0.13], *p=0.003, $I^2$=37% |
| **CD4+/CD8+** | | | |
| Total group | 17 (1238) | 0.32 [0.27, 0.360], *p<0.00001, $I^2$=75% | 0.32 [0.22, 0.43], *p<0.00001, $I^2$=75% |
| Excluding outliers | 15 (1110) | 0.28 [0.23, 0.32], *p<0.00001, $I^2$=17% | 0.26 [0.21, 0.32], *p<0.00001, $I^2$=17% |
| **CD4+CD25+ Tregs (%)** | | | |
| Total group | 2 (78) | -0.23 [-0.60, 0.15], p=0.23, $I^2$=87% | -1.24 [-3.73, 1.25], p=0.33, $I^2$=87% |
| Ma M 2010 | 1 (40) | -2.67 [-4.42, -0.92], *p=0.003 | na |
| Zhang Y 2010a | 1 (38) | -0.11 [-0.49, 0.27], p=0.58 | na |
| **NK cell activity (%)** | | | |
| Total group | 18 (1175) | 9.13 [8.49, 9.77], *p<0.00001, $I^2$=97% | 8.89 [5.11, 12.67], *p<0.00001, $I^2$=97% |
| Excluding outliers | 11 (790) | 5.57 [4.73, 6.41], *p<0.00001, $I^2$=29% | 5.71 [4.65, 6.77], *p<0.00001, $I^2$=29% |

*statistically significant; MD: mean difference; FE: fixed effect; RE: random effect; $I^2$: proportion of heterogeneity; na: not applicable.

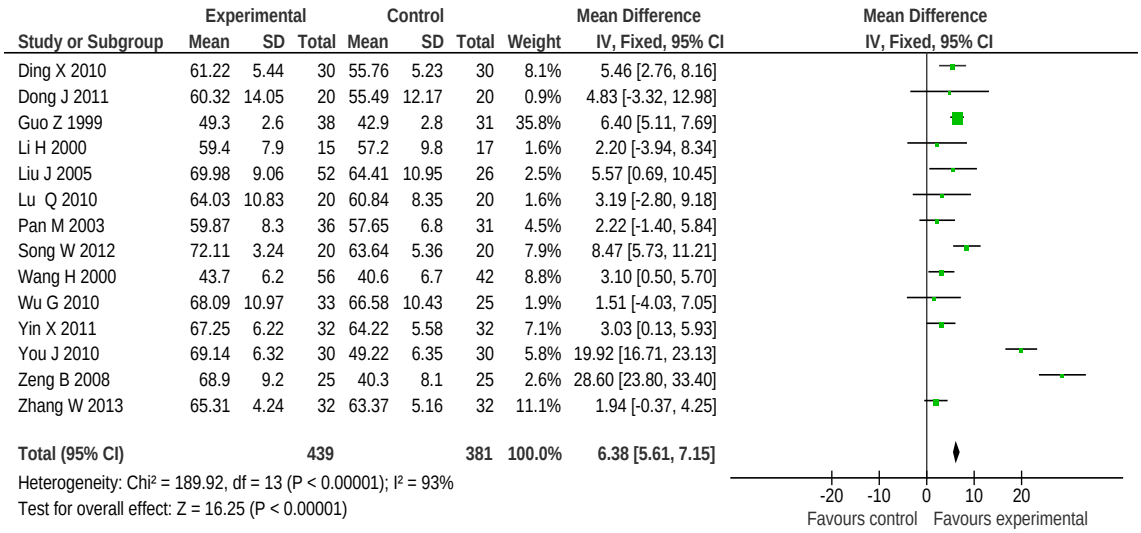


**Figure 4.59: Forest plot of mean difference for CD+3 (n=14)**
control: chemotherapy alone; experimental: HM + chemotherapy

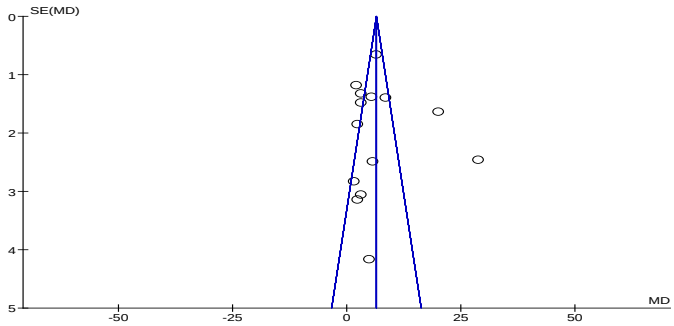


**Figure 4.60: Funnel plot of 14 studies that reported CD+3**

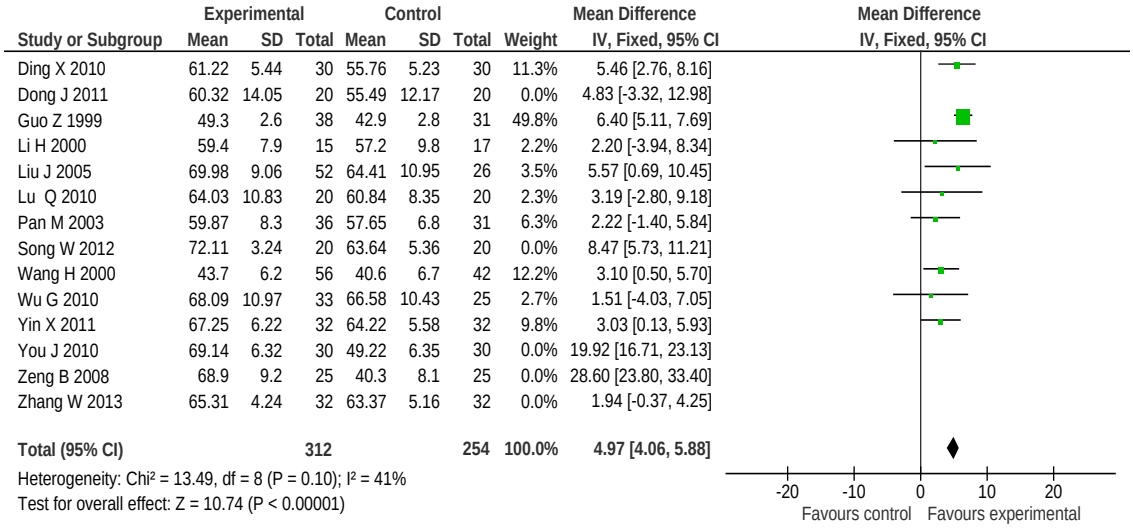


**Figure 4.61: Forest plot of mean difference for CD+3 after removal of outliers (n=9)**
control: chemotherapy alone; experimental: HM + chemotherapy

The pooled result for this sub-group of studies, showed that the CHM interventions significantly increased the percentage of CD3+ cells (MD 4.97, 95% CI [4.06, 5.88], $I^2$=41%) with a reduction in the effect size but no important heterogeneity (Figure 4.61).

#### *4.8.5.2 CD4+ cells*

Fifteen studies reported assessable data for CD4+ cells. Thirteen out of the 15 studies reported that the additional CHM interventions improved the percentage of CD4+ cells in serum. Wang et al. (2000) and Zeng et al. (2013) reported there were no differences before and after the CHM treatments. In the CMT control groups, only Wu et al. (2010) reported the CD4+ cells percentage were raised after treatment (Wu et al., 2010). The other studies all reported that the CD4+ cells were either not changed or reduced.

In the comparison between the two treatment groups after treatment, the pooled CD4+ percentage was significantly higher in the CHM plus CMT groups (MD 7.27, 95% CI [6.73, 7.82], p<0.00001, $I^2$=97%), and there was important heterogeneity. The effect size was similar in the two analysis models (Table 4.12; Figure 4.62). The funnel plot found that six studies were outside of the 95% confidence zone (Ding et al., 2010; Zeng et al., 2013; Zeng et al., 2008; Song et al., 2012; Zhang et al., 2013; Zhou et al., 2012, Figure 4.63), so these studies were removed from the pool. The pooled result for the remaining nine studies was still significantly in favour of the combination therapy group (MD 4.82, 95% CI [4.11, 5.53], p<0.00001, $I^2$=39%), with reduced effect size but no important heterogeneity (Table 4.12; Figure 4.64).

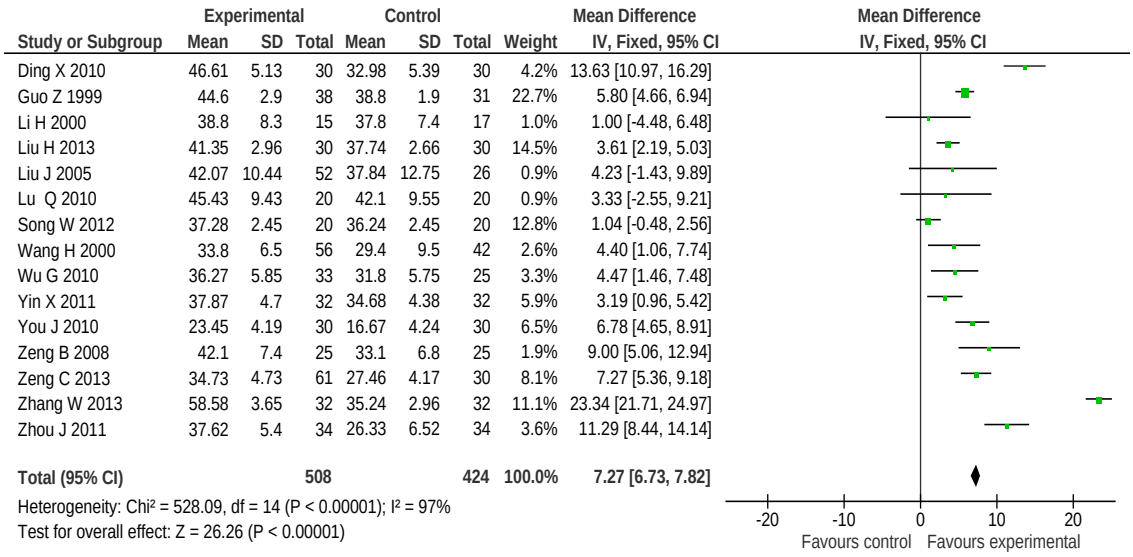


**Figure 4.62: Forest plot of mean difference for CD+4 (n=15)**
control: chemotherapy alone; experimental: HM + chemotherapy

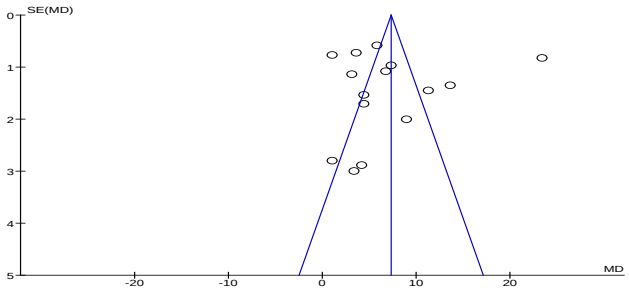


**Figure 4.63: Funnel plot of 15 studies that reported CD4+**

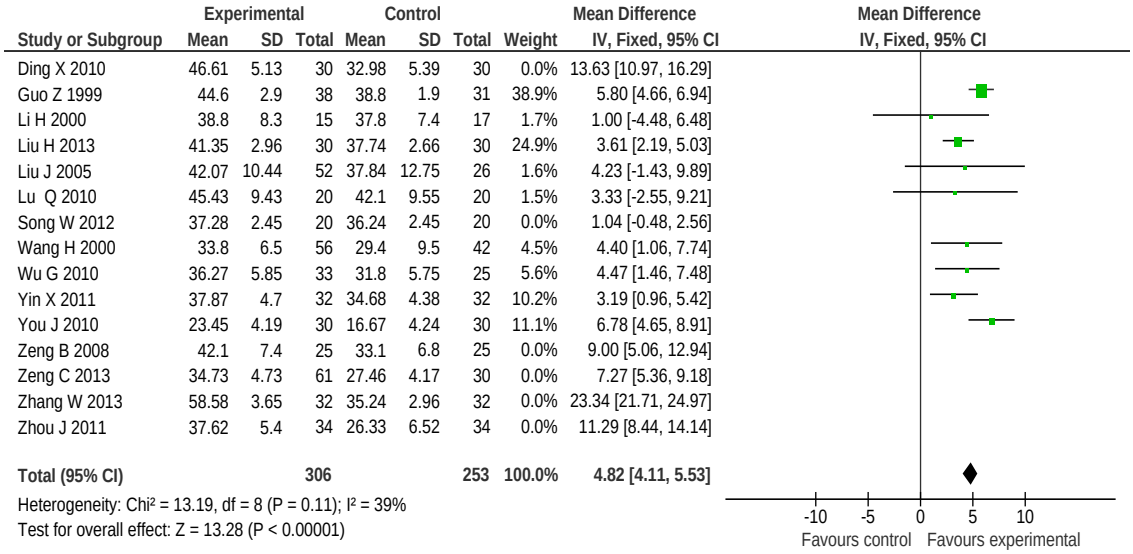


**Figure 4.64: Forest plot of MD for CD+4 after outlier removal (n=9)**
control: chemotherapy alone; experimental: HM + chemotherapy

### *4.8.5.3 CD8+ cells*

Fourteen studies reported assessable data for CD8+ cells. The majority of the studies (n=8) reported the CHM interventions decreased CD8+ cell percentages compared to baseline. There was a statistically significant reduction in CD8+ cells when CHMs were added to the CMT interventions (MD 1.68, 95% CI [-1.07, 2.28], p<0.00001, $I^2$=99%) compared with CMT alone, with important heterogeneity (Figure 4.65). The two analysis models produced similar outcomes (Table 4.12).

The funnel plot showed that five studies that were outliers (Ding et al., 2010; Guo, 1999; Zeng et al., 2013; Zhang et al., 2013; Zhou et al., 2012; Figure 4.66), so they were removed from the pooled analysis. The pooled result of the 9 remaining studies still showed a statistically significant reduction in favour of the combination therapy groups (MD -1.39, 95% CI [-2.30, 0.48], p=0.003, $I^2$=37%), and there was no important heterogeneity (Figure 4.67).

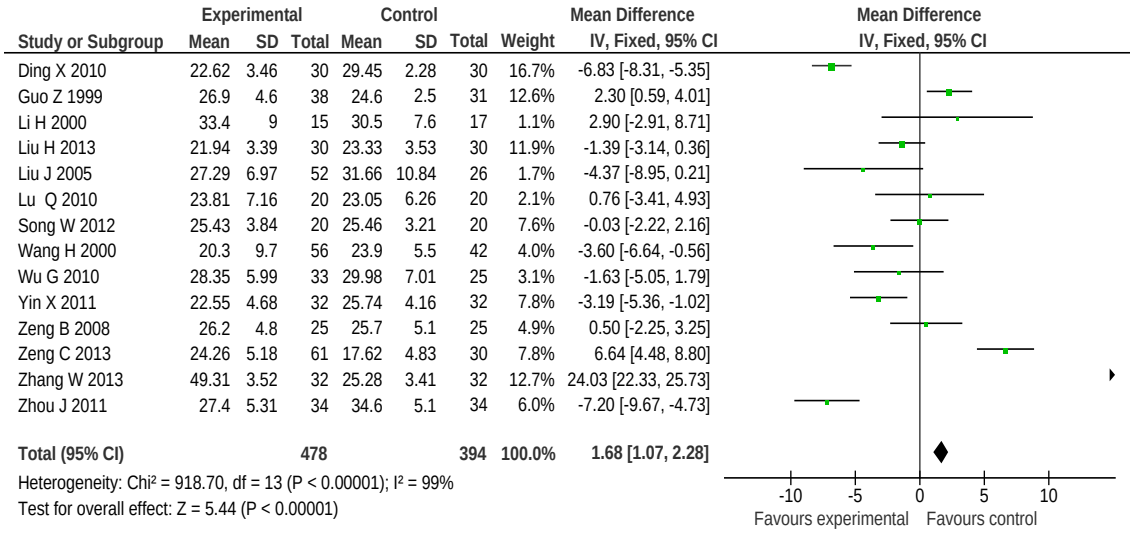


**Figure 4.65: Forest plot of mean difference for CD+8 (n=14)**
control: chemotherapy alone; experimental: HM + chemotherapy

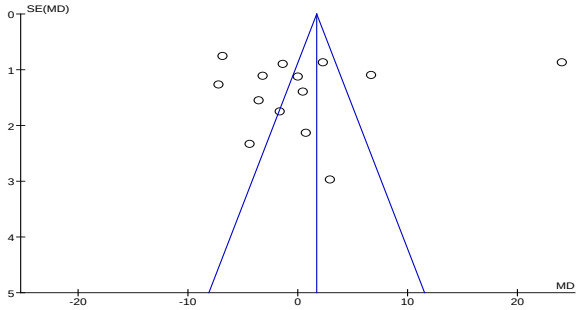


**Figure 4.66: Funnel plot of 14 studies that reported CD+8**

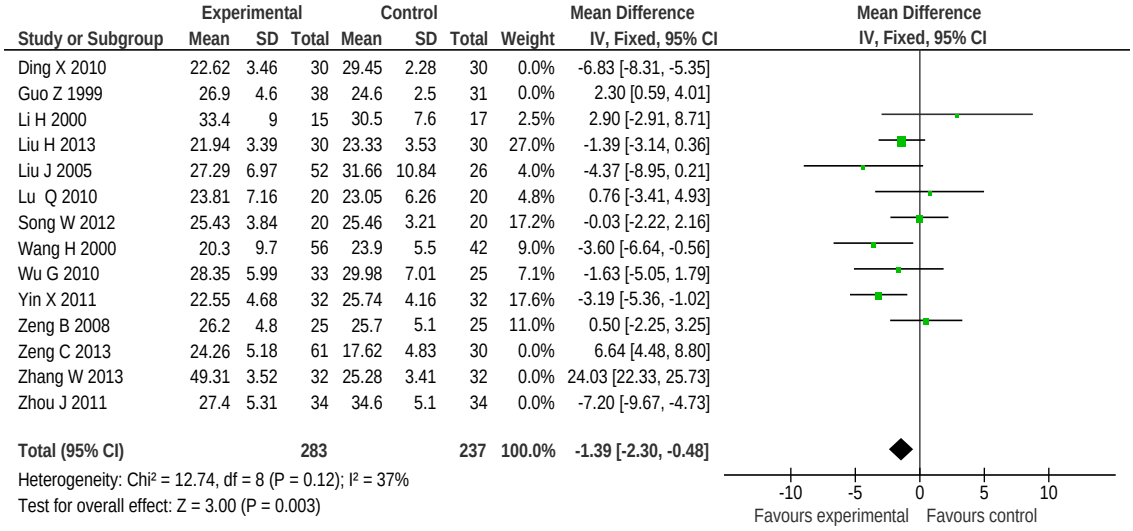


**Figure 4.67: Forest plot of mean difference for CD+4 after outlier removal (n=9)**
control: chemotherapy alone; experimental: HM + chemotherapy

### *4.8.5.4 CD4+/CD8+*

Seventeen studies reported assessable data for analyzing the ratio of CD4+ to CD8+ cells. All studies reported the additional CHM interventions increased the ratio of CD4+ to CD8+ compared with

baselines. In the comparison with the CMT control groups after treatment, the ratio of CD4+ to CD8+ was statistically significantly higher when CHMs were added to the interventions (MD 0.32, 95% CI [0.27, 0.36], p<0.00001, $I^2$=75%), and there was important heterogeneity (Figure 4.68).

The funnel plot found that two studies were the outliers (You et al., 2010; Zhou et al., 2012; Figure 4.69), so they were removed from the pooled analysis. The pooled result of 15 studies still showed a significant difference in favour of the combination therapy (MD 0.28, 95% CI [0.23, 0.32], p<0.00001, $I^2$=17%), and there was no important heterogeneity (Figure 4.70). The fixed model and random model showed the same effect sizes and $I^2$ values (Table 4.12).

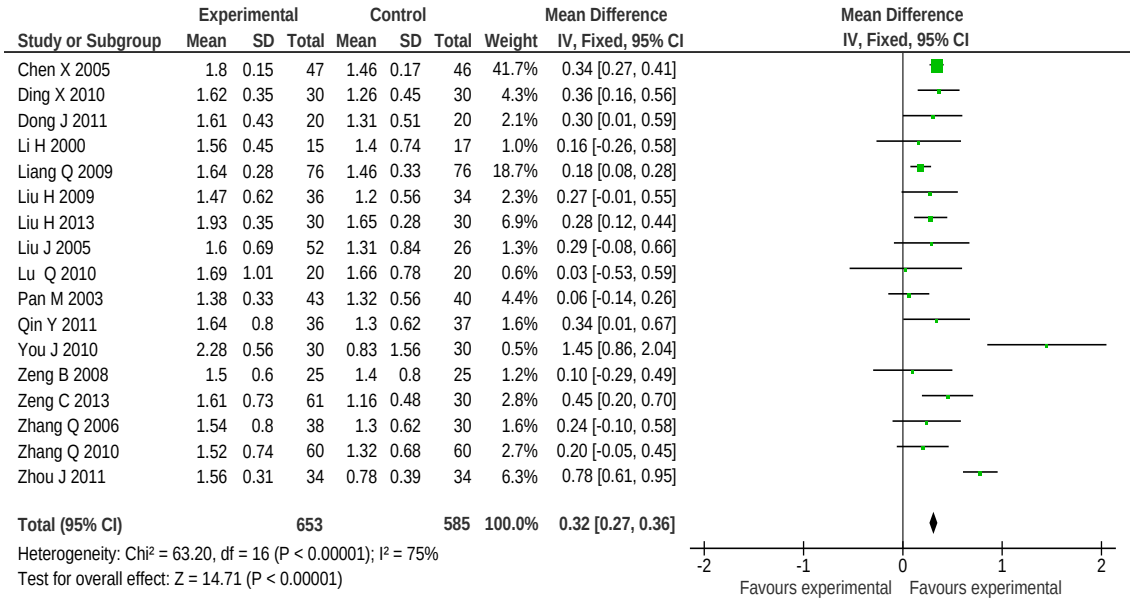


**Figure 4.68: Forest plot of mean difference for CD4+/CD+8 (n=17)**
control: chemotherapy alone; experimental: HM + chemotherapy

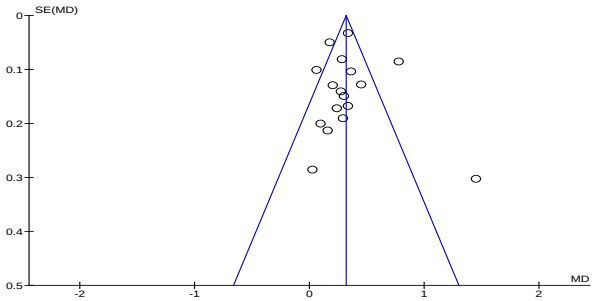


**Figure 4.69: Funnel plot of 17 studies that reported CD4+/CD8+**

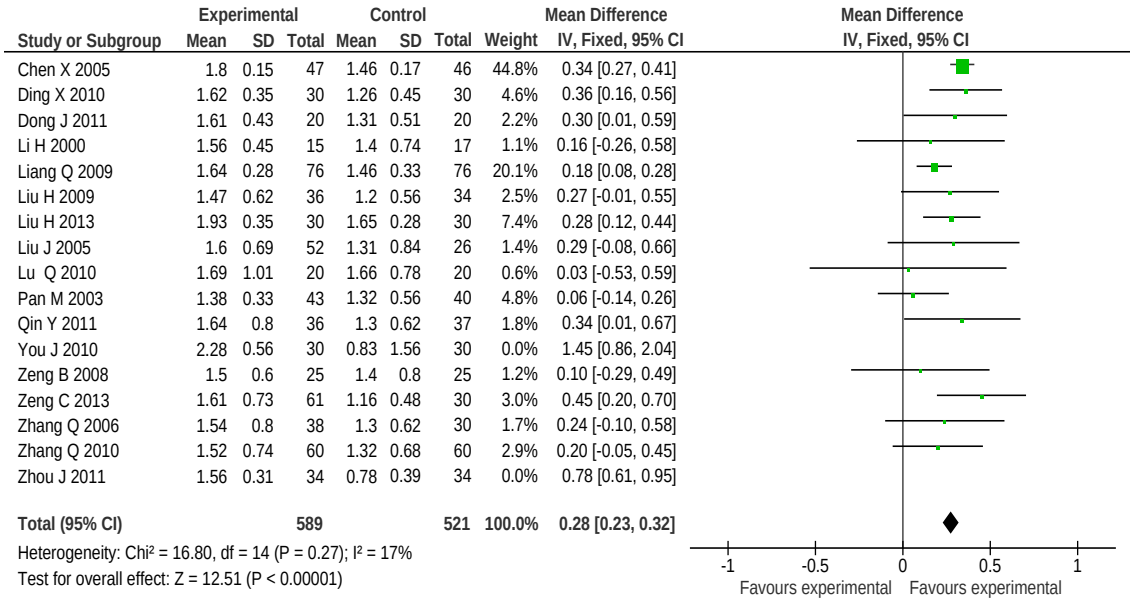


**Figure 4.70: Forest plot of mean difference for CD+4/CD8+ after outlier removal (n=15)**
control: chemotherapy alone; experimental: HM + chemotherapy

### *4.8.5.5 NK cell activity*

Eighteen studies reported assessable data for percentage of NK cell activity (%) in serum. Fifteen out of 18 studies reported the CHMs increased NK cell activity. The other three studies reported no change in NK cell activity after the CHM interventions compared with the baseline values. In the comparison with the CMT control groups, the NK cell activity was statistically significantly higher when the additional CHMs were included in the intervention (MD 9.13, 95% CI [8.49, 9.77], $p<0.00001$, $I^2=97\%$), and there was important heterogeneity (Figure 4.71).

The funnel plot found that seven studies that were outliers (Li et al., 2000; Qin et al., 2011; Song et al., 2012; Wu et al., 2010; Zeng et al., 2008; Zhang et al., 2013; Zhou, 2012) (Figure 4.72), so they were removed from the pooled analysis. The sensitivity analysis involved 11 studies. The pooled result still found that the combination treatment groups showed statistically significant increases in NK cell activity compared with the control chemotherapy groups (MD 5.57, 95% CI [4.73, 6.41], $p<0.00001$, $I^2=29\%$), with reduced effect size but no important heterogeneity, and the funnel plot was symmetric (Figure 4.73 and 4.74). Similar results were found in the fixed model and random model analyses (Table 4.12).

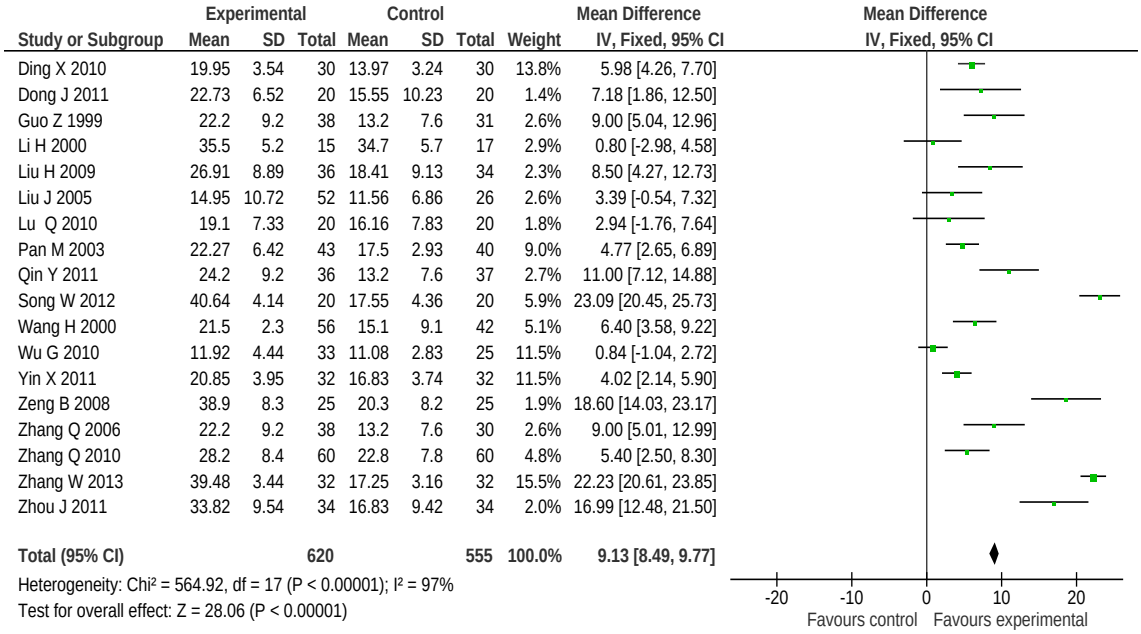


**Figure 4.71: Forest plot of mean difference for NK cell activity (n=18)**
control: chemotherapy alone; experimental: HM + chemotherapy

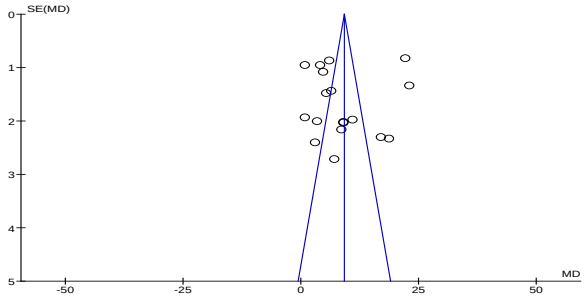


**Figure 4.72: Funnel plot of 18 studies that reported NK cell activity**

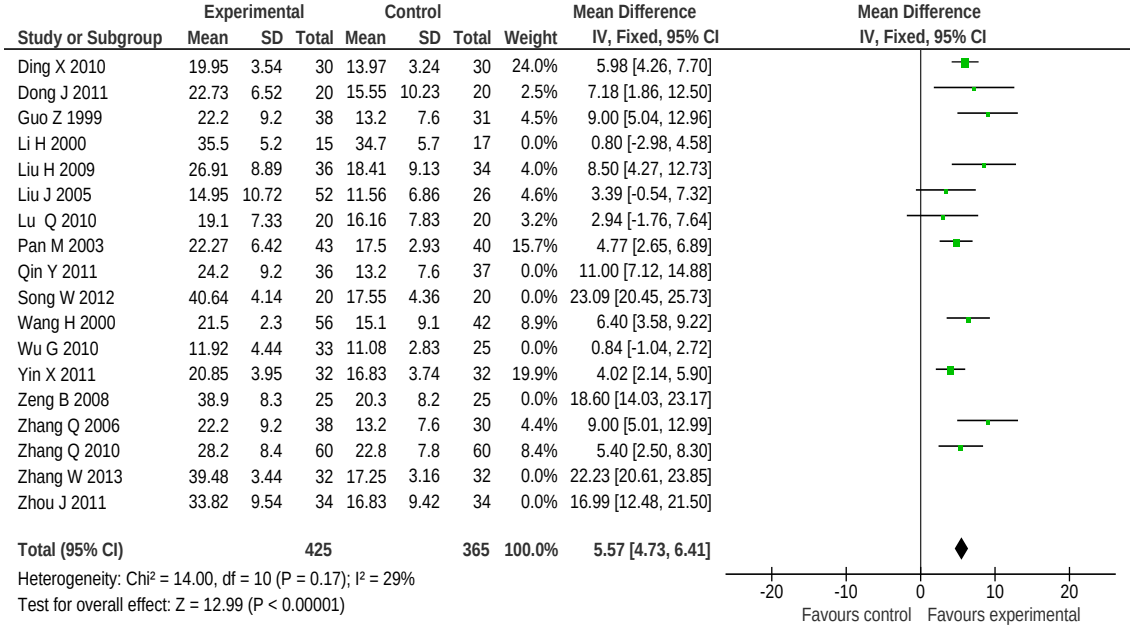


**Figure 4.73: Forest plot of mean difference for NK cell activity after outlier removal (n=11)**
control: chemotherapy alone; experimental: HM + chemotherapy

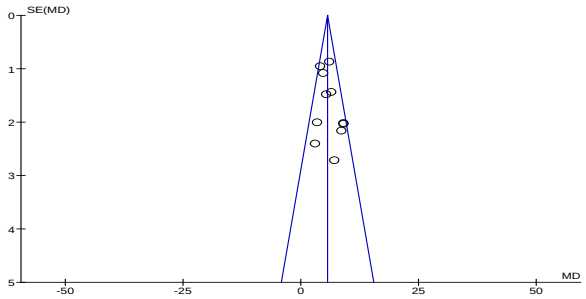


**Figure 4.74: Funnel plot of 11 studies that reported NK cell activity after outlier removal**

### *4.8.5.6 CD4+CD25+ Tregs*

Two studies reported data on the percentage of CD4+CD25+ Tregs in serum. The pooled result showed there was no statistically significant difference between the two treatment groups in CD4+CD25+ Tregs (MD -2.23, 95% CI [-0.60, 0.15], p=0.23, $I^2$=87%), and there was important heterogeneity (Figure 4.75). The results of these two studies were inconsistent, so the individul studies were analysed separately. Zhang et al. (2010) was in in adjuvant setting and found no difference between the two treatment groups (MD -0.11, 95% CI [-0.49, 0.27], p=0.58). Ma et al. (2010) included patients in adjuvant or palliative settings, and showed a significant difference in favour of the combination treatment (MD -2.67, 95% CI [-4.42, -0.92], p=0.003).

The expression of CD4+ CD25+ Tregs is higher in cancer tissues and peripheral blood of cancer patients than in healthy cohorts. Over-expression of FoxP3+, CD4+ CD25+ Tregs suppressed tumour-specific T cell immunity in cancer patents and was correlated with poorer survival (Curiel et al. 2004). The reason for inconsistent results of these two studies may be due to participants being at different stages of the disease.

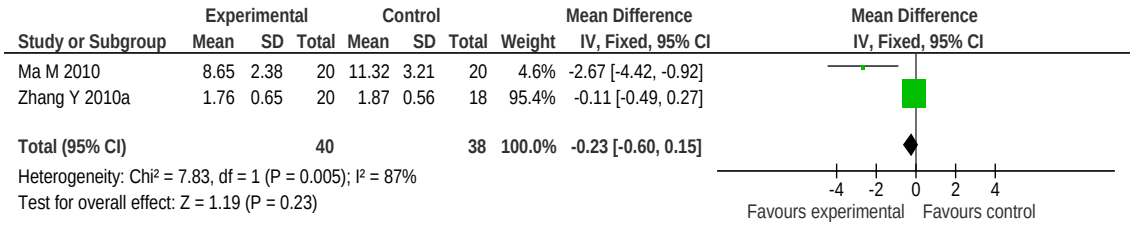


**Figure 4.75: Forest plot of mean difference for CD4+CD25+ Tregs (n=2)**

### *4.8.5.7 Discussion of effects on the immune system*

In general, CRC patients have lower cell-mediated immunity (CMI) characterised by lower percentages of CD3+, CD4+, CD4+/CD8+ and NK cells, but higher percentages of CD8+ cells in serum. The low CMI status is correlated with the stage of the disease and the tumour load (Ji et al., 2010). Chemotherapy can induce myosuppression that will further damage the patients' immunity, including the CMI. The results showed that the combination of CHMs with CMT improved the CMI, in terms of increased CD3+, CD4+, CD4+/CD8+, and NK cell activity in serum compared with CMT

alone groups. The finding of increased CD3+, CD4+, CD4+/CD8+ was consistent with the systematic review by Zhong et al. (2012) which included considerably fewer studies.

However, the results of these meta-analyses showed important heterogeneity. The reasons may have been associated with base-line factors such as stage of the disease and tumour burden, the CHM and CMT interventions, treatment duration, and methods of measuring the T cell subsets. Due to the important heterogeneity in these meta-analysis results, there was the question of how to select an appropriate analysis model. In the fixed effect model, in which the assumption is that every study bears the same value, the analysis tries to estimate the true effect of the intervention in both magnitude and direction. That is, it aims to determine the best estimate of the effect of the treatment. The random effect model is based on the assumption that the effect in every study is not the same. It describes the average effect of the treatment (Higgins et al., 2011). In dealing with similar data, different systematic reviews have used different analysis models. Zhong et al. (2012), in the systematic review of HM combined CMT for CRC, used a random effect model in their meta-analysis, whereas the systematic review of CHM combined CMT for lung cancer by Chen et al. (2009) applied the fixed effect model. In this project, the goal was to assess the true effect of CHMs in the management of CRC. The majority of included studies had reported the addition of CHMs improved CMI and the data from the studies showed the direction of the effect on immunity generally to be positive. Therefore, the fixed effect model was adopted in the first place.

In general, the approach for dealing with important heterogeneity was based on clinical aspects by grouping studies by type of CMT, and previously treated or untreated patients. However, in this section, the relatively fewer studies made this approach less applicable and when grouping by CMT type was tried but it did not reduce heterogeneity. Since the heterogeneity was unexplainable based on clinical factors, an alternative approach was used. Firstly, the analysis incorporated both fixed and random effect models. However, the comparison of the results between the fixed effect and the random effect models showed they were similar in effect sizes and $I^2$ values so variation in the model did not resolve the issue. It was evident that there was asymmetry in the funnel plots with the obvious presence of outliers, so the sensitivity analysis method of excluding outliers to obtain a better measure of effect size was adopted. When there was substantial funnel plot asymmetry, then the outliers that were outside of the 95% confidence zone and any small studies that were isolated at the bottom of the funnel plot were excluded from the meta-analysis, to determine whether the outliers affected the overall result. After this process, the pooled results generally showed less heterogeneity, the effect sizes were reduced while the directions of the effect were not changed. Also, there was little difference between the fixed and random effect models after this procedure. Consequently, the resultant comparisons provided higher degrees of certainty in the estimates of treatment effects. Also, these analyses suggest that any effect of reporting or publication bias was not substantial enough to affect the overall result.

Nevertheless, the issue of possible reporting or publication bias remains. The heterogeneity in results could have been due to positive reporting bias or it could have been due the multiple factors that could have impacted on the results for CMI parameters including: participants being at different stages of the disease and or having differing tumour loads, for example partipants receiving Stage II and III patients receiving adjuvant chemotherapy could be expect to show very different CMI profiles to stage IV patient receiving palliative treatment; different chemotherapy regimens could have different effects on CMI; and the intensity and duration of chemotherapy is also likely to have influenced immunity with more cycles of chemotherapy resulting in more immunosuppression.

In this review, non-individual participant data was used, so it was not possible to perform detailed analysis based on these clinical factors. Future studies of this topic could consider these factors.

### 4.8.6 **Adverse events associated with Chinese herbal medicines**

In total, ten studies reported on adverse events due the the CHM interventions. There were no serious adverse events reported that were associated with the CHMs. Common CHM adverse events were mild gastrointestinal symptoms such as nausea and diarrhoea (Table 4.13). Other HM reviews reported similar findings (Zhong et al., 2012; Guo et al., 2012).

**Table 4.13: Ten studies that reported herbal medicine (HM) adverse events**

| Study | Treatment | Adverse events (AEs) |
|---|---|---|
| Cao B (2011) | Yiqi zhuyu decoction | Hypertension, bleeding: not different between groups |
| Hou A (2009) | Fuzhengxiaoai decoction-I | Two cases of mild vomiting. |
| Kono T (2013) | TJ-107 (goshajinkigan) | Well tolerated. None of AEs were considered TJ-107 related. |
| Schink M (2007) | Mistletoe extract | None of the AEs in the treatment group were related to the Mistletoe extract. |
| Shen H (2003) | Changbi'an Capsule | No HM related AEs were observed. |
| Torisu M (1990) | YunZhi powder *Coriolus vesiocolor* spores | Pigmentation of nail, cough when taking the PSK, mild diahrroea, constipation. |
| Xion S (2003) | Changkangfu capsule | Mild gastrointestinal symptoms were reorted. |
| Yang Y (2008) | Quxie capsule | Mild diarrhoea. |
| Yang Y (2007) | Quxie capsule | Mild diarrhoea. |
| Yang Y (2008a) | Kang'ai Injection | No HM related AEs were observed. |
| Zheng Y (2011) | Shenqisan | No HM related AEs were observed. |

## 4.9 Chapter 4 summary and conclusions

Most of the RCTs of HM for CRC were conducted in China. Potential bias due to methodological issues was evident amongst the included studies, so any conclusions must be tentative. The HM interventions were diverse and included oral decoctions, tablets, powders, HM extracts for intravenous infusion, decoctions for enema, and HM extracts for TACE. The 78 CHM formulas included 146 distinct plant-based HMs plus 20 items of insect or animal origin. Nevertheless, the same or similar

ingredients were included in multiple studies, so the degree of variation was less than these numbers suggest.

In the section on HM treatments used alone, the HMs:

- improved the immunosuppression status induced by surgical treatment during the peri-operative period;
- may have beneficial effects on OS and DFS for stage III CRC post surgery;
- appeared not significantly different to the 5-FU regimens in treating ACRC in terms of tRR and OS; and
- improved KPS in ACRC patients.

Therefore these HMs may provide an additional therapeutic option for ACRC patients in the terminal stage of the disease. Also, Javanica oil emulsion (extracted from *Brucea javanica* seed) is a potential surrogate for chemo-therapeutic agents for liver tumours included those resulting from CRC liver metastasis.

In the section on integrative therapy, the meta-analyses found that CMTs including oxaliplatin and non-oxaliplatin regimens, combined with CHMs:

- significantly improved the tRR in CRC compared to the same chemotherapy alone;
- elevated the one-year, two-year, three-year, and five-year OS;
- the mean OS in the combination therapy groups was significantly better than in the CMT alone groups.

These results suggest that the additional CHM treatments may have synergetic anti-cancer effects to the CMT, enhance the anti-cancer effects of the CMT, or provide additional anticancer effects. The results were broadly consistent with the study by Zhong et al 2012 for tRR and survival.

Also, the combination of CMT and CHM treatment significantly:

- improved KPS and other meaures of QoL;
- alleviated the chemotherapy-related adverse events of neutropenia, thrombocytopenia, anaemia, nausea and vomiting, diarrhoea, neurotoxicity, aloplecia, stomatitis, and impairment of liver function; and
- improved cell-mediated immunity status.

In terms of the safety of the HMs, most of the AEs were mild gastrointestinal symptoms and there was no report of death, vital organ damage or undesirable herb-drug interactions caused by the application of HMs for CRC management in the studies included in this review.

Previous reviews included fewer studies, with four studies in Wu 2005 and 20 studies in Zhong et al 2012, all of which were of integrative medicine. In this systematic review and meta-analysis the

number of included studies (88 RCTs) and consequently the sample sizes in the meta-analyses were considerably larger, and the scope of the review was not restricted to Chinese herbal medicine or to integrative medicine. Nevertheless, most of the 88 RCTs of HM for CRC were conducted in China and most were of integrative medicine. In addition, the meta-analyses were sub-grouped into non-oxaliplatin group, oxaliplatin group, adjuvant setting group, palliative setting group, previously treated group, and previously untreated group. This process improved the validity of data sets and reduced the statistical heterogeneity of the meta-analysis results. Moreover, the effects of various HM administration routes were examined. These included oral administration, intravenous drip, transcatheter arterial chemoembolization (TACE), intraperitoneal hyperthermic perfusion and enema.

However, the studies tended to have small sample sizes, be of short durations, and were mainly single-centre RCTs. There were number of reporting issues, such as over-simplified or incomplete reporting of outcomes that may have been associated with a lack of clear guidelines for trial reporting in the journals published in China, or inadequacies in the original trial protocols. Since the analyses are based on published data, the original patient-level data are unavailable, and few studies had available protocols, it is difficult to judge the accuracy and methodological rigor of the original studies. In this chapter, the results are estimations based on the pooled results from multiple studies so the results summaried above and the associated conclusions must be tentative. Since some of the results are promising further investigations should be conducted using sufficiently powered, rigorously designed RCTs.

# Chapter 5. FOLFOX4 Combined with Herbal Medicine for Advanced Colorectal Cancer

## 5.1 Introduction to chapter five

This chapter is based on the published paper: Chen M, May BH, Zhou IW, Xue CC, Zhang AL. (2014). FOLFOX 4 Combined with herbal medicine for advanced colorectal cancer: A systematic review. *Phytother Res*. Jul, 28(7): 976-91.

It also includes one additional study (Cao et al., 2011) which was identified in the update search (December 2013), so there are some differences to the published version.

The FOLFOX regimen refers to 5-fluorouracil (5-FU) plus leucovorin (LV) combined with oxaliplatin. It is a standard first line combination chemotherapy setting for advanced CRC (ACRC) (Lee & Chu, 2007; Prescrire Editorial Staff, 2010). A number of modalities of FOLFOX regimens, which consist of varying doses and schedules of 5-FU, LV and oxaliplatin, have been studied in palliative settings. Of these, FOLFOX4 has been the most widely investigated (Waddell & Solimando, 2005). The FOLFOX4 regimen has a fixed dosage and schedule in each cycle, so in studies that employed FOLFOX4, there is lesser variation in the chemotherapy that could contribute to heterogeneity in results. Also, in this systematic review all participants were at the advanced stage of CRC, so variation due to differences between participants was reduced.

FOLFOX4 comprises a 2-hour infusion of LV (200 mg/$m^2$/d), followed by a 5-FU bolus (400mg/$m^2$/d) and a 22-hour infusion (600 mg/$m^2$/d) for 2 consecutive days every 2 weeks, together with oxaliplatin 85mg/$m^2$ as a 2-hour infusion on day 1. It is used in conjunction with anti-nausea medications. The regimen is repeated every 14 days until disease progression or unacceptable toxicities force cessation (De Gramont et al., 2000).

This section focuses on CHMs as adjuvants to FOLFOX4 in the treatment of ACRC. It aims to determine if CHMs demonstrate evidence of efficacy and safety in the treatment of ACRC and/or the management of the side effects of FOLFOX4. Since the FOLFOX4 regimen has fixed dosage and schedule, and the participants were all in the late stage of CRC, this sub-group of studies shows elevated similarity between studies and reduced heterogeneity in treatment and participant characteristics. Consequently, this meta-analysis was suitable for identifying the best available evidence for the effects of CHM in ACRC (Research question 6).

## 5.2 Search results

Fourteen studies (Cao et al., 2011; Ding et al., 2010; Fang & Li, 2008; Li et al., 2007b; Li et al., 2007c; Qiu, 2011; Wu et al., 2010; Xu & Wang, 2010; Yang, 2008; Zeng et al., 2009; Zeng et al., 2008; Zhang et al., 2008; Zhang et al., 2010a; Zhang et al.,2010b) that investigated a combination of CHM plus FOLFOX4 versus FOLFOX4 for ACRC were included in this review.

The 14 studies enrolled 1,060 assessable in-patient participants with 546 participants in the CHM plus FOLFOX4 test groups and 514 participants in the FOLFOX4 alone control groups. All studies were conducted in China and published in Chinese medical journals from 2007 to 2011. Participant characteristics, interventions and outcome measurements are summarised in Table F1 in Appendix F.

Thirteen different test interventions were used. Seven studies employed commercially available CHM extracts. *Kang'ai* Injection was used in two studies. Compound *Kushen* injection, Ginsenoside Rg3 capsules, *Aidi* injection, *Gubenxiaoliu* Capsule and Javanica oil injection were each used in one study. Seven studies used multi-herbal decoctions. In total, 61 different herbs and/or their extracts were used, with the six most frequent being:

- *Huang qi: Astragalus membranaceus* (Fisch.) Bge. root (eight studies) (Cao et al., 2011; Li et al., 2007b; Li et al., 2007c; Qiu, 2011; Yang, 2008; Zeng et al., 2008; Zhang et al., 2008; Zhang et al., 2010b);
- *Yi yi ren: Coix lachryma-jobi* L. seed (six studies) (Li et al., 2007c; Wu et al., 2010; Xu & Wang, 2010; Zeng et al., 2008; Zhang et al., 2008; Zhang et al., 2010a);
- *Ren shen: Panax ginseng* C.A. Mey. root (six studies) (Cao et al., 2011; Li et al., 2007b; Qiu, 2011; Yang, 2008; Zeng et al., 2009; Zhang et al., 2010a);
- *Ku shen: Sophora flavescens* Ait. root (six studies) (Cao et al., 2011; Ding et al., 2010; Qiu, 2011; Yang, 2008; Zeng et al.,2008; Zhang et al., 2008);
- *Bai zhu: Atractylodes macrocephala* Koidz. Root (six studies) (Cao et al., 2011; Li et al., 2007c; Xu and Wang, 2010; Zeng et al., 2008; Zhang et al., 2008; Zhang et al., 2010b); and
- *Fu ling: Poria cocos* (Schw) Wolf sclerotium (four studies) (Li et al., 2007c; Wu et al., 2010; Xu & Wang, 2010; Zhang et al., 2008).

## 5.3 Meta-analysis results

Meta-analyses were performed for each of the following outcomes. The numerical data are presented in Table 5.1.

### 5.3.1 **Effect on tumour response**

The tumour response rate (tRR) ranged from 30% to 54.1% for the test groups and from 20% to 46.6% for the control groups in the 13 studies (1,000 participants). There was a significant improvement in tRR for CHMs plus FOLFOX4 (test groups) compared to FOLFOX4 alone (Table 5.1; Figure 5.1). The pooled tRR in the test groups was elevated to 43.8% (RD 9.1%). The total numbers of complete remissions (CRs) in the test and control groups were 19 and 8 patients, respectively, but this difference was not significant (RD 2.3%).

The tRR data included 234 (24.9%) participants who were previously treated with chemotherapy for ACRC, but separate results were reported for only 126 of these. Previously treated ACRC patients have been found to be less responsive to current first-line settings of chemotherapy (Giantonio et al., 2007). When the sub-groups of previously treated (two studies) and previously untreated (four studies) patients were analysed, the RD of the pooled tRR was 8.7% for previously treated and 9.0% was previously untreated but there was no significant difference between test and control groups. For the two studies of *Kang'ai* Injection, the RD was 3.0% but there was no significant difference between groups (Table 5.1).

Thirteen studies reported on tRR so a Funnel Plot was used to assess publication bias (Figure 5.2). The symmetry of the Funnel Plot suggests the risk of publication bias was low for these studies.

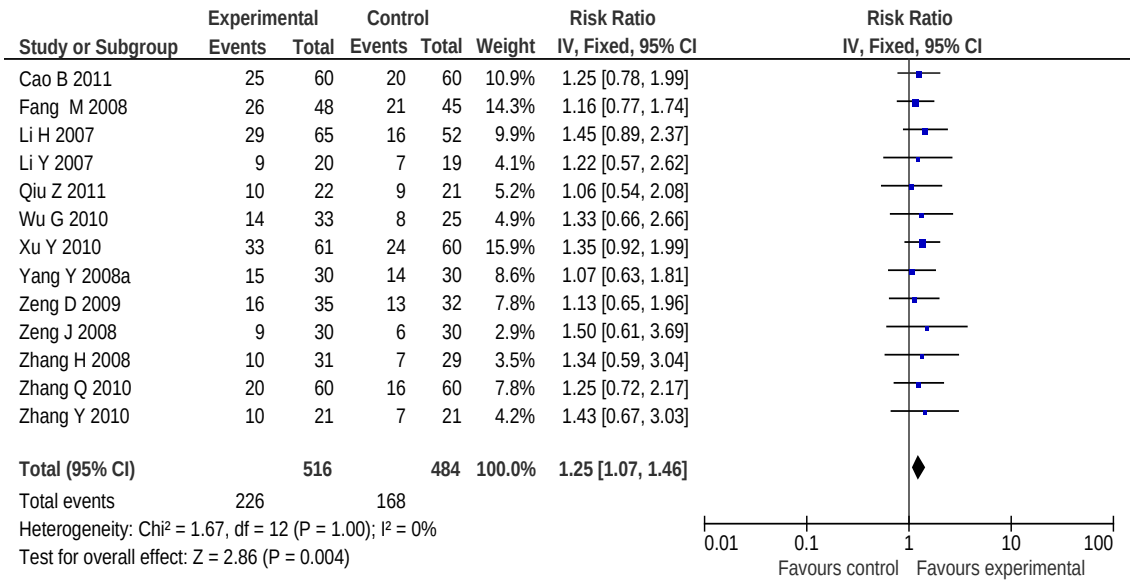


**Figure 5.1: Forest plot of risk ratio for tumour response rate in advanced colorectal cancer (n=13)**

control: chemotherapy alone; experimental: HM + chemotherapy

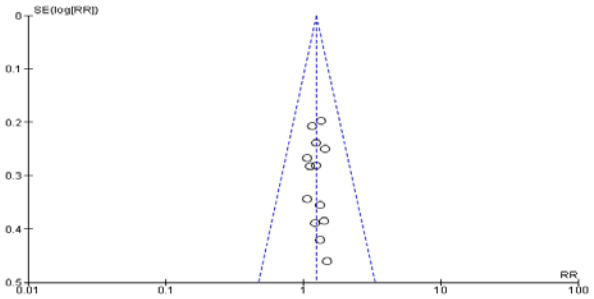


**Figure 5.2: Funnel plot of the 13 studies that reported tumour response rate in advanced colorectal cancer**

**Table 5.1: Results of meta-analyses for each outcome measure in Chapter 5**

| **Outcomes** | **No Studies*[SN] (participants)** | **Meta-analysis results (between groups at end of treatment): 95%CI** | **Incidence % (n/N) or MD ± SD** | **RD (%)** |
|---|---|---|---|---|
| **Tumour response** | | | | |
| Tumour response rate (tRR) | 13[1-13] (1000) | RR 1.25[1.07, 1.46], *p=0.004, $I^2$=0%, FE | T: 43.8% (226/516)<br>C: 34.7% (168/484) | 9.1 |
| Complete remission (CR) | 12[2-13] (880) | RR 1.94 [0.89, 4.25], p =0.10, $I^2$=0%, FE | T: 4.2% (19/456)<br>C: 1.9% (8/424) | 2.3 |
| tRR (previously treated patients) | 2[3,10] (126) | RR 1.41 [ 0.77, 2.61], p =0.27, $I^2$=0%, FE | T: 29.7% (19/64)<br>C: 21.0% (13/62) | 8.7 |
| tRR (previously untreated patients) | 3[1,3,5,9] (221) | RR 1.21 [ 0.92, 1.59], p =0.17, $I^2$=0%, FE | T: 47.3% (70/148)<br>C: 38.3% (51/133) | 9.0 |
| tRR (Kang'ai Injection sub-group) | 2[5,8] (103) | RR 1.07 [ 0.71, 1.61], p =0.76, $I^2$=0%, FE | T: 48.1% (25/52)<br>C: 45.1% (23/51) | 3.0 |
| **Overall survival (OS)** | | | | |
| One year OS | 3[3,12,13] (279) | RR 1.51 [1.19, 1.90], *p =0.0006, $I^2$=0%, FE | T: 62.3% (91/146)<br>C: 42.1% (56/133) | 20.2 |
| mOS | 4[1,2,4, 14] (339) | MD 2.33 [39, 3.27], *p<0.00001. | NA | NA |
| mTTP | 4[1,2,8,14] (343) | MD 2.25 [63, 2.87], *p<0.00001. | NA | NA |
| **Quality of life** | | | | |
| Karnofsky Performance Status (KPS) | 9[3-5,7,9-13] (669) | RR 1.84 [1.54, 2.19], p<0.00001, $I^2$=0%, FE | T: 60.9% (210/345)<br>C: 32.1% (104/324) | 28.8 |
| mean KPS (Wu 2010) | 1[6] (58) | MD 12.18 [96, 16.40], *p<0.00001 | T: before 74.38 ± 11.16, after 73.94 ± 6.35<br>C: before 75.16 ± 12.78, after 61.76 ± 9.23 | NA |
| Su Ying's QoL Questionnaire (Yang 2008a) | 1[8] (60) | MD 4.10 [2.36, 5.84], *p<0.00001 | T: before 40.73 ± 3.49; after 42.30 ± 3.88<br>C: before 40.90 ± 2.44; after 38.20 ± 2.91 | NA |
| Body weight (BW) | 3[8,9,13] (169) | RR 1.88 [1.14, 3.08], *p =0.01, $I^2$=0%, FE | T: 38.4% (33/86)<br>C: 19.5% (16/82) | 18.9 |
| **Chemotherapy toxicity** | | | | |
| Neutropenia events (grade 3/4) | 10[1-6,8,10,12,13] (692) | RR 0.33 [0.18, 0.60], *p=0.0003, $I^2$=0%, FE | T: 3.9% (14/359)<br>C: 12.6% (42/333) | 8.7 |

| **Outcomes** | **No Studies*[SN] (participants)** | **Meta-analysis results (between groups at end of treatment): 95%CI** | **Incidence % (n/N) or MD ± SD** | **RD (%)** |
|---|---|---|---|---|
| Nausea & vomiting (grade 3/4) | 9[1-8,13] (633) | RR 0.34 [0.18, 0.66], *p=0.001, $I^2$=0%, FE | T: 3.0% (10/330); C: 12.5% (38/303) | 9.5 |
| Neurotoxicity (grade 3/4) | 7[1-4,12,13] (529) | RR 0.39 [0.15, 1.00], *p =0.05, $I^2$=0%, FE | T: 2.2% (6/277) C: 6.0% (15/252) | 3.8 |
| Diarrhoea (grade 3/4) | 5[2,3,6,8,12] (448) | RR 0.39[0.11, 1.42], p =0.15, $I^2$=0% FE | T: 0.9% (2/236) C: 3.3% (7/212) | 2.4 |
| Anaemia (grade 3/4) | 3[6,12,13] (220) | RR 0.30 [0.05, 1.89], *p =0.20, $I^2$=0% FE | T: 0% (0/114) C: 2.9% (3/105) | 2.9 |
| Thrombocytopenia (grade 3/4) | 1[13] (42) | RR 1.00, [0.07, 14.95], p =1.00, FE | T: 4.8% (1/21) C: 5.0% (1/20) | NA |
| Stomatitis (grade 3/4) | 2[2,3] (210) | RR 0.43 [0.08, 2.31], p =0.33, $I^2$=0%, FE | T: 1.8% (2/113) C: 4.1% (4/97) | 2.3 |
| Constipation (grade 3/4) | 1[3] (117) | RR 0.40 [0.04, 4.29], p =0.45, FE | T: 1.5% (1/65) C: 3.8% (2/52) | NA |
| *Kang'ai* Injection Sub-group | | | | |
| Neutropenia events (grade 3/4) | 2[5,8] (103) | RR 0.19[0.04, 0.83], *p =0.03, $I^2$=0%, FE | T: 3.8% (2/52) C: 21.6% (11/51) | 17.8 |
| Nausea & vomiting (grade 3/4) | 2[5,8] (103) | RR 0.27[0.08, 0.89], *p =0.03, $I^2$=0%, FE | T: 5.8% (3/52) C: 21.6% (11/51) | 15.8 |
| **Zhang et al. (2008)** | | | | |
| Myolesuppression (grade 3) | 1[11] (60) | RR 0.47[0.04, 4.89], p =0.53, FE | T: 3.23% (1/31) C: 6.90% (2/29) | NA |
| **Zeng et al. (2009)** | | | | |
| Neutropenia (all grades) | 1[10] (67) | RR 0.72 [0.54, 0.96], *p=0.02, FE | T: 62. 9% (22/35) C: 87. 5% (28/32) | NA |
| Neurotoxicity (all grades) | 1[10] (67) | RR 0.87 [0.58, 1.30], p=0.5, FE | T: 54.3% (19/35) C: 62.5% (20/32) | NA |
| Nausea, vomiting, diarrhoea (all grades) | 1[10] (67) | RR 0.82 [ 0.65, 1.03], p=0.08, FE | T: 74.3% (26/35) C: 90.6% (29/32) | NA |
| **Immune function** | | | | |
| CD3+ cells (%) | 2[1,6] (118) | MD 4.70 [2.27, 7.13], *p=0.0001, $I^2$=37%, FE | NA | NA |
| CD4+ cells (%) | 2[1,6] (118) | MD 9.08 [0.10, 18.05], *p=0.05, $I^2$=95%, RE | NA | NA |
| CD8+ cells (%) | 2[1,6] (118) | MD -4.47 [-9.54, 0.61], p=0.08, $I^2$=87% RE | NA | NA |
| Ratio CD4+/CD8+ | 2[1,12] (180) | MD 0.28 [0.14, 0.43], *p=0.0002, $I^2$=13%, FE | NA | NA |
| NK cells (%) | 3[1,6,12] (238) | MD 4.03 [0.51, 7.55], *p=0.02, $I^2$=88%, RE | NA | NA |
| **Zeng et al. (2009)** | | | | |
| CD3+ cells (%) | 1[10] (67) | MD 8.80 [7.34, 10.26], *p<0.00001, FE | T*: 3.6±2.31, C: -5.2±3.60 | NA |
| CD4+ cells (%) | 1[10] (67) | MD 9.40 [8.51, 10.29], *p<0.00001, FE | T*: 3.2±1.42, C: -6.2±2.18 | NA |
| CD8+ cells (%) | 1[10] (67) | MD 7.80 [6.62, 8.98], *p<0.00001, FE | T*: 1.3±1.92, C: -6.5±2.86 | NA |
| Ratio CD4+/CD8+ | 1[10] (67) | MD 0.22 [0.16, 0.28], *p<0.00001, FE | T*: 0.04±0.09, C: -0.18±0.28 | NA |

T: test group; C: control group; RR: risk ratio; N: total number of participants in group(s); n: number of incidence(s) in group(s); $I^2$: test of heterogeneity of meta-analysis of pooled data, over 50% represents substantial heterogeneity; MD: mean difference; SD standard deviation; RD: Risk difference; NA: not applicable; T*: Mean change; FE: Fixed Effect model; RE: Random Effect model; *$p \leq 0.05$: statistic significant.
*__SN__: study No (superscript) (1st author (year)). 1. Cao B 2011; 2. Ding X 2010; 3. Fang M 2008; 4. Li H 2007; 5.Li Y 2007; 6. Qiu Z 2011; 7.Wu G 2010; 8. Xu Y 2010; 9. Yang Y 2008a; 10. Zeng D 2009; 11. Zeng J 2008; 12. Zhang H 2008; 13. Zhang Q 2010; 14. Zhang Y 2010.

### 5.3.2 **Effect on overall survival and time to progression**

Three studies reported one-year overall survival (OS). There was a significant difference in favour of the test groups (RD 20.2%) (Table 5.1). No long-term (two-year or more) OS data were reported. Median OS and median time to progression (TTP) were each reported in four studies. The estimated mean values of median OS and median TTP were significantly increased when FOLFOX4 was combined with CHMs compared with the FOLFOX4 treatment alone (Table 5.1).

### 5.3.3 **Effect on quality of life and body weight**

Ten studies used Karnofsky Performance Status (KPS) to measure quality of life (QoL) (Li et al., 2007b; Li et al., 2007c; Qiu, 2011;Wu et al., 2010; Xu & Wang, 2010; Zeng et al., 2009; Zeng et al., 2008; Zhang et al., 2008; Zhang et al., 2010a; Zhang et al., 2010b). Three studies (Yang, 2008; Zeng et al., 2009; Zhang et al., 2010b) included body weight (BW) measurement.

Clinical effectiveness was defined as ‘improved’ for KPS and as ‘improved’ for BW. To minimise bias resulting from small changes in scores, the meta-analyses only included patients who recorded a KPS score that was 10 or more points higher after the intervention compared to prior to the intervention and patients who had gained 1 kg or more. Patients who achieved a stable KPS score and/or stable BW after the intervention were excluded from the analyses.

The KPS improvement was significantly greater in the test groups based on nine studies (RD 28.8%) (Table 5.1). In Wu et al. (2010), KPS was presented as mean plus standard deviation (SD). There was no statistically significant difference before and after the treatment in the test group (MD 0.44, 95% CI -3.94 to 4.82, $p = 0.84$), but there was a significant decline after treatment in the control group (MD 13.40, 95% CI 7.22 to 19.58, $p<0.0001$), so the mean KPS after treatment was significantly higher in the test group, indicating a relative improvement in the test group (Table 5.1).

Yang (2008) reported Su Ying’s QoL questionnaire which comprised 12 items that are scored from 5 to 1 according to increase in severity, so the more severe the symptoms the lower the score. In the test group, the total score was not significantly different before and after the treatment (MD 1.57, 95% CI -3.44 to 0.30, $p = 0.10$), but it significantly decreased in the control group after treatment (MD 2.70, 95% CI 1.34 to 4.06, $p<0.0001$). The between-group scores after treatment were significantly different in favour of the test group (Table 5.1).

For body weight, there was a significant difference in body weight improvement in favour of the test group based on three studies (RD 18.9%) (Table 5.1).

### 5.3.4 **Effect on alleviation of chemotherapy-related adverse events**

All 14 studies reported that the CHMs alleviated chemotherapy toxicity. Zhang et al. (2008) reported events as gastrointestinal reactions or myelosuppression, while Zeng et al. (2009) and Cao et al. (2011) reported toxicity events as totals (all grades). Therefore, these three studies were not included in the meta-analyses.

Most of the AEs were mild (grades 1–2). The three most commonly reported AEs were nausea and vomiting, neutropenia and neurotoxicity. The meta-analyses included toxic events at grades 3 and 4 only (i.e. severe toxicity) (Table 5.1). The pooled grade 3 and 4 events for neutropenia (n = 10, RD 8.7%), nausea and vomiting (n = 9, RD 9.5%) and neurotoxicity (n = 7, RD 3.8%) were significantly fewer in the test groups. For the *Kang'ai* Injection sub-group (n = 2), significant reductions were found for neutropenia (RD 17.8%) and nausea and vomiting events (RD 15.8%). No significant difference between groups was found for the pooled grade 3 and 4 events for: diarrhoea (five studies, RD 2.4%); anaemia (three studies, RD 2.9%); stomatitis (two studies, RD 2.3%); thrombocytopenia (one study); or constipation (one study). Adverse events specific to the CHMs were not reported in any of the 14 studies.

### 5.3.5 **Effect on immune function**

Four studies reported effects on immune function, in terms of the percentage of T-lymphocyte subsets and Natural Killer (NK) cell activity in serum. One study reported benefits for T cells and NK cells but data were presented as difference scores, so these could not be included in pooling (Zeng et al., 2009).

There was a significant improvement in the pooled data for CD3+ cells (Wu et al., 2010) and ratio CD4+/CD8+ cells (Ding et al., 2010; Zhang et al., 2010a) (Table 5.1). For CD4+, CD8+ cells and NK cells, there were benefits reported in some studies but the pooled results were too heterogeneous to be meaningful.

## 5.4 Discussion of CHMs combined with FOLFOX4 for advanced colorectal cancer

All 14 studies included in this meta-analysis were published after 2007 and all employed FOLFOX4 as a single regimen. Since this is currently the most commonly used first-line chemotherapy regimen for ACRC, the results of this section are of direct clinical relevance.

Internationally recognised measurement systems were used in all included studies, including the TNM staging system, WHO criteria for solid tumour response and grading of acute and subacute toxicity, and the KPS scoring system. Participants' age was in the range 48–60 years in nine studies and one study enrolled participants whose mean age was 72.7 years (Li et al., 2007). The majority of the cohort was younger than the general population of CRC patients internationally, whose median age is over 70 years. A tendency for trial participants to be younger than the average CRC patient has been found in other ACRC trials but younger patients did not appear to respond better or experience less toxicity than older patients (Hind et al., 2008), so this difference was judged as not likely to affect the generalizability of results. Overall, the heterogeneity of meta-analyses was low for the clinical outcome measures.

The pooled data indicate the addition of the CHMs significantly improved tRR when compared to FOLFOX4 alone (Table 5.1). In the sub-group analysis of tRR for previously treated and untreated ACRC participants, the results showed a similar benefit for the CHM in both groups but this did not reach significance (Table 5.1). A non-significant result was also found in the CR pooling. It is possible that the small number of participants in these sub-groups meant there was insufficient statistical power to detect a difference. It is also notable that FOLFOX4 itself has not yielded high CRs in ACRC (Cassidy et al., 2008; De Gramont et al., 2000; Goldberg et al., 2004; Goldberg et al., 2006). Overall, these results suggested that the CHMs conferred an additional benefit to tRR when combined with FOLFOX4 for ACRC, but these results need to be verified by a large scale clinical trial.

One year-OS, median OS and median TTP were greater in the CHM plus FOLFOX4 groups compared to FOLFOX4 alone (Table 5.1). Participants in the test groups were more likely to show a KPS improvement than in the control groups (RD 28.8%).

For chemotherapy-related AEs, significant reductions were found in the test groups for grade 3 and 4 neutropenia (T: 3.9% vs. C: 12.6%), nausea and vomiting (T: 3.0% vs. C: 12.5%) and neurotoxicity (T: 2.2% vs. C: 6.0%) (Table 5.1). These findings were generally consistent with the results in Chapter 4 and earlier reviews of CHM adjuvant to chemotherapy for CRC (Liu and Zhu, 2009; Wu et al., 2005; Zhong et al., 2012).

The results for T-lymphocyte subsets suggest that some CHMs may have immune enhancing effects but these data were only available for a few studies and there was substantial heterogeneity in some of the meta-analyses (Table 5.1). The substantial heterogeneity was similar to that found in the Chapter 4 meta-analyses of T-lymphocyte subsets. It is notable that the following CHMs that were used in the studies, *Astragalus*, *P. ginseng*, *Atractylodes*, *Poria* and *Coix*, have been reported to have immunomodulatory effects (Gong, 2010; Yang et al., 2002; Yang et al., 2011).

From the clinical perspective, these findings suggest that combining CHMs, which contain the above main herbs, with FOLFOX4 was clinically beneficial in advanced CRC since the addition of the CHMs to this commonly used chemotherapy regimen appeared to reduce a number of the adverse events associated with FOLFOX4. Also, adding the CHMs did not appear to reduce the effectiveness of FOLFOX4 since tRR and survival were not reduced, rather they appear to have been enhanced. In addition, these CHMs are in common use and did not appear to produce additional severe AEs.

### 5.4.1 **Comparison with other studies of FOLFOX4**

The improvement in tRR of 20.0% to 46.6 % (average 34.9%) for the control groups in the 12 studies was consistent with the results of a review by Lu et al. (2010) that pooled 27 clinical studies of FOLFOX4 for ACRC conducted in China (879 participants), none of which involved a comparison with CHM. The review by Lu et al. found the following tRRs: total 26.10 to 57.14% (27 studies); previously untreated patients 30.8 to 65.0% (12 studies); and previously treated patients 16.6 to 47.6% (12 studies). In this section, the pooled tRRs of 34.7% for all control group participants, 38.3 % for previously untreated participants and 21.0% for previously treated participants all fell within these ranges, so the results for the control groups in this section were broadly consistent with non-CHM studies conducted in China on FOLFOX4. In the control groups in this review, the median OS was 10.2 to 18.6 months and the TTP was 6.9 to 10.2 months, which were similar to the ranges found in the review by Lu et al. (OS 9.0 to 17.7 months, TTP 5.47 to 9.00 months).

It was evident that the average tRR (34.7%) in the control groups in this review and in the review of Chinese FOLFOX4 studies (Lu et al., 2010) were both relatively low compared to large international trials (Cassidy et al., 2008; De Gramont et al., 2000; Goldberg et al., 2004; Goldberg et al., 2006) in which the tRRs were in the range 45.0 to 58.5%. These international trials only included previously untreated ACRC, whereas in this review the trial participants were both previously treated and untreated ACRC participants and there were no second line treatments in the Chinese trials. Both of these factors could adversely affect the outcomes for tRR (de Gramont et al., 2000; Giantonio et al., 2007).

In the included studies, the most common FOLFOX4-related grade 3 and 4 AEs were neutropenia, nausea and vomiting, diarrhoea and neurotoxicity, which are the same as in the international studies (Cassidy et al., 2008; de Gramont et al., 2000; Goldberg et al., 2004; Goldberg et al., 2006). However, the incidence rates for grade 3/4 neutropenia, neurotoxicity and diarrhoea in the pooled control group data were less than in the international studies. A likely reason for this difference is the relatively shorter duration of treatment in the studies in this review, since neutropenia and neurotoxicity tend to become more severe the longer the chemotherapy continues (Boisdron-Celle et al., 2002; de Gramont et al., 2000).

The grade 3/4 nausea and vomiting rate was comparable to the international studies. This may be due to the preventive use of anti-emetic drugs and dose modification in FOLFOX4 protocols which enable control of the severity of nausea and vomiting even with longer treatment, or inclusion of previously treated and untreated participants without reporting separate results for these two groups who could be expected to show differential responses to interventions.

### 5.4.2 **How the Chinese herbal medicines might work**

Except for *Kang'ai* injection, which was tested in two studies, different multi-herb decoctions or manufactured products were tested in each study. Therefore, on the basis of the clinical trial results alone, it was difficult to determine which individual herbs could have contributed to the reported effects. The main rationale for combining CHMs with chemotherapy was the alleviation of AEs due to chemotherapy. Of the six most commonly used herbs in these RCTs, *Astragalus membranaceous*, *Panax ginseng*, *Atractylodes lanceolata*, *Poria cocos* and *Coix lachry-jojobi* are traditionally used for fatigue, poor appetite, diarrhoea and other gastrointestinal disorders (Bensky et al., 2004) and each of these herbs has been reported to have immunomodulatory effects (Gong, 2010; Shergis et al., 2012; Yang et al., 2011). These actions may at least partially account for the reported improvements in AEs.

In addition, recent experimental research into each of the six herbs used most frequently in the 13 studies indicates that each has effects that may contribute to the suppression of tumour growth. The findings of some of these studies are discussed briefly below.

Astragalus polysaccharides have been shown to have anti-proliferative effects in cell-line studies (Zong et al., 2012). Astragalus saponins inhibited proliferation in a human colorectal cancer HT-29 cell line regardless of the p53 status, demonstrated tumour suppressive effects in a nude mice xenograft model, enhanced the cytotoxic effect of 5-FU (Tin et al., 2007) and have demonstrated anti-angiogenic effects (Law et al., 2012). Astragalus flavonoids have also been reported to have pro-apoptopic effects in colon cancer HCT-116 cells (Auyeung & Ko, 2010).

A number of studies have found ginsenosides and ginseng polysaccharides to have anti-proliferative and pro-apoptopic effects (Nag et al., 2012; Zong et al., 2012). The ginsenoside Rg3 inhibited growth of tumours *in-vivo* in HCT-116 cells (He et al., 2011) and has shown anti-angiogenic activities (Wang et al., 2009b). Also, the ginseng saponin metabolite, compound K, has been reported to inhibit metastatic growth in hepatocellular carcinoma both *in vitro* and *in vivo* (Ming et al., 2011).

Coix seed has a long history as an anti-cancer agent in China and has been developed into an injectable product (*Kanglaite* Coix oil extract) (Li, 2007; Woo et al., 2007). Coix extracts have been shown to have anti-inflammatory and anti-carcinogenic effects in animal models of CRC (Chung et al., 2010; Li et al., 2011). Polysaccharides from *Poria cocos* appear to potentiate immune response by

up-regulating immune stimulators and down-regulating immune suppressors and have shown anti-tumour activity in various cancer cell lines by suppressing tumour angiogenesis (Rios, 2011).

Compounds derived from Atractylodes have shown bio-activity in vitro. Anti-inflammatory effects have been reported for atractylenolide I and atractylenolide III (Li et al., 2007a) and atractylenolide II inhibited proliferation of B16 cells, induced G1 cell cycle arrest and induced apoptosis (Ye et al., 2011). In mouse splenocytes, Atractylodes glycoproteins stimulated both Th1 and Th2 lymphocyte proliferation with a greater effect on Th1 lymphocytes (Lee et al., 2007). In an RCT of cachetic cancer patients (n = 64), the administration of a lactone from Atractylodes improved mid-arm muscle circumference, reduced the serum levels of interleukin-1 (IL-1) and tumour necrosis factor alpha (TNF-alpha) and reduced urine proteolysis-inducing factors (PIF) (Liu et al., 2005).

The alkaloids matrine and oxymatrine found in Sophora root have been developed into anti-cancer agents in China and Sophora flavonoids appear to have anti-tumour activity (Sun et al., 2012); for example, sophoflavescenol has shown cytotoxicity in a number of cancer cell lines as well as having antioxidant, anti-inflammatory and apoptotic activities (Jung et al., 2011).

While it appears possible that at least some of the CHMs used in these studies may have contributed to tumour response directly, it is also possible that this effect was indirect via alleviating AEs and thereby enabling patients to better tolerate the chemotherapy. Further research is needed to investigate these issues.

### 5.4.3 **Limitations of this meta-analysis**

In interpreting the findings of these meta-analyses a number of factors need to be considered including methodological issues, trial duration, the nature and size of study samples and variation in the CHMs used. All studies claimed to be randomised but only six stated an appropriate method of sequence generation and none provided information on allocation concealment procedures, so there was potential for selection bias. Lack of blinding increases the risk of bias for subjective outcomes such as KPS and AEs such as nausea, so the results for these outcomes should be considered less reliable than for more objective measures such as tRR and OS. Three studies did not report reasons for dropouts or loss to follow-up. Methodological issues of these types have been found in trials of both conventional medicine and CHM conducted in China (Wu et al., 2009). Clinical trial reporting needs to be clear, complete and transparent (Moher et al., 2010), so there is a pressing need for improvements in the conduct of clinical trials in China and for proper reporting of methods and results in Chinese journals.

Most the studies were relatively small and small trials appear to overestimate true effects (Higgins & Green, 2011). Some of the trials appear to have included both previously treated and untreated

participants without reporting separate results for these two groups who could be expected to show differential responses to interventions.

Other issues were quality control of the medicines prepared by the investigators' hospitals and the use of test medicines that comprise multiple ingredients which may vary in quality from batch to batch and may be affected by decoction conditions. These factors limit the comparability of studies. While there was no apparent publication bias based on the Funnel Plot for tRR, there may have been bias in favour of positive trials for other outcomes.

## 5.5 Chapter 5 summary and conclusions

Whereas previous reviews included a range of regimens in the meta-analysis pools (Wu et al 2005, Zhong et al 2012), this chapter focussed on FOLFOX4 to provide the best available clinical evidence for CHM in the treatment or management of ACRC. The meta-analysis results suggest the addition of these CHMs to a FOLFOX4 regimen increased tumour response rate (tRR) and one-year survival but evidence was lacking for longer term effects. Six individual plants were frequently used as ingredients of the CHMs, and each has shown bioactivity of relevance to cancer, but it was not possible to determine which of these made the greatest contributions to the improvements in tumour response or other outcomes. Overall, the addition of the CHM interventions appears to have improved quality of life and reduced the incidence of severe neutropenia, nausea, vomiting, and neurotoxicity associated with FOLFOX4 chemotherapy.

# Chapter 6. Contributions of Specific Plants to Tumour Response, Neutropenia, Nausea and Vomiting

## 6.1 Introduction to Chapter 6

In the previous two chapters, the meta-analysis found that when HMs were combined with chemotherapy (including oxaliplatin regimens and non-oxaliplatin regimen groups), the tRRs were improved, and there were reductions in a number of the adverse events associated with chemotherapy, including nausea and vomiting, diarrhoea, neutropenia, thrombocytopenia, and neurotoxicity. This chapter aims to answer the research question: Which herbs and herbal combinations are most frequently used and effective in CRC treatment and for alleviation of the side effects of conventional CRC treatments?

The largest group of interventions in this review were oxaliplatin regimens combined with CHM. The main oxaliplatin regimens included: FOLFOX regimens, which all combine oxaliplatin, 5-fluorouracil (5-FU) and leucovorin (LV); and XELOX, which is oxaliplatin combined with oral capecitabine (Hirsch and Zafar, 2011). Capecitabine converts to 5-FU in the body and was found to be as effective as intravenous 5-FU/LV (Twelves, et al., 2005; Cassidy, et al., 2008). FOLFOX regimens have several modifications but their effectiveness and their AEs were reported to be similar (Hind et al., 2008).

The sensitivity analyses undertaken in chapter required a relatively large data set for multi-level grouping, and this data set needed to have low heterogeneity. In the meta-analyses in Chapter 4 the oxaliplatin regimens showed low heterogeneity, as did the FOLFOX4 for ACRC meta-analyses in Chapter 5. The approach taken in Chapter 5 reduced heterogeneity associated with the interventions and participants, and this was reflected in the lack of important statistical heterogeneity. However, this approach also reduced the size of the data set. Therefore, the groups of studies that combined oxaliplatin regimens with CHMs for CRC was selected for the analyses of the contributions of individual HMs presented in this chapter.

This chapter provides results of a series of meta-analyses of oxaliplatin regimens combined with CHMs for:

1. Tumour response rate (tRR) (42 included studies);
2. Alleviation of nausea and vomiting (27 included studies); and
3. Alleviation of neutropenia (29 included studies).

Each meta-analysis includes a sensitivity analysis aimed to select short lists of HMs and combinations of HMs that are potentially effective for each of these outcomes. It also reviewed the experimental literature on the short-listed herbs for tumour response rate, neutropenia and nausea and vomiting. Other outcomes such as thrombocytopenia and neurotoxicity were not included due to insufficient data availability. For the methods used in the sensitivity analyses, see Chapter 3.

The results reported in this chapter have been published in three articles:

- Chen MH, May BH, Zhou IW, Xue CC, Zhang AL. Meta-Analysis of Oxaliplatin-Based Chemotherapy Combined with Traditional Medicines for Colorectal Cancer: Contributions of Specific Plants to Tumour Response. *Integr Cancer Ther*. 2016a;15(1):40-59.
- Chen MH, May BH, Zhou IW, Zhang AL, Xue CC. Integrative Medicine for Relief of Nausea and Vomiting in the Treatment of Colorectal Cancer Using Oxaliplatin-Based Chemotherapy: A Systematic Review and Meta-Analysis. *Phytother Res.* 2016b; 30(5):741-53.
- Chen MH, May BH, Zhou IW, Sze DM, Xue CC, Zhang AL. Oxaliplatin-based chemotherapy combined with traditional medicines for neutropenia in colorectal cancer: A meta-analysis of the contributions of specific plants. *Crit Rev Oncol Hematol*. 2016c; 105:18-34.

This chapter represents an amalgam of these three papers. The methods are detailed in Chapter 3. In this chapter the abbreviation CHM refers to a Chinese herbal medicine formula or commercial product; HM refers to a single herbal medicine – most of which are plants.

## 6.2 Meta-analysis of oxaliplatin-based chemotherapy combined with herbal medicine for tumour response rate

### 6.2.1 Background and rationale

In Chapter 5, the meta-analyses of CHMs combined with FOLFOX4 found that the systemic CHMs conferred benefits to ACRC patients in terms of tumour response rate (tRR), quality of life and some chemotherapy-induced adverse events, when compared to FOLFOX4 alone. Although it is likely that the most frequent HMs in the multi-component formulae were contributors to the pooled outcomes, it is possible that other lower frequency HMs may be of research interest, particularly with regard to their effect on tumour response rate (tRR), which is the most frequently reported primary outcome in cancer trials (Saad & Katz, 2009). In addition, one traditional rationale for using multi-ingredient HMs is the concept of synergetic action, so it was possible that certain combinations of HMs may be more effective than these HMs individually.

In this section, the aim was to identify which HMs, and which combinations of HMs, were associated with elevated tRRs in the clinical trials of the integrated treatment of CRC. These sensitivity analyses of RCTs of HMs combined with oxaliplatin regimens for CRC were conducted in order to select HMs for further clinical and experimental research regarding their effects on tumour growth.

### 6.2.2 Included studies and characteristics

Fourty-two studies that reported tRR were included in the meta-analyses. These 42 studies enrolled 3,070 assessable participants with 1,613 in the test groups and 1,457 in the controls. All studies were published from 2005 to 2013. Forty-one studies were conducted in China and one in Japan (Kono, et

al., 2013). Participant characteristics and interventions are summarised in Table G1 in Appendix G. Thirty-one studies used the CHMs orally. Eleven studies employed commercially available CHM injections. The oxaliplatin regimens included the following: FOLFOX regimens in 39 studies, and XELOX in 3 studies.

### 6.2.3 **Meta-Analysis of Tumour Response**

Meta-analyses were conducted for complete response (CR) and tRR. When risk ratio (RR) is more than +1 (IV model, fixed, 95% CI), it favours the test group. Meta-analyses were performed for the following groups:

1. Total (42 studies);
2. Non-oral (injection) group (11 studies); and
3. Oral administration group (31 studies).

#### *6.2.3.1 Total group*

In the 42 studies (n = 3,070), the test groups showed significantly improved tRR (RR 1.30 [1.20, 1.42], $I^2$ = 0%), without heterogeneity (Figure 6.1).

#### *6.2.3.2 Oral administration group*

In 31 studies (n = 2,145), the CHMs were administered orally as decoctions, capsules, or tablets. Two studies by the same author used the same multi-ingredient CHMs (Liu et al., 2005a, Liu et al., 2005b). The pooled tRR showed significant improvement (RR 1.27 [1.15, 1.41], $I^2$ = 0%), without heterogeneity (Figure 6.1).

#### *6.2.3.3 Non-oral group*

Ten different injection products were tested in 11 studies (n = 938). There were significant improvements for tRR (RR 1.36 [1.18-1.57], $I^2$ = 0%) $I^2$ = 0%) compared to controls, without heterogeneity (Figure 6.1).

### 6.2.4 **Sensitivity analyses for selection of herbs for tumour response rate**

A series of multi-level sensitivity analyses were conducted for the multi-ingredient oral CHM interventions. The multi-ingredient CHM formulae tended to differ in name but there was considerable similarity in their main ingredients. The effects on tRR of the HMs used in multiple studies were reported below at the level of the single HM, pair of HMs, and groups of 3 or more HMs. Since the aim was to select HMs for further research, only HMs with significant tRR results that were equal or greater than the pooled tRR were reported in this chapter. For details of the method, refer to section 3.3. See Table 6.1 for all significant tRR results.

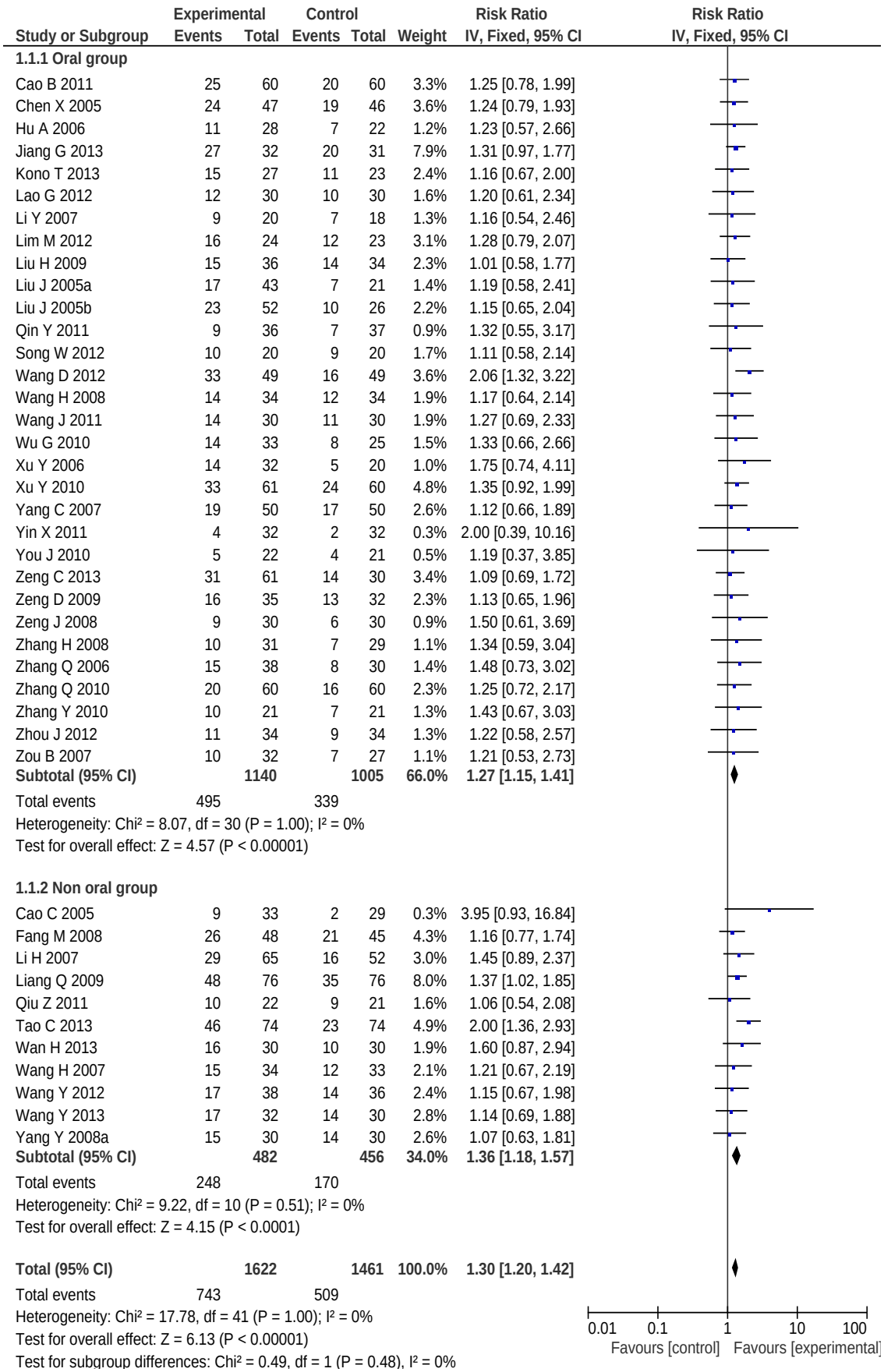


**Figure 6.1: Forest plot of risk ratio for tumour response rate of oxaliplatin-based regimens combined with Chinese herbal medicines (included oral and non-oral sub-groups)**

### *6.2.4.1 Level 1: Single herbal medicines*

All plant-based ingredients (n=87) in the formulae were recorded in a spread-sheet. The number of HMs per formula averaged 12 and ranged from 2 to 25. Thirty one out of 87 HMs were used in two or more formulae. For each HM, the full botanical name was given in the first instance together with the plant part used and the Chinese name in *pinyin*. Thereafter, the name was shortened to the genus only.

**Table 6.1: Effects of specific herbal medicines on tumour response: single herbal medicines and combinations**

| Level | Chinese herbal Medicine (CHM) | RR | 95% CI | N. studies (part.) | $I^2$% |
|---|---|---|---|---|---|
| 1 | *Coptis* | 1.49 | [1.18, 1.89] | 3 (221) | 29 |
| 1 | *Sanguisorba* | 1.49 | [1.18, 1.89] | 3 (221) | 29 |
| 1 | *Aucklandia* | 1.45 | [1.16, 1.80] | 5 (340) | 0 |
| 1 | *Paeonia* | 1.44 | [1.18, 1.77] | 5 (409) | 0 |
| 1 | *Sophora* | 1.44 | [1.17, 1.77] | 5 (401) | 0 |
| 1 | *Akebia* | 1.41 | [1.09, 1.83] | 5 (310) | 16 |
| 1 | *Sparganium* | 1.36 | [1.13, 1.64] | 6 (491) | 0 |
| 1 | *Curcuma* | 1.34 | [1.14, 1.58] | 11 (797) | 0 |
| 1 | *Citrus* | 1.3 | [1.07, 1.59] | 6 (420) | 0 |
| 1 | *Pinellia* | 1.28 | [1.02, 1.59] | 7 (514) | 0 |
| 1 | *Coix* | 1.25 | [1.08, 1.46] | 19 (1283) | 0 |
| 1 | *Hedyotis* | 1.25 | [1.06, 1.49] | 10 (687) | 0 |
| 1 | *Astragalus* | 1.24 | [1.08, 1.43] | 18 (1194) | 0 |
| 1 | *Scutellaria* | 1.24 | [1.04, 1.49] | 9 (599) | 0 |
| 1 | *Atractylodes* | 1.23 | [1.08, 1.41] | 23 (1549) | 0 |
| 1 | *Poria* | 1.23 | [1.06, 1.43] | 19 (1213) | 0 |
| 1 | *Codonopsis* | 1.23 | [1.07, 1.41] | 17 (1206) | 0 |
| 2 | *Sophora + Aucklandia* | 1.51 | [1.18, 1.92] | 3 (221) | 27 |
| 2 | *Sparganium + Curcuma* | 1.36 | [1.13, 1.64] | 6 (491) | 0 |
| 2 | *Paeonia + Astragalus* | 1.31 | [1.04, 1.65] | 4 (311) | 0 |
| 2 | *Codonopsis + Citrus* | 1.3 | [1.07, 1.59] | 6 (420) | 0 |
| 2 | *Astragalus + Hedyotis* | 1.26 | [1.06, 1.51] | 9 (647) | 0 |
| 2 | *Poria + Coix* | 1.26 | [1.06, 1.49] | 15 (981) | 0 |
| 2 | *Curcuma + Astragalus* | 1.25 | [1.04, 1.49] | 9 (635) | 0 |
| 2 | *Coix + Atractylodes* | 1.25 | [1.06, 1.47] | 17 (1105) | 0 |
| 2 | *Astragalus + Scutellaria* | 1.24 | [1.04, 1.49] | 9 (599) | 0 |
| 2 | *Codonopsis* + Hedyotis | 1.23 | [1.03, 1.46] | 8 (575) | 0 |
| 2 | *Atractylodes + Hedyotis* | 1.23 | [1.00, 1.51] | 9 (624) | 0 |
| 2 | Astragalus + *Codonopsis* | 1.23 | [1.05, 1.43] | 13 (902) | 0 |
| 2 | *Poria +Atractylodes* | 1.23 | [1.05, 1.44] | 17 (1105) | 0 |
| 2 | *Atractylodes + Astragalus* | 1.23 | [1.05, 1.44] | 17 (1131) | 0 |
| 2 | *Poria + Codonopsis* | 1.21 | [1.02, 1.43] | 14 (923) | 0 |
| 2 | *Atractylode + Codonopsis* | 1.21 | [1.04, 1.41] | 16 (1143) | 0 |
| 3 | *Sophora + Paeonia + Curcuma* | 1.44 | [1.16, 1.78] | 4 (341) | 8 |
| 3 | *Sophora + Astragalus + Scutellaria* | 1.31 | [1.04, 1.65] | 4 (303) | 0 |
| 3 | *Curcuma + Astragalus + Hedyotis* | 1.3 | [1.05, 1.60] | 5 (363) | 0 |
| 3 | *Astragalus + Hedyotis + Scutellaria* | 1.28 | [1.04, 1.56] | 6 (431) | 0 |
| 3 | *Pinellia + Coix + Poria* | 1.28 | [1.02, 1.59] | 7 (514) | 0 |
| 3 | *Codonopsis + Scutellaria + Hedyotis* | 1.26 | [1.03, 1.56] | 5 (371) | 0 |
| 3 | *Astragalus + Codonopsis + Scutellaria* | 1.26 | [1.04, 1.53] | 7 (469) | 0 |
| 3 | *Curcuma + Astragalus + Codonopsis* | 1.25 | [1.04, 1.52] | 7 (513) | 0 |
| 3 | *Coix + Poria + Atractylodes* | 1.25 | [1.05, 1.49] | 14 (923) | 0 |
| 3 | *Curcuma + Astragalus + Scutellaria* | 1.24 | [1.02, 1.51] | 6 (441) | 0 |
| 3 | *Astragalus + Codonopsis + Hedyotis* | 1.24 | [1.03, 1.49] | 7 (535) | 0 |
| 3 | *Atractylodes + Astragalus + Hedyotis* | 1.24 | [1.00, 1.54] | 8 (584) | 0 |
| 3 | *Coix + Poria + Astragalus* | 1.22 | [1.01, 1.48] | 13 (827) | 0 |

| Level | Chinese herbal Medicine (CHM) | RR | 95% CI | N. studies (part.) | $I^2$% |
|---|---|---|---|---|---|
| 3 | *Poria+ Atractylodes+ Astragalus* | 1.21 | [1.00, 1.46] | 12 (787) | 0 |
| 3 | *Poria+ Atractylodes+ Codonopsis* | 1.21 | [1.02, 1.43] | 14 (923) | 0 |
| 3 | *Atractylodes + Astragalus + Codonopsis* | 1.2 | [1.00, 1.44] | 12 (839) | 0 |
| 4 | *Sophora + Paeonia + Sparganium + Curcuma* | 1.45 | [1.16, 1.80] | 3 (281) | 38 |
| 4 | *Astragalus + Hedyotis* + Aucklandia + Scutellaria | 1.31 | [1.00, 1.70] | 3 (183) | 0 |
| 4 | *Sophora + Scutellaria + Hedyotis + Astragalus* | 1.3 | [1.02, 1.66] | 3 (243) | 0 |
| 4 | *Astragalus + Codonopsis + Citrus + Hedyotis* | 1.29 | [1.01, 1.64] | 4 (256) | 0 |
| 4 | *Curcuma + Astragalus + Scutellaria + Codonopsis* | 1.28 | [1.03, 1.57] | 5 (371) | 0 |
| 4 | *Pinellia + Coix + Atractylodes + Poria* | 1.27 | [1.00, 1.60] | 6 (456) | 0 |
| 4 | *Sparganium + Curcuma + Astragalus + Codonopsis* | 1.25 | [1.01, 1.53] | 5 (393) | 0 |
| 4 | *Coix + Atractylodes + Poria + Codonopsis* | 1.23 | [1.02, 1.49 | 11 (741) | 0 |
| 5 | *Sanguisorba + Coptis + Sophora + Paeonia + Curcuma* | 1.49 | [1.18, 1.89] | 3 (221) | 29 |
| 5 | *Sophora + Scutellaria + Aucklandia + Astragalus + Hedyotis* | 1.33 | [1.00, 1.76] | 2 (123) | 0 |
| 5 | *Codonopsis + Scutellaria + Citrus + Hedyotis + Astragalus* | 1.29 | [1.00, 1.65] | 3 (183) | 0 |
| 5 | *Curcuma + Codonopsis + Hedyotis + Scutellaria + Astragalus* | 1.27 | [1.02, 1.58] | 4 (311) | 0 |
| 6 | *Sophora + Curcuma + Scutellaria + Astragalus + Codonopsis + Paeonia* | 1.29 | [1.02, 1.65] | 3 (243) | 0 |
| 6 | *Sparganium + Curcuma + Hedyotis + Astragalus + Scutellaria + Codonopsis* | 1.27 | [1.01, 1.61] | 3 (251) | 0 |
| 8 | *Paeonia + Curcuma + Hedyotis + Sophora + Sparganium + Codonopsis + Astragalus + Scutellaria* | 1.29 | [1.00, 1.66] | 2 (183) | 0 |

RR: Risk Ratio for tumour response; 95% CI: 95% Confidence Interval; N. studies (part.): number of studies (participants); $I^2$ %: measure of heterogeneity of result

The most frequently used HMs are listed below as: species. plant part [*pinyin* name] family (frequency).

- *Atractylodes macrocephala* Koidz. root [*bai zhu*] Asteraceae (n=23);
- *Coix lacryma-jobi* L. seed [*yi ren*] Gramineae (n=19);
- *Poria cocos* (Schw) Wolf sclerotium [*fu ling*] Polyporaceae (n=19);
- *Astragalus membranaceus* (Fisch.) Bge.root [*huang qi*] Fabaceae (n=18);
- *Codonopsis pilosula* (Franch.). Nannf.root [*dang shen*] Campanulaceae (n=17);
- *Curcuma zedoaria* (Berg.) Rosc. or *C. phaeocaulis* Val. rhizome [*e zhu*] Zingiberaceae (n=11);
- *Hedyotis diffusa Willd.* Aerial parts [*she she cao*] Rubiaceae (n=10);
- *Scutellaria barbata* D. Don. aerial parts [*ban zhi lian*] Labiatae (n=9); and
- *Pinellia ternata* (Thunb.) Breit. tuber [*ban xia*] Araceae (n=7).

The tRRs of the group of studies that included each particular HM were calculated. These tRRs were sorted from high to low, significant tRRs were identified (n=25), and groups with moderate heterogeneity ($I^2$> 30%) were excluded (n= 8), leaving 17 different HMs in the following analyses (Table 6.1). The pooled tRR results were divided into three groups: 1. tRR significant and greater or

equal to the tRR of the total pool (RR 1.27); 2. tRR significant but less than the total pool; and 3. tRR no significant (results not reported).

The first group, in descending order of tRR, included 10 HMs: *Sanguisorba officinalis* L. root [*di yu*] Rosaceae (n=3); *Coptis chinensis* Franch. root [*huang lian*] Ranunculaceae (n=3); *Aucklandia lappa* Decne. root [*mu xiang*] Asteraceae (n=5); *Sophora flavescens* Ait. root [*ku shen*] Fabaceae (n=5); *Paeonia lactiflora* Pall. or *P. veitchii* Lynch. Root [*chi shao*] Ranunculaceae (n=5); *Akebia quinata* (Thunb.) Decne. fruit [*ba yue zha*] Lardizabalaceae (n=5); *Sparganium stoloniferum* Buch.-Hamil. root [*san leng*] Sparganiaceae (n=6); *Curcuma* (n=11); *Citrus reticulata* Blanco. peel [*chen pi*] Rutaceae (n=6); and *Pinellia* (n=7) (Table 6.1).

In the second group, the following seven HMs showed significant tRRs that were slightly lower than the average tRR of the pool: *Coix*, *Hedyotis*, *Astragalus*, *Scutellaria*, *Atractylodes*, *Poria* and *Codonopsis* (Table 6.1). The frequency of each HM was plotted against the tRR to explore any relationships. It was evident that the high frequency HMs tended to be closer the tRR of the pool, while the lower frequency HMs showed a broader distribution above and below the pool (Figure 6.2). Non-significant HMs had frequencies of 6 or less, and all HMs excluded due to heterogeneity had frequencies of 2 or 3. Therefore, all the higher frequency HMs remained in the analysis.

#### *6.2.4.2 Level 2: Pairs of herbal medicines*

HMs that showed significant tRR results (n=17) were paired with other HMs from groups 1 or 2 above. When HMs from group 3 were included in pairings or higher level combinations, the results were always at or below the tRR for the pool so these combinations are not reported. The four pairs that showed tRRs that were above or equal to the total pool, in descending order of tRR were: *Sophora* + *Aucklandia* (n=3), *Sparganium* + *Curcuma* (n=6), *Paeonia* + *Astragalus* (n=4), and *Codonopsis* + *Citrus* (n=6). A further 12 pairs were significant but had tRRs lower than the total for the pool (Table 6.2).

#### *6.2.4.3 Level 3: Combinations of three herbal medicines*

The significant pairs from level 2 were combined with other HMs that showed significant tRRs at level 1. The five triplets that showed tRR values above or equal to the total pool, in descending order of tRR were: *Sophora* + *Curcuma* + *Paeonia* (n=4), *Sophora* + *Astragalus* + *Scutellaria* (n=4), *Curcuma* + *Astragalus* + *Hedyotis* (n=5), *Pinellia* + *Poria* + *Coix* (n=7), and *Astragalus* + *Hedyotis* + *Scutellaria* (n=6). An additional 11 triplets showed significant tRRs that were lower than the total for the pool (Table 6.2).

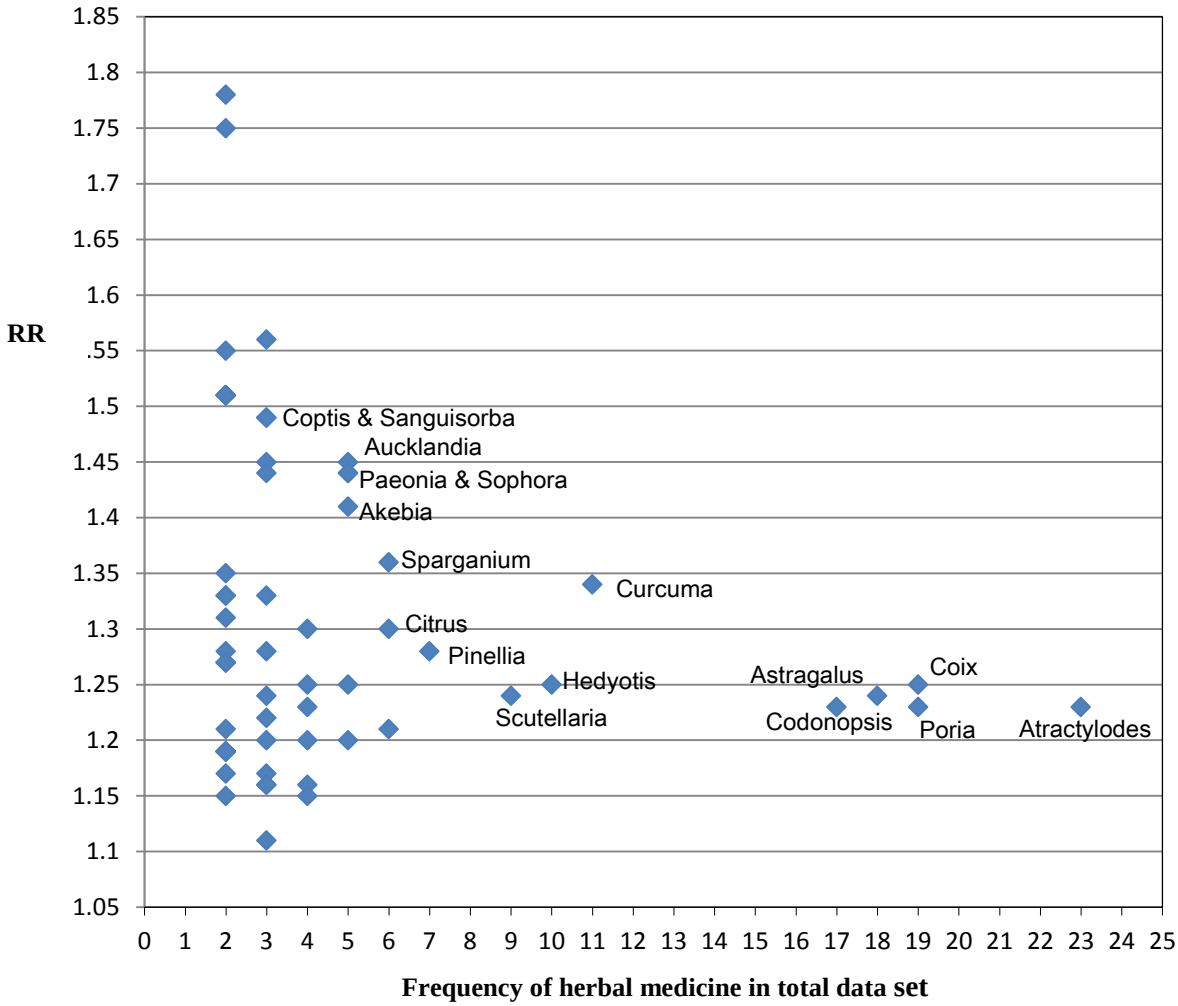


**Figure 6.2: Frequency of Chinese herbal medicine in data set versus risk ratio (RR) for tumour response rate (tRR)**

#### *6.2.4.4 Level 4: Combinations of four herbal medicines*

The significant combinations from level 3 were combined into groups of four. Six combinations showed tRRs above or equal to the total pool: *Sophora* + *Paeonia* + *Sparganium* + *Curcuma* (n=3), *Astragalus* + *Hedyotis* + *Aucklandia* + *Scutellaria* (n=3), *Sophora* + *Scutellaria* + *Hedyotis* + *Astragalus* (n=3), *Astragalus* + *Codonopsis* + *Citrus* + *Hedyotis* (n=4), *Curcuma* + *Astragalus* + *Scutellaria* + *Codonopsis* (n=5), and *Pinellia* + *Coix* + *Atractylodes* + *Poria* (n=6). An additional two combinations showed significant tRRs that were lower than the pool total (Table 6.2).

#### *6.2.4.5 Level 5: Combinations of five herbal medicines*

The significant combinations from level 4 were combined into groups of five. Four combinations showed tRRs equal or higher than the pool: *Sanguisorba* + *Coptis* + *Sophora* + *Paeonia* + *Curcuma* (n=3), *Sophora* + *Scutellaria* + *Aucklandia* + *Astragalus* + *Hedyotis* (n=2), *Codonopsis* + *Scutellaria* +

*Citrus* + *Hedyotis* + *Astragalus* (n=3), and *Curcuma* + *Codonopsis* + *Hedyotis* + *Scutellaria* + *Astragalus* (n=4) (Table 6.2).

#### *6.2.4.6 Level 6: Combinations of six or more herbal medicines*

The significant combinations from level 5 were further combined. There were two combinations of six HMs. One showed a tRR higher than the pool: *Sophora* + *Curcuma*+ *Scutellaria* + *Astragalus* + *Codonopsis* + *Paeonia* (n=3), and the other was equal to the pool: *Sparganium* + *Curcuma* + *Hedyotis* + *Astragalus* + *Scutellaria* + *Codonopsis* (n=3). There were no combinations of seven HMs and one combination of eight which showed a tRR equal to the pool: *Paeonia* + *Curcuma* + *Hedyotis* + *Sophora* + *Sparganium* + *Codonopsis* + *Astragalus* + *Scutellaria* (n=2) (Table 6.2).

### 6.2.5 **Herbal medicines with consistent results at multiple levels**

Combinations of up to eight HMs produced tRR results that were equal or higher than the total for the pool. Seven HMs appeared at all levels: *Astragalus*, *Codonopsis*, *Scutellaria*, *Hedyotis*, *Sophora*, *Curcuma* and *Paeonia*. Of these, *Sophora*, *Curcuma* and *Paeonia* showed significant tRR results that were equal or higher than the total for the pool at each level. This suggested that when these HM were included in a formulation, the tRR tended to be elevated.

### 6.2.6 **Potential synergistic effects of herbal medicines**

Three HM pairs showed higher tRRs as pairs than for the HMs singly: *Sophora* + *Aucklandia*, *Coix* + *Poria*, and *Astragalus* + *Hedyotis* (Table 6.2). Of these, the first pair was also had a tRR higher than the pool.

Three HM triplets showed potential synergistic effects: *Astragalus* + *Hedyotis* + *Scutellaria*, *Astragalus* + *Codonopsis* + *Scutellaria*, and *Codonopsis* + *Hedyotis* + *Scutellaria*. Of these, the tRR of the first triplet was also higher than the pool (Table 6.2).

The combination *Sophora* + *Paeonia* + *Sparganium* + *Curcuma* showed an improved tRR as a group, compared to the pooled results of the single HMs but there was moderate heterogeneity ($I^2$=38%). The group of five HMs *Sanguisorba* + *Coptis* + *Sophora* + *Paeonia* + *Curcuma* had a tRR that was equal or superior to the single HMs (Table 6.2).

### 6.2.7 **Discussion of the results for tumour response rate**

All 42 included studies employed oxaliplatin regimens in the test and control groups. These are currently first-line chemotherapy regimens for CRC in the palliative setting, so the results of these meta-analyses are of direct clinical relevance. The heterogeneity of all meta-analyses was low for the

tRR. The pooled data indicated the addition of the HMs significantly improved tRR when compared to oxaliplatin regimens alone. Benefits were evident in the sub-groups for injections and orally administered CHMs (Figure 6.1). These results were consistent with the results for CHM combined with FOLFOX4 in Chapter 5 and the CHM combined oxaliplatin group in Chapter 4, which included the same studies.

In this section, the most frequently used herbs were *Atractylodes*, *Coix*, *Poria* and *Astragalus* but the specific aim of this section was to identify HMs that showed promise for further research into their effects on tumour growth, so the contribution of each individual HM to the meta-analysis results for tRR was used as the basis for selection. It was reasoned that some CHM formulae may not have aimed at improving tRR, and may have been focussed on improving outcomes relating to adverse effects of chemotherapy and/or improving quality of life. Therefore, some HMs would not be expected to make individual contributions to the tRR results.

The results found that the most frequent HMs, such as *Astragalus*, showed tRRs that were significant but slightly lower than the tRR of pool. This is likely to be a statistical effect since these frequent HMs were the main contributors to the pooled result. It is notable that there were no significant negative tRRs for any of the HMs or their combinations, which suggests that the HMs were not inhibiting the actions of the chemotherapy.

With regard to selecting the most promising HMs for further research, it was evident that many HMs showed significant tRRs, either singly or in combination with other HMs. For example, *Coptis* and *Sanguisorba* appeared the most promising at level 1 based on their individual tRRs (Table 6.2) but these HMs were infrequent overall and did not appear at each level, so they could not show consistent results at multiple levels. In contrast, *Sophora*, *Paeonia* and *Curcuma* all appeared at seven levels of combination, with tRRs that were significant and equal or above the pool at each level without heterogeneity, hence they showed consistent benefit and were selected as the most promising for further research. Nevertheless, this does not mean that *Coptis* and *Sanguisorba* and other HMs with high tRRs at level 1 show no promise.

The results suggest synergistic actions for some HM combinations. *Hedyotis*, *Astragalus* and *Scutellaria* all appeared frequently in the formulae and each has been reported to inhibit CRC *in vitro* and *in vivo* (Tin et al., 2007; Lin et al., 2013; Cai et al., 2012). These HMs showed significant tRRs but all were slightly lower than the pool at level 1. However, these HMs showed higher tRRs in combinations at levels 3 to 6 and all are in the final group of eight HMs. These results suggest that this grouping should also be subject to further research to explore potential synergistic effects between these plants and their compounds on tumour response as well as their effects when combined with oxaliplatin.

An advantage of this approach to the identification of HMs for further research is that it was based directly on the effects of CHM formulae that contain the individual HMs on tumour response rather than on the overall frequency of the HM in the formulae contained in the meta-analysis pool. This procedure allowed identification of potentially effective HMs which appeared at relatively lower frequencies within the total data set. Had frequency been used as the criterion for selection, *Atractylodes*, *Poria* and *Astragalus* would have been identified but *Sophora*, *Paeonia* and *Curcuma* may have been missed.

Conversely, a limitation to this method for selecting HMs and HM combinations is that the data set provided a restricted number of actual HM combinations at each level. Therefore, all the possible combinations could not be assessed. Also, as the levels increased, the number of significant combinations that remained in the data set declined and so did the number of studies from which the data were derived. Consequently, the procedures used removed very low frequency HMs from the data set. Also, the number of levels used in assessing combinations of HM was arbitrary. It was based on what was possible given the available data. This also had the effect of limiting the HM available for selection and may have eliminated promising HM.

#### *6.2.7.1 Experimental studies of Paeonia, Curcuma and Sophora*

These three HMs have all shown evidence of anti-tumour activity in experimental studies. These are summarised briefly below.

Anti-cancer actions of Paeonia (chi shao)

The HM *chi shao* can be derived from the roots of *Paeonia lactiflora* and *Paeonia veitchii*, both of which can contain paeonol and paeoniflorin (Xu et al., 2009; Zhu et al., 2015). In human colon cancer LoVo cells, paeonol blocked cell cycle at the G1 to S transition and induced apoptosis (Li et al., 2013). In HT-29 CRC cells, paeonol inhibited proliferation (Ye et al., 2009) and showed a synergetic antiproliferative effect when combined with 5-FU (Ji et al., 2005). In human oesophageal adenocarcinoma cells, paeonol showed a dose-dependent growth-inhibitory effect and this effect was synergistic when combined with cisplatin (Wan et al., 2008). Paeonol appears to influence multi-drug resistance. It showed reversal of resistance in a paclitaxel-resistant human breast cancer cell line (Cai et al., 2014) and reversed endoplasmic reticulum (ER) stress induced resistance to doxorubicin in human hepatocellular carcinoma cells (Fan et al., 2013). Paeoniflorin has shown growth-inhibitory, pro-apoptotic effects in human cervical cancer HeLa cells (Zhang and Zhang, 2011) as well as anti-inflammatory effects in a colitis model (Zhang et al., 2014) and in human umbilical vein endothelial cells (Xu et al., 2013).

The combination of extracts of *Paeonia* and *Astragalus* were found to synergistically induce the expression of leukotriene B4-12-hydroxydehydrogenase (LTB4DH) in a dose- and time-dependent manner leading to cell cycle arrest in HepG2 cells by controlling the leukotriene B4 pathway (Wei et al., 2011). Although this combination has not been investigated in colon cancer, the leukotriene B4 pathway plays an important role in the proliferation of colon cancer cells (Ihara et al., 2007).

Anti-cancer actions of Curcuma (e zhu)

The official sources of *e zhu* are *Curcuma wenyujin* Y. H. Chen et C. Ling, *C. phaeocaulis* Val., and *C. kwangsiensis* S. G. Lee et C. F. Liang (Lu et al., 2012), but older references use the name *C. zedoaria* Roscoe (Jiangsu New Medical Academy, 1986). The rhizomes contain multiple aromatic compounds including elemenes and non-volatile compounds such as curcumins (Lu et al., 2012).

Curcumin has been investigated in multiple cancer cell-lines (Lu et al., 2011). In human colon cancer HT-29 and HCT-15 cells it dose-dependently inhibited proliferation (Hanif et al., 1997) and induced apoptosis in colorectal carcinoma HCT116 cells and human colorectal cancer LoVo Cells (Lu et al., 2011; Guo et al., 2013). A study of curcumin combined with FOLFOX in two colon cancer cell-lines (HCT-116 and HT-29) reported a synergistic growth-inhibitory effect which appeared to involve the EGFR and IGF-1R growth factor pathways (Patel et al., 2008).

Delta-elemene was found to dose-and time-dependently induce apoptosis in colorectal adenocarcinoma (DLD-1) cells (Xie et al., 2009). Beta-elemene has shown growth-inhibitory activity in multiple cancer cell-lines including CCL-222 and CCL-225 colon carcinoma cells (Li et al., 2010). In colo-205 colorectal adenocarcinoma cells it also increased cisplatin cytotoxicity (Li et al., 2013). Beta-elemene exhibited low toxicity in normal cells, having much weaker anti-proliferative effects in normal human lung fibroblast CCD-19 Lu cells, human bronchial epithelial NL20 cells, and human ovary epithelial IOSE-397 cells, compared to the corresponding cancer cell-lines ( Lu et al., 2012).

Anti-cancer actions of *Sophora flavescens* (*ku shen*)

The dried roots of *Sophora flavescens* Aiton. contain a number of alkaloids, including matrine, oxymatrine, sophoridine and sophocarpine, and flavonoids such as kurarinone (Miao et al., 2001). It is traditionally used to treat solid tumours and inflammatory diseases (Sun et al., 2007). Since 1992, products containing total Sophora alkaloids, oxymatrine and matrine have been approved by the Chinese State Food and Drug Administration (SFDA) for the treatment of various types of solid tumours (Sun et al., 2012).

In human colon cancer HT-29 cells, Xiao et al (2013) reported that a range of ethanol and aqueous extracts of Sophora roots inhibited the cell growth *in-vitro* (Xiao et al., 2013). In a mouse model of

cancer cachexia, sophocarpine and matrine both reduced cachexia symptoms in BALB/c mice inoculated with colon-26 adenocarcinoma cells and suppressed the expression of the pro-inflammatory cytokines TNF-α and IL-6 (Zhang et al., 2008).

Huang et al. (2007) reported that matrine dose-dependently inhibited human colon cancer HT-29 cell proliferation by promoting apoptosis (Huang et al., 2007). Chang et al. (2013) also reported that matrine inhibited proliferation of HT-29 cells. Matrine appeared to activate caspase-3 and caspase-9 and release cytochrome-C to induce apoptosis (Chang et al., 2013). In human colon cancer LoVo cells, Zhang et al. (2014) reported that matrine inhibited proliferation in a time- and dose- dependent manner. They found the mechanisms of action of matrine were via inducing cell cycle arrest at the G1 phase by down-regulation of cyclin D1 and up-regulation of p27 and p21. Apoptosis was induced by reduction of the Bcl-2/Bax ratio and caspase-9 activation. Matrine was reported to have an upstream effect on these proteins by inactivating Akt (Zhang et al., 2014).

In a mouse model using transplanted colon tumour SW480 cells, sophoridine reduced tumour weight and volume and reduced expression of p53 and VEGF67 (Wang et al., 2010). In xenografts of SW480 cells in mice, Liang et al (2012) reported that sophoridine inhibited tumour growth with no apparent toxicity, and in an SW480 cell-line study its action was via caspase-9, caspase-3, caspase-7 and PARP (Liang et al., 2012).

Matrine and oxymatrine have shown synergistic effects with different anti-cancer agents (Liu et al., 2014). Matrine showed synergistic effects when combined with celecoxib, trichostatin A and rosiglitazone against proliferation and VEGF expression in MDA-MB-231 breast cancer cells (Yu et al., 2009), and enhanced the activity of trichostatin A in human non-small cell lung cancer A549 cells (Zhang et al., 2009). In a transplanted human gastric cancer SGC-7901 model in nude mice, the inhibitory effect of matrine combined with 5-FU was greater than either compound used individually, without increasing bone marrow inhibition (Hu et al., 2005).

In a study that compared the antitumour activities of total Sophora alkaloids (KS-As) and flavonoids (KS-Fs), Sun et al. (2007) reported higher growth inhibitory effects for KS-Fs, and for kurarinone in particular, than for KS-As in multiple cancer cell-lines including human colorectal cancer CaCo-2 cells. There was little effect on the peripheral blood cell numbers in normal mice treated with KS-Fs. KS-Fs also enhanced the cytotoxicity of taxol and adriamycin (Sun et al., 2007).

### 6.2.8 **Summary of the results for tumour response rate**

The meta-analyses suggested that the combination of the CHMs with oxaliplatin-based regimens significantly increased tRR in the treatment of CRC. Benefits were evident for both injection products and orally administered CHMs. Detailed sensitivity analyses of specific plant-based HM ingredients of

the oral CHMs found that *Paeonia*, *Curcuma*, and *Sophora* produced consistent contributions to the tRR results. Compounds in each of these HMs have shown growth-inhibitory effects in CRC cell-lines. There were no instances of HMs reducing the tRR of the chemotherapy. Specific combinations of HMs appear to produce higher contributions to tRR than the HMs individually. Notable among these is the combination of *Hedyotis*, *Astragalus*, and *Scutellaria*. Further studies are required to investigate the effects of the HMs identified in this study and the possible synergistic effects of the HM combinations.

## 6.3 Meta-analysis of oxaliplatin-based chemotherapy combined with herbal medicine for relief of nausea and vomiting

### 6.3.1 Background and rationale

The management of chemotherapy-induced nausea and vomiting (CINV) remains an issue in the treatment of colorectal cancer using oxaliplatin based regimens. Risk factors for CINV following chemotherapy include type of chemotherapeutic drug, patient's age less than 50, female, history of low prior chronic alcohol intake, and history of previous chemotherapy-induced emesis. Over seventy percent of patients receiving oxaliplatin regimens experience CINV (Navari, 2009). CINV tends to get worse as the number of treatment cycles increases. This significantly reduces the quality of life of patients, can result in poor compliance with their chemotherapy schedule and can lead to deterioration of physical and mental status (Lohr, 2008).

The mechanisms of CINV are complex. CINV can be initiated by enterochomaffin cells in the gastrointestinal tract releasing serotonin (5-HT) in response to damage of gastrointestinal epithelium and activation of the chemoreceptor trigger zone which detects potential toxins. Activation of vagal afferent fibres stimulates the vomiting centre in the medulla, which in turn sends impulses via efferent fibres to activate the vomiting response. Antiemetic drugs act by blocking neuronal pathways involved at various stages in the emetic response, mainly via antagonism of 5-hydroxytryptamine (5-HT3) receptors, dopamine receptors, neurokinin-1 (NK-1) and/or acetylcholine, corticosteroid, histamine, cannabinoid, and/or opiate receptors (Lohr, 2008; Navari, 2009).

Oxaliplatin regimens used in colorectal cancer (CRC) are considered to have moderate emetic risk and the preventative use of 5-HT3 antagonists combined with dexamethasone is recommended, with the additional use of NK-1 antagonists in selected patients (NCCN, 2012). Despite the introduction of these effective anti-emetic agents, CINV remains a significant issue for people undergoing chemotherapy (Navari, 2009).

Certain HMs with histories of use for nausea and vomiting have been integrated with conventional therapies for CINV. Ginger (*Zingiber officinale* Roscoe) has been used for nausea and evidence from

animal studies suggests anti-CINV effects (Handiadka et al., 2012a). However, a systematic review of seven randomised controlled trials (RCTs) of ginger in CINV management in various cancers found inconsistent results between studies (Marx et al., 2013). Other HMs that have been reported to alleviate CINV in animal models include: *Panax ginseng* C. A. Mey.; *Panax quinquefolius* L.; *Panax notoginseng* (Burk.) F. H. Chen; *Scutellaria baicalensis* Georgi; *Ganoderma lucidum* (Fr.) Karst.; Mint oil (Mentha *spp*); and grape seed extract (Handiadka et al., 2012b).

A number of possible mechanisms for the reported anti-CINV actions of these HMs have been proposed. These include inhibition of 5-HT3 receptors; substance P and NK1 receptors; antioxidant and free radical scavenging activity; anti-inflammatory actions; chemo and radio-protective effects; immunomodulation; neuromodulation; anti-spasmodic effects; and regulation of gastrointestinal motility. Since these HMs may contain multiple bioactive compounds it appears likely that multiple mechanisms are involved (Handiadka et al., 2012b, Suzuki et al., 2013).

In this section, the meta-analyses aimed to assess whether integrative management of CRC, in which CHMs were added to oxaliplatin regimens, reduced the incidence of CINV. The sensitivity analysis aimed to determine whether any particular HMs showed promise for further research into their anti-emetic and/or nausea alleviating effects.

### 6.3.2 **Included studies and characteristics**

Twenty-seven studies published from 2005 to 2013, which enrolled 1,843 (T: 960/C: 883) assessable participants, were included in the meta-analyses. Six studies employed commercially available injections and 21 studies (1,322 participants) used orally administered CHMs (Table G2, in Appendix G).

### 6.3.3 **Meta-analysis of alleviation of chemotherapy-induced nausea and vomiting**

Meta-analysis was conducted for all grades of nausea and vomiting combined since severe nausea and vomiting (grade 3/4) was relatively infrequent. Also, nausea and vomiting are unpleasant symptoms at any grade, so allevation of any grade would be of clinical relevance. A lower RR indicated a lower risk of nausea and vomiting. When RR was less than +1 (IV model, fixed, 95% CI), it favoured the test group.

#### *6.3.3.1 Total group*

For all 27 studies, the test groups showed significantly reduced nausea and vomiting (RR 0.65 [0.59, 0.71], $I^2$=28%) without important heterogeneity (Figure 6.3). The absolute risk reduction was 24% compared to controls (RD= -0.24 [-0.28, -0.19], $I^2$=48%). In the 17 studies for which anti-emetic drugs, such as ondansetron or granisetron, were used in both groups, the RR was 0.68 [0.60, 0.77],

$I^2$=32%, whereas in the studies that did not mention use of anti-emetic drugs (n=10) the RR was 0.60 [0.51, 0.70], $I^2$=19%, which was similar.

#### *6.3.3.2 Injection group*

Four different injection products were tested in 6 studies (Table 6.3). There was a significant reduction in nausea and vomiting (RR 0.73 [0.61, 0.86], $I^2$=60%, RD= -0.20 [-0.29, -0.12], $I^2$=50%) in the HM plus oxaliplatin groups. The heterogeneity was moderate to substantial (Figure 6.3). Co-kushen Injection (n=2) (Ding et al., 2010; Tao and Xu, 2013) showed significantly reduced nausea and vomiting (RR 0.66 [0.50, 0.86], $I^2$ =64%), but there was substantial heterogeneity. *Kang'ai* Injection (n=2) (Qiu 2011; Yang, 2008) showed a significant reduction with no important heterogeneity (RR 0.47 [0.29, 0.77], $I^2$ =28%).

#### *6.3.3.3 Oral administration group*

The CHMs were administered orally as decoctions, capsules or tablets in 21 studies. The combination of CHMs plus oxaliplatin showed a significant reduction in nausea and vomiting incidence compared to the same oxaliplatin regimens (RR 0.62 [0.55, 0.69], $I^2$=5%) without important heterogeneity (Figure 6.3). The absolute risk reduction was 25% (RD -0.25 [-0.30, -0.20], $I^2$=49%).

### 6.3.4 **Sensitivity analyses for selection of herbs for chemotherapy-induced nausea and vomiting**

The orally administered CHMs contained 98 different plant-based HMs with an average of 12 HMs per CHM intervention. The 48 HMs that were used in two or more studies were included in the following sensitivity analyses. Thirty of these HMs showed significant RRs for reduction of incidence of CINV with low heterogeneity ($I^2$ < 30%). The effects of these herbs were also assessed when they appeared as pairs, triplets and higher level combinations in the HM interventions. Significant RR results with low heterogeneity that were equal or lower than the total pool for the oral interventions RR 0.62 [0.55, 0.69] are reported in Table 6.2. The full botanical name and Chinese name in *pinyin* of each herb is given the first time it appears in this chapter. Subsequently, the name is shortened to genus only.

#### *6.3.4.1 Level 1: Single herbal medicines*

The following seven herbs did not always appear in association with another particular herb, so they were suitable for further analysis. Of these, *Panax G* (n=4) had the lowest RR (0.51 [0.39, 0.66], $I^2$=0%); followed by *Poria* (n=16) (RR 0.61 [0.54, 0.69], $I^2$=15%); *Coix* (n=14) (RR 0.61 [0.53, 0.70], $I^2$=29%); *Codonopsis* (n=12) (RR 0.61 [0.52, 0.72], $I^2$=28%); *Panax N* (n=4) (RR 0.61 [0.43, 0.87], $I^2$=0%); *Atractylodes* (n=16) (RR 0.62 [0.54, 0.71], $I^2$=13%); and *Astragalus* (n=13) (RR 0.65 [0.55, 0.76], $I^2$=0%) (Table 6.2). The following herbs were the most frequently used in the CHM

interventions: *Poria* (n=16); *Atractylodes* (n=16); *Coix* (n=14); *Astragalus* (n=13); and *Codonopsis* (n=12).

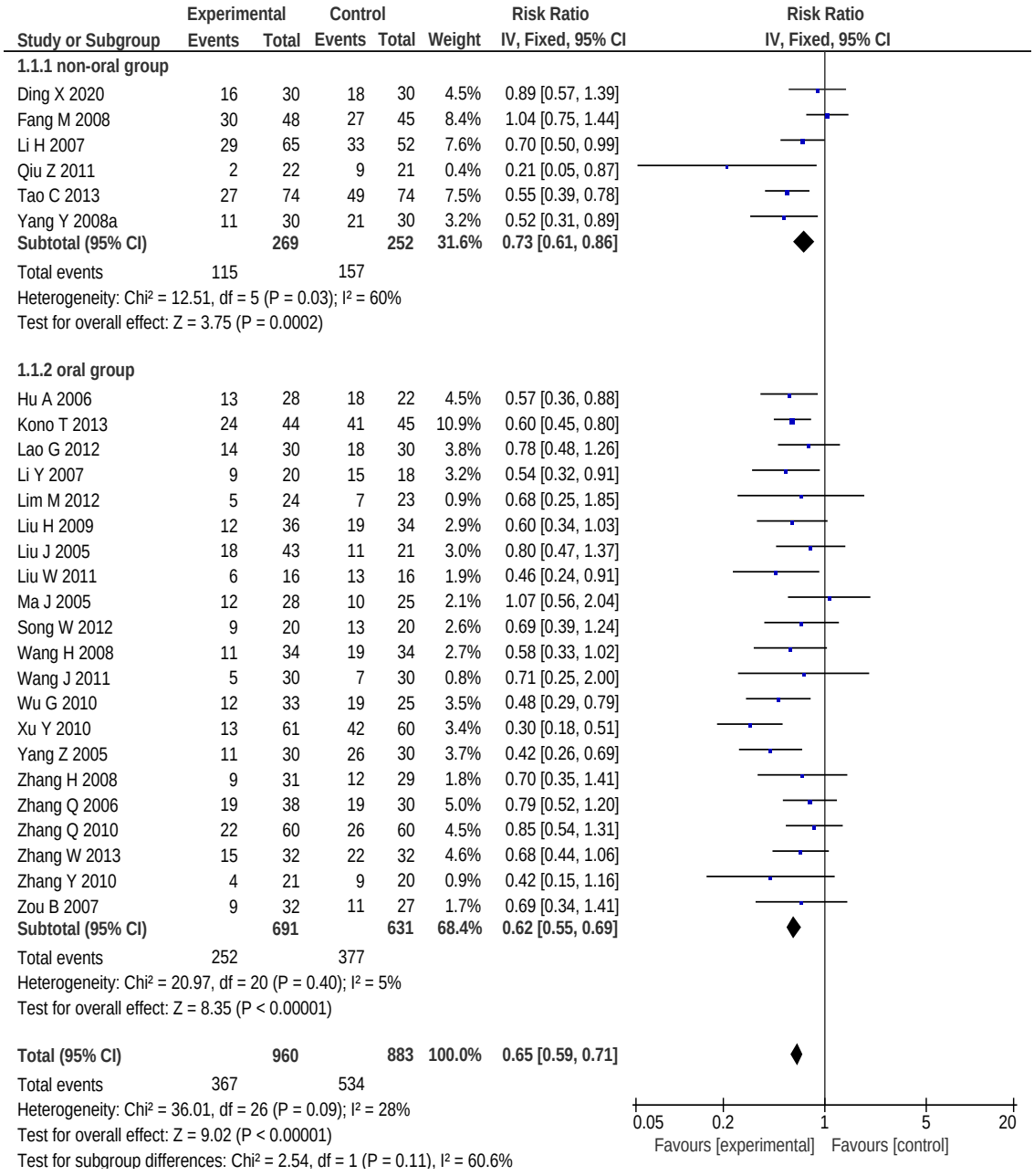


**Figure 6.3: Forest plot of risk ratio for Chinese herbal medicine alleviation of chemotherapy-induced nausea and vomiting (included oral and non-oral sub-groups)**

### *6.3.4.2 Level 2: Pairs of herbal medicines*

Seven pairs of herbs showed RRs that were lower than the total pool (Table 6.2). The lowest RRs were for: *Panax G.* + *Astragalus* (n=4) (RR 0.49 [0.35, 0.67], $I^2$=0%); followed by *Poria* + *Dioscorea* (n=5) (RR 0.56 [0.47, 0.67], $I^2$=0%).

#### *6.3.4.3 Level 3: Combinations of three herbal medicines*

Six different triplets showed significant RRs that were lower than the total pool (Table 6.2). The combination of *Dioscorea* + *Coix* + *Poria* (n=3) had the lowest RR (0.49 [0.37, 0.65], $I^2$=0%); followed by *Panax G* + *Atractylodes* + *Coix* (n=3) (RR 0.52 [0.39, 0.69], $I^2$=0%).

#### *6.3.4.4 Level 4: Combinations of four herbal medicines*

*Atractylodes* + *Poria* + *Coix* + *Glycyrrhiza* was the only combination, among nine combinations, that was significant and lower than or equal to the pool (RR 0.51 [0.38, 0.70], $I^2$=0%, n=3) (Table 6.2).

#### *6.3.4.5 Level 5: Combinations of five herbal medicines*

Three combinations of five herbs showed RRs lower than the total pool. *Astragalus* + *Atractylodes* + *Coix* + *Lycium barbarum* L. (*gou qi zi*) + *Scutellaria barbata* D. Don. (*ban zhi lian*) (n=3) had the lowest RR (0.58 [0.41, 0.83], $I^2$=0%) (Table 6.2).

#### *6.3.4.6 Level 6: Combinations of six herbal medicines*

Six combinations of six HMs showed significant RRs lower than the total pool. The lowest RR (0.50 [0.36, 0.69], $I^2$=0%) was for *Panax G* + *Dioscorea* + *Coix* + *Glycyrrhiza* + *Atractylodes* + *Poria* (n=2).

**Table 6.2: Effects of specific combinations of herbal medicines on chemotherapy induced nausea and vomiting: Levels 1-9**

| Level | HM botanical name (pinyin) | N studies (part.) | RR [95% CI] | $I^2$ |
|---|---|---|---|---|
| 1 | *Hordeum vulgare* L. (*mai ya*) | 2 (98) | 0.47 [0.33, 0.68] | 0 |
| 1 | *Crataegus pinnatifida* Bge (*shan zha*) | 2 (98) | 0.47 [0.33, 0.68] | 0 |
| 1 | *Massa medica fermentata* (*shen qu*) | 2 (120) | 0.47 [0.30, 0.73] | 0 |
| 1 | *Panax ginseng* C. A. Mey. (*ren shen*)* | 4 (222) | 0.51 [0.39, 0.66] | 0 |
| 1 | *Glycyrrhizauralensis*Fisch (*gan cao*)* | 3 (170) | 0.51 [0.38, 0.70] | 0 |
| 1 | *Magnolia officinalis* Rehd. et Wils (*hou po*) | 3 (179) | 0.54 [0.38, 0.77] | 0 |
| 1 | *Amomum kravanh Pierre* ex. Gagnep. (*bai dou kou*) | 2 (117) | 0.54 [0.36, 0.81] | 0 |
| 1 | *Dioscorea opposita* Thunb. (*shan yao*) | 5 (321) | 0.56 [0.47, 0.67] | 0 |
| 1 | *Sophora flavescens* Ait. (*ku shen*) | 2 (92) | 0.56 [0.35, 0.92] | 0 |
| 1 | *Lycium barbarum* L. (*gou qi zi*) | 3 (168) | 0.58 [0.41, 0.83] | 0 |
| 1 | *Cornus officinalis* Sieb. Et Zucc. (*shan zhu yu*) | 2 (139) | 0.59 [0.46, 0.75] | 0 |
| 1 | *Paeonia suffruticosa* Andr. (*mu dan pi*) | 2 (139) | 0.59 [0.46, 0.75] | 0 |
| 1 | *Alisma orientalis* (Sam.) Juzep. (*ze xie*) | 2 (139) | 0.59 [0.46, 0.75] | 0 |
| 1 | *Rehmannia glutinosa* Libosch. (*shu di huang*) | 3 (199) | 0.60 [0.47, 0.75] | 0 |
| 1 | *Nelumbo nucifera* Gaertn. (*lian zi*) | 2 (110) | 0.60 [0.41, 0.88] | 0 |
| 1 | *Poria cocos* (Schw) Wolf (*fu ling*)* | 16 (1012) | 0.61 [0.54, 0.69] | 15 |
| 1 | *Coix lacryma-jobi* L. (*yi ren*)* | 14 (945) | 0.61 [0.53, 0.70] | 29 |

| Level | HM botanical name (pinyin) | N studies (part.) | RR [95% CI] | $I^2$ |
|---|---|---|---|---|
| 1 | *Codonopsis pilosula* (Franch.). Nannf. (*dang shen*) | 12 (747) | 0.61 [0.52, 0.72] | 28 |
| 1 | *Paeonia lactiflora* Pall. (*bai shao*) | 5 (272) | 0.61 [0.48, 0.76] | 0 |
| 1 | *Panax notoginseng* (Burk.) F.H. Chen (*tian qi*) | 4 (245) | 0.61 [0.43, 0.87] | 0 |
| 1 | *Atractylodes macrocephala* Koidz. (*bai zhu*)* | 16 (976) | 0.62 [0.54, 0.71] | 13 |
| 1 | *Eclipta prostrata* L. (*mo han lian*) | 2 (129) | 0.63 [0.41, 0.97] | 0 |
| 1 | *Sophora japonica* L. (*huai hua*) | 3 (150) | 0.63 [0.46, 0.86] | 0 |
| 1 | *Scutellariabarbata* D. Don. (*ban zhi lian*) | 6 (356) | 0.64 [0.50, 0.81] | 0 |
| 1 | *Astragalus membranaceus* (Fisch.) Bge. (*huang qi*)* | 13 (733) | 0.65 [0.55, 0.76] | 0 |
| 1 | *Ligusticum chuanxiong* Hort. (*chuan xiong*) | 2 (114) | 0.65 [0.46, 0.92] | 0 |
| 1 | *Angelica sinensis* (Oliv.) Diels. (*dang gui*) | 2 (118) | 0.68 [0.50, 0.92] | 11 |
| 1 | *Hedyotis diffusa* Willd. (*she she cao*) | 4 (228) | 0.69 [0.51, 0.93] | 0 |
| 1 | *Akebia quinata* (Thunb.) Decne. (*ba yue zha*) | 5 (264) | 0.70 [0.51, 0.95] | 0 |
| 1 | *Curcuma zedoaria* (Berg.) Rosc. or *C. phaeocaulis* Val. (*e zhu*) | 7 (439) | 0.71 [0.57, 0.88] | 0 |
| 1 | *Spatholobus suberectus* Dunn (*ji xue teng*) | 3 (192) | 0.73 [0.55, 0.98] | 0 |
| 2 | *Panax G + Astragalus* | 3 (162) | 0.49 [0.35, 0.67] | 0 |
| 2 | *Poria + Dioscorea* | 5 (321) | 0.56 [0.47, 0.67] | 0 |
| 2 | *Sophora F +Astragalus* | 2 (92) | 0.56 [0.35, 0.92] | 0 |
| 2 | *Glycyrrhiza + Rehmannia* | 2 (110) | 0.59 [0.39, 0.88] | 0 |
| 2 | *Poria + Coix* | 12 (755) | 0.59 [0.50, 0.69] | 31 |
| 2 | *Poria + Rehmannia* | 3 (199) | 0.60 [0.47, 0.75] | 0 |
| 2 | *Atractylodes + Coix* | 12 (767) | 0.60 [0.51, 0.70] | 28 |
| 2 | *Poria + Paeonia* | 5 (272) | 0.61 [0.48, 0.76] | 0 |
| 2 | *Poria + Atractylodes* | 14 (865) | 0.63 [0.54, 0.72] | 22 |
| 2 | *Atractylodes + Astragalus* | 12 (701) | 0.66 [0.56, 0.78] | 0 |
| 2 | *Atractylodes + Akebia quinata* | 5 (264) | 0.70 [0.51, 0.95] | 0 |
| 2 | *Atractylodes + Curcuma* | 7 (439) | 0.71 [0.57, 0.88] | 0 |
| 2 | *Astracgalus + Curcuma* | 6 (375) | 0.72 [0.56, 0.93] | 0 |
| 3 | *Dioscorea + Coix +Poria* | 3 (168) | 0.49 [0.37, 0.65] | 0 |
| 3 | *Panax G + Atractylodes + Coix* | 3 (180) | 0.52 [0.39, 0.69] | 0 |
| 3 | *Amomum + Poria + Coix* | 2 (117) | 0.54 [0.36, 0.81] | 0 |
| 3 | *Dioscorea + Poria + Atractylodes* | 3 (174) | 0.56 [0.43, 0.73] | 2 |
| 3 | *Coix* + Paeonia + Poria | 3 (168) | 0.56 [0.41, 0.75] | 0 |
| 3 | *Dioscorea + Paeonia + Poria* | 3 (172) | 0.58 [0.44, 0.75] | 0 |
| 3 | *Poria + Codonopsis + Artactylodes* | 12 (751) | 0.63 [0.53, 0.74] | 32 |
| 3 | *Poria + Atractylodes + Ligusticum* | 2 (114) | 0.65 [0.46, 0.92] | 0 |
| 3 | *Atractylodes + Paeonia + Poria* | 4 (213) | 0.65 [0.50, 0.83] | 0 |
| 3 | *Coix + Astragalus + Atractylodes* | 10 (596) | 0.66 [0.55, 0.79] | 0 |
| 3 | *Poria + Astragalus + Atractylodes* | 10 (590) | 0.68 [0.57, 0.81] | 0 |
| 3 | *Poria + Atractylodes + Spatholobus* | 3 (192) | 0.73 [0.55, 0.98] | 0 |
| 4 | *Glycyrrhiza + Atractylodes + Poria + Coix* | 3 (170) | 0.51 [0.38, 0.70] | 0 |
| 4 | *Coix + Astragalus + Atractylodes + Eclipta prostrata* | 2 (129) | 0.63 [0.41, 0.97] | 0 |
| 4 | *Sophora J + Atractylodes + Paeonia + Poria* | 3 (150) | 0.63 [0.46, 0.86] | 0 |
| 4 | *Coix + Scutellaria + Atractylodes + Astragalus* | 6 (356) | 0.64 [0.50, 0.81] | 0 |
| 4 | *Codonopsis + Astragalus + Atractylodes + Poria* | 9 (522) | 0.66 [0.54, 0.80] | 0 |

| Level | HM botanical name (pinyin) | N studies (part.) | RR [95% CI] | $I^2$ |
|---|---|---|---|---|
| 4 | *Poria* + *Angelica sinensis* + *Atractylodes* + *Coix* | 2 (118) | 0.68 [0.50, 0.92] | 11 |
| 4 | *Poria* + *Atractylodes* + *Codonopsis* + *Hedyotis* | 4 (228) | 0.69 [0.51, 0.93] | 0 |
| 4 | *Codonopsis* + *Atractylodes* + *Poria* + *Paeonia* | 3 (164) | 0.69 [0.50, 0.94] | 0 |
| 4 | *Atractylodes* + *Astragalus* + *Coix* + *Curcuma* | 5 (3110 | 0.70 [0.52, 0.93] | 0 |
| 4 | *Codonopsis* + *Atractylodes* + *Poria* + *Curcuma* | 6 (369) | 0.73 [0.58, 0.93] | 0 |
| 5 | *Scutellaria* +*Lycium* + *Astragalus* + *Atractylodes* + *Coix* | 3 (168) | 0.58 [0.41, 0.83] | 0 |
| 5 | *Rehmannia* + *Atractylodes* + *Glycyrrhiza* + *Poria* + *Coix* | 2 (110) | 0.59 [0.39, 0.88] | 0 |
| 5 | *Scutellaria* + *Curcuma* + *Atractylodes* + *Astragalus* + *Coix* | 4 (158) | 0.62 [0.45, 0.86] | 0 |
| 6 | *Panax G* + *glycyrrhiza* + *Atractyludes* + *Coix* + *Poria* +*Dioscorea* | 2 (110) | 0.50 [0.36, 0.69] | 0 |
| 6 | *Magnolia* + *Atractylodes* + *Poria* + *Coix* + *Astragalus* + *Codonopsis* | 3 (179) | 0.54 [0.38, 0.77] | 0 |
| 6 | *Lycium* + *Astragalus* + *Atractylodes* + *Coix* + *Scutellaria* + *Poria* | 2 (98) | 0.57 [0.36, 0.91] | 0 |
| 6 | *Dioscorea* + *Cornus* + *Poria* + *Rehmannia* + *Paeonia S* + *Alisma* | 2 (139) | 0.59 [0.46, 0.75] | 0 |
| 6 | *Coix* + *Panax N* + *Curcuma* + *Astragalus* + *Atractylodes* + *Scutellaria* | 3 (198) | 0.60 [0.42, 0.87] | 0 |
| 6 | *Sophora J* + *Atractylodes* + *Paeonia L* + *Poria* + *Coix* + *Nelumbo* | 2 (110) | 0.60 [0.41, 0.88] | 0 |
| 6 | *Poria* + *Scutellaria* + *Astragalus* + *Atractylodes* + *Coix* + Codonopsis | 5 (286) | 0.65 [0.50, 0.85] | 0 |
| 7 | *Hordeum* + *Astragalus* + *Atractylodes* + *Codonopsis* + Coix + *Poria* + *Crataegus* | 2 (98) | 0.47 [0.33, 0.68] | 0 |
| 7 | *Glycyrrhiza* + *Atractylodes* + *Poria* + Coix + *Astragalus* + *Codonopsis* + *Massa medica* | 2 (120) | 0.47 [0.30, 0.73] | 0 |
| 7 | *Coix* + *Hedyotis* + *Scutellaria* + *Astragalus* + *Atractylodes* + *Poria* + *Codonopsis* | 3 (188) | 0.69 [0.49, 0.98] | 0 |
| 9 | *Codonopsis* + *Panax N* + *Astragalus* + *Atractylodes* + *Poria* + *Coix* + *Hedyotis* + *Scutellaria* + *Curcuma* | 2 (128) | 0.61 [0.37, 1.00] | 0 |

*Included in the final six CHMs; RR: Risk Ratio for CINV; 95% CI: 95% Confidence Interval; N. studies (part.): number of studies (participants); $I^2$ %: measure of heterogeneity of result

#### *6.3.4.7 Level 7: Combinations of seven herbal medicines and more*

Two combinations of seven HMs were lower than the total pool: *Codonopsis* + *Atractylodes* + *Astragalus* + *Coix* + *Poria* + *Crataegus pinnatifida* Bge (*shan zha*) + *Hordeum vulgare* L. (*mai ya*) (n=2) (RR 0.47 [0.33, 0.68], $I^2$=0%), *Glycyrrhiza* + *Atractylodes* + *Poria* + *Coix* + *Astragalus* + *Codonopsis* + *Massa medica* (n=2) (RR 0.47 [0.30, 0.73], $I^2$=0%). In only one combination of nine HMs was the RR higher than the total pool (Table 6.2).

### 6.3.5 **Herbal medicines with consistent results at multiple levels**

Six herbs showed significantly reduced RRs lower than or equal to the pool, with low heterogeneity at multiple levels. *Atractylodes*, *Poria* and *Coix* appeared at all seven levels when used as components of various HMs interventions; *Glycyrrhiza* appeared at five levels; while *Astragalus* and *Panax G* appeared at four levels.

### 6.3.6 **Potential synergistic effects of herbal medicines**

Combinations of HMs that showed RRs that were lower than those of the HMs singly included two pairs: *Panax G* + *Astragalus*; and *Coix* + *Atractylodes*; two triplets *Poria* + *Coix* + *Dioscorea*, *Poria* + *Coix* + *Paeonia lactiflora* Pall (*bai shao*); and one group of six HMs *Panax G* + *Coix* + *Atractylodes* + *Poria* + *Dioscorea* + *Glycyrrhiza* (Table 6.2).

### 6.3.7 **Discussion of the results for chemotherapy-induced nausea and vomiting**

The meta-analyses showed reductions in CINV in both the injection and oral groups but there was substantial heterogeneity in the injection group ($I^2$=60%) compared to the oral group ($I^2$=5%). In the oral intervention studies the absolute risk reduction was 25%, which was higher than for the injection group (20%). In a previous meta-analysis of tumour response rate, the injection groups appeared more effective than the oral groups (Figure 6.1). One likely reason for these differences is the injection products were mainly aimed at aiding tumour response rather than reducing CINV. Nevertheless, the result for the two studies of *Kang'ai* injection, which is composed of *Panax ginseng*, *Astragalus* and *Sophora flavescens* Ait., showed a significant reduction in CINV incidence, without important heterogeneity ($I^2$=28%).

It has been suggested that combining certain HMs with anti-emetic drugs results in greater benefit (Dong, 2012). In the total group, there was a slightly reduced benefit in the 17 studies that used anti-emetic drugs compared to the ten that did not. However, it is possible that some studies did not report the use of anti-emesis medications since these are in routine use. Therefore this result is difficult to interpret. This issue warrants further investigation.

The following six herbs appeared at multiple levels of combination in the oral interventions: *Atractylodes* (n=16), *Poria* (n=16), *Coix* (n=14), *Astragalus* (n=13*), Glycyrrhiza* (n=5), and *Panax G* (n=4). This list contains the herbs with the highest overall frequencies, such as *Atractylodes* and *Poria*, and also some lower frequency plants such as *Glycyrrhiza* and *Panax G*. Conversely, some relatively frequent herbs such as *Curcuma* (n=7) did not show a reduced RR for CINV. Therefore, the selection process did not simply reflect overall frequency of the herb within the data set.

Ginger was not included in the final analyses, although it appeared to significantly reduce CINV (RR 0.43 [0.31, 0.61]), since it was used in only two studies and the heterogeneity was substantial ($I^2$=69%), leading to its exclusion from Table 6.2.

The effects of extracts and compounds derived from the six herbs identified as potentially reducing CINV have received research attention in experimental models in animals and cell-lines to assess their effects on emesis, pica, gastrointestinal motility and gastro-protection. The volume of published

research is variable, with *Ginseng*, *Atractylodes*, and *Poria* having received the most attention. This research is briefly reviewed for each of the six herbs below.

#### *6.3.7.1 Actions of Panax ginseng relevant to nausea/vomiting*

The anti-emetic effect of Korean red *ginseng* total extract (KRGE) on nausea and vomiting was investigated in ferrets administered intraperitoneal cisplatin (7.5mg/kg) which induced both nausea and vomiting with one-hour latency. The animals were monitored every 30 mins and the total number of episodes of nausea and vomiting were marked. Pre-treatment with orally administered KRGE, one hour and two hours before cisplatin, significantly attenuated the cisplatin-induced nausea and vomiting in a dose-dependent manner. No significant effect was evident when KRGE was administered 4 hours prior to cisplatin (Kim et al., 2005).

In rodents, emetics do not produce vomiting but instead induce pica (the eating of kaolin). In rats, the effects of an extract of Korean *ginseng* (KG) administered before and after cisplatin, on pica, food intake, body weight, haematological parameters and histopathology was investigated by Raghavendran *et al* (2011). Pre-treatment with KG one hour before cisplatin significantly reduced kaolin intake at 24, 48, and 72 hours post-cisplatin. Normal food intake significantly improved compared to the group that received cisplatin alone and there was less reduction in body weight. Post-treatment KG showed similar effects. The increases in the levels of white blood cells, neutrophils, lymphocytes induced by cisplatin were significantly lower in the rats pre-treated with KG, suggesting that KG reduced cisplatin-induced inflammation. Cisplatin-induced damage to the gastric mucosa and small intestine was reduced by pre-treatment, but not by post-treatment with KG (Raghavendran et al., 2011). A similar result was obtained using American *ginseng* berry extract (AGBE) and ginsenoside Re, which is one of its constituents. Pre-treatment reduced cisplatin-induced pica and improved food intake. When tested for antioxidant actions, both AGBE and ginsenoside Re were found to scavenge superoxide and hydroxyl radicals (Mehendale et al., 2005).

Pre-treatment with ginsenoside Rg2 has been reported to have an inhibitory effect on human 5-HT3A receptors expressed in *Xenopus* oocytes that was dose dependent and reversible (Choi et al., 2003). Using the same model, similar effects have been reported for two ginsenoside metabolites: compound K (CK) or M4, which is derived from protopanaxadiol (PD) ginsenosides, and protopanaxatriol (PT) ginsenosides, respectively (Lee et al., 2004). These studies suggest that the reported effects of *ginseng* on nausea and vomiting may be via antagonism of the 5-HT3A receptor.

#### *6.3.7.2 Actions of Poria cocos relevant to nausea/vomiting*

Tai et al. (1995) investigated the effects of a range of triterpenes extracted from *Poria* in frogs orally administered copper sulphate as an emetic. The latency to first emesis was significantly prolonged

compared to controls by some, but not all, of the triterpenes. Those showing a significant anti-emetic effect had an exo-methylene group at C24 in their side chain.

The effects of three *Poria*-derived triterpenoids [PA: Pachymic acid; DA: dehydroeburicoic acid; and HA: 3β-hydroxylanosta-7,9 (11), 24-trien-21-oic acid] on human 5-HT3A receptors was investigated in *Xenopus* oocytes using a two electrode voltage-clamp technique. Each triterpenoid showed concentration dependent, reversible, inhibition on 5HT-induced inward current, with HA showing the highest potency (Lee et al., 2009).

#### *6.3.7.3 Actions of Atractylodes macrocephala relevant to nausea/vomiting*

The effects of an extract of *Atractylodes* on restitution of the intestinal mucosa after damage was investigated in a cell migration model using intestinal epithelial (IEC-6) cells treated with: *Atractylodes* extract; spermidine (SPD, 5 μmol/L) as the positive control; the polyamine inhibitor alpha-difluoromethylornithine (DFMO, 2.5mmol/L) as the negative control; and a no treatment control. At doses of 100mg/L and 200mg/L, *Atractylodes* significantly increased IEC-6 cell migration after wounding compared to no treatment, and the effect was comparable to that of SPD. The effect of *Atractylodes* was retained when combined with DFMO. *Atractylodes* exposure increased cellular polyamine content and other markers indicating a polyamine dependent mechanism (Song et al., 2015). In human gastric mucosa epithelium, *Atractylodes* extract promoted the growth of human gastric mucosa cells, DNA synthesis and pepsin secretion, but had no effect on acid secretion (Zhu et al., 2003).

*Atractylodes* has been reported to enhance gastric emptying and small intestinal motility in mice fed *Atractylodes* extract plus the marker Blue dextran 2000, compared to a saline control (Li et al., 1996). This prokinetic effect could be blocked by atropine in a study of isolated mouse ileum, which indicated the effect may be mediated via muscarinic receptors (Ma et al., 1996). In guinea pig colon sections, *Atractylodes* extract was reported to increase smooth muscle contraction (Ding et al., 2005).

#### *6.3.7.4 Actions of Astragalus membranaceus relevant to nausea/vomiting*

A number of studies have investigated the effect of *Astragalus* on gastrointestinal motility. In healthy dogs, the investigators measured the myoelectric activity in the duodenum and jejunum after a 25% concentrated solution (1 mL/kg) of *Astragalus* extract was injected into the dog's empty stomach. The duration of each myoelectric cycle, each phase of the cycle and the electrical potential were measured. The results showed *Astragalus* could significantly extend the duration of myoeletric cycles in the duodenum and jejunum but the motility enhancing effect was most pronounced in the jejunum (Yang et al., 1993). In normal mice, *Astragalus* significantly enhanced small intestine motility and antagonized the inhibitive effects of atropine and the non-selective beta-adrenergic agonist

isoproterenol. In the stomach, *Astragalus* also antagonized inhibition of gastric emptying induced by atropine (3mg/kg), but did not antagonize the dopamine-serotonin receptor antagonist metoclopramide (0.8mg/kg) (Zheng et al., 2003).

In healthy humans, small intestine transmission time was measured by using a hydrogen breath test to determine the peak value of lactose absorption after taking 18 g lactose orally. After taking *Astragalus* for one week, the time to the peak value of lactose absorption was significantly shortened, compared to before administration of *Astragalus*, suggesting increased motility (Qiaoet al., 2001).

#### *6.3.7.5 Actions of Glycyrrhiza (liquorice) relevant to nausea/vomiting*

The effects of aqueous extracts of several HMs, including *Glycyrrhiza*, *Astragalus* and *Atractylodes*, were tested in isolated smooth muscle strips taken from different gastric regions of the rat. *Glycyrrhiza*, *Astragalus*, and *Atractylodes* increased longitudinal and circular fundic muscle tension; *Glycyrrhiza* and *Atractylodes* enhanced longitudinal muscle tension in strips from the gastric body; while *Glycyrrhiza* increased the motility index of pyloric circular muscle (Zheng et al., 1998).

A study that investigated the effects of isoliquiritigenin (a flavonoid in *Glycyrrhiza* spp.) on gastrointestinal motility in mice fed a charcoal meal, found an inhibitory effect at low doses (0.003, 0.03 mg/kg) and a prokinetic effect at high doses (3 and 30 mg/kg). Subsequent *in vitro* studies indicated that the spasmogenic effect involved activation of muscarinic receptors, while the spasmolytic effect was associated with blockade of calcium channels (Chen et al., 2009).

Sato et al. (2006) investigated the effect of glycycoumarin, a compound from Glycyrrhiza, on carbamylcholine (CCh)-induced contraction of mouse jejunum and reported an antispasmodic effect related to the inhibition of the phosphodiesterase 3 pathway.

#### *6.3.7.6 Actions of Coix relevant to nausea/vomiting*

The effect of de-hulled *Coix* seed was examined in an indomethacin-induced gastric lesion model in rats. Erosion of the gastric mucosa was examined by imaging and by histopathological observation. *Coix* extract was found to produce dose-dependent gastroprotection against indomethacin. This effect was at least partially due to antioxidant actions of the phenolic acids in *Coix* (Chung et al., 2011). A methanol extract of *Coix* seeds was found to reduce nitric oxide and superoxide production in RAW 264.7 macrophages (Seo et al., 2000).

### 6.3.8 **Safety of the herbal medicine interventions**

The included studies did not report any serious adverse events associated with the CHMs and the meta-analyses results did not show increased CINV in any of the studies. When combined with anti-

emetic drugs the CHMs did not appear to reduce their effectiveness rather, the results were suggestive of enhanced effect (tRR: RR 1.29 [1.14, 1.46], $I^2$ 0%).

### 6.3.9 **Summary of the results for chemotherapy-induced nausea and vomiting**

In nausea and vomiting associated with oxaliplatin-based chemotherapy for CRC, the addition of CHMs appeared to significantly reduce incidence based on a meta-analysis of 27 studies. This effect was most pronounced in the group of 21 studies that administered the CHMs orally. There was low statistical heterogeneity in this group, the oxaliplatin regimens and CINV measurements were consistent across studies, and there was considerable similarity in the HMs used. Further sensitivity analysis based on individual HM ingredients, identified six herbs (*Atractylodes*, *Poria*, *Coix*, *Astragalus*, *Glycyrrhiza*, and *Panax G*) that were associated with significant reductions in CINV without important heterogeneity in the sensitivity analysis results. Experimental studies of these six herbs have reported inhibitory effects on nausea and vomiting (or its animal equivalent), regulation of gastrointestinal motility, gastro-protective effects, and/or antioxidant actions which may at least partially explain the effects identified in the meta-analyses of the clinical trial results. These herbs warrant further experimental and/or clinical research into their efficacy as anti-emetics, the underlying anti-emetic mechanism, and the safety of concurrent administration with chemotherapeutic drugs to explore their possible addition to chemotherapy regimens in patients whose CINV is not sufficiently well-controlled by conventional therapies.

## 6.4 Meta-analysis of oxaliplatin-based chemotherapy combined with herbal medicine for chemotherapy induced neutropenia

### 6.4.1 **Background and rationale**

Chemotherapy induced neutropenia (CIN) is a decline in absolute neutrophil count (ANC) as a result of myelosuppression induced by systematic chemotherapy (Dale, 2002). Neutropenia can lead to life-threatening infections so it is common practice to decrease dose intensity or cut short treatment cycles when serious neutropenia is evident, but this will directly affect the effectiveness of the chemotherapy (Crawford et al., 2004). CIN severity is classified into four grades based on decline in ANC (Koini et al., 2015). Febrile neutropenia (FN) is a serious condition that combines elevated body temperature, sepsis and severe neutropenia (Koini et al., 2015, Aapro et al., 2010). Risk factors for CIN include age, performance status, nutritional status, chemotherapy dose intensity, and low baseline blood cell counts (Lyman et al., 2005).

CIN is one of the most common adverse reactions in CRC treatment using oxaliplatin regimens (Hind et al., 2008). In CRC clinical trials the incidence of grade 3/4 CIN was found to vary from 37% to 56% in different populations (Sugihara et al., 2012), and CIN incidence has been found to be higher in

regimens that combine oxaliplatin with 5-FU compared to single 5-FU regimens (Becouarn et al., 1998; de Gramont et al., 2000). This may be associated with oxaliplatin reducing 5-FU plasma clearance by inhibiting 5-FU catabolism (Boisdron et al., 2002).

Granulocyte colony-stimulating factor (G-CSF) initiates the proliferation of granulocyte precursor cells and their differentiation into mature granulocytes in bone marrow. Clinical trials have found that recombinant G-CSF significantly reduced the incidence of grade 3/4 CIN and accelerated cancer patients' recovery from CIN after chemotherapy, thereby making higher-intensity treatment regimens possible (Saarinen et al.,1992; Lydaki et al., 1995; Gomez et al., 2006; Hecht et al., 2010). In the past, G-CSF treatment was expensive (Hendler et al., 2011) but the advent of biosimilars has made G-CSF support for CIN/FN prophylaxis more cost-effective (Gascón et al., 2011).

In advanced CRC (Chapter 5), the meta-analysis of RCT results showed FOLFOX4 combined with CHMs reduced grade 3/4 CIN by 8.7% compared to FOLFOX4 alone (Chen et al., 2014). In this meta-analysis, the aims were: 1. assessment of the clinical trial literature on the effects of HMs on CIN associated with the use of oxaliplatin regimens for CRC; 2. identification of which HMs, or combinations of HMs, were associated with CIN alleviation when used in combination with oxaliplatin regimens in the clinical trials; and 3. identification of directions for further clinical and experimental research.

Meta-analysis of RCTs of CHMs combined with oxaliplatin regimens for CRC which reported data on CIN incidence was conducted. Then sensitivity analyses were used for sub-groups of studies that used the same or similar HM interventions to assess the potential contributions of individual plant-based HMs to the meta-analysis results. For the HMs that appeared most likely to be contributing to reduced CIN incidence, the experimental literature was reviewed to identify possible mechanisms of action.

Sub-group analyses were planned for: route of HM administration – intravenous (IV) or oral; and studies employing G-CSF. Data analysis for CIN was stratified by: FN, CIN grade 4, CIN grade 3, CIN grade 3/4, CIN grade 1/2, and CIN all grades. To determine if the addition of the HMs interfered with the effectiveness of the chemotherapy, meta-analysis of tumour response rate (tRR) was conducted for the studies in the groups for CIN grade 3/4 and all grades.

### 6.4.2 **Included studies and characteristics**

Thirty-two studies (2,224 participants) that combined an oxaliplatin regimen with a CHM versus the same oxaliplatin regimen and reported outcome data for CIN, were included. Of these, two studies (Deng & Shen, 2010; Fang & Li, 2008) used G-CSF, and two studies (Xu & Wang, 2010; Zeng et al., 2013) reported neutrophil count as mean difference, so these studies were analysed separately.

All studies were published from 2005 to 2013. Thirty-one studies were conducted in China and one in Japan (Kono et al., 2013). The studies reported all participants were diagnosed as CRC based on pathology tests, and had adequate liver and kidney function and peripheral blood count or no contraindication to chemotherapy pre-study. Twenty-six studies specified life expectancy of three months or longer and 17 studies specified no use of chemotherapy for at least one month prior to study commencement (Table G3, in Appendex G).

Oxaliplatin regimens included FOLFOX in 30 studies and XELOX in two studies (Deng & Shen, 2010; Wang, 2013). The CHMs and chemotherapy were used concurrently. Orally administered CHMs were used in 24 studies (1,319 particpants) and 8 studies employed commercially available CHM injections (Table G3, in Appendex G).

All studies used the WHO system or National Cancer Institute Common Toxicity Criteria (NCI-CTC) for grading acute and sub-acute toxicity which divides CIN into 4 grades (Miller et al., 1981; National Cancer Institute, 1999). Data were reported as dichotomous in 30 studies. Data reported as neutrophil counts (2 studies) were analysed separately using MD. When RR was less than +1 or MD was less than zero it favoured the test group. Only one study reported FN data (see oral administration group).

### 6.4.3 **Meta-analysis of change in chemotherapy induced neutropenia**

The overall results showed significantly reduced CIN in the test groups compared to the control groups for grade 4, grade 3, grade 3/4, grade 1/2 and all grades, with no statistical heterogeneity ($I^2$ =0%) (Figure 6.4; Table 6.3). Results were also reported for the following sub-groups:

1. Injection group without G-CSF (7 studies);
2. Oral administration group without G-CSF (21 studies);
3. Studies that used G-CSF (2 studies); and
4. Neutrophil count group (2 studies).

#### *6.4.3.1 Injection group (no G-CSF)*

Seven studies tested five different injection products, three of which were tested in two studies each (Table 6.5). There was no difference between groups in the single study that reported grade 4 CIN or in the four studies that reported grade 3. There were significant differences in grade 3/4, grade 1/2, and all grades, with no heterogeneity. Incidence rates for grade 3/4 CIN were 4.5% in the test groups versus 11.1% in the controls (Figure 6.4; Table 6.3).

#### *6.4.3.2 Oral administration group (no granulocyte colony-stimulating factor)*

In 21 studies, CHMs were administered orally as decoctions, capsules or tablets. In the four studies that reported grade 4 CIN, there was no significant difference between groups. Significant differences

were found for grade 3, grade 3/4, grade 1/2, and all grades with no heterogeneity. Incidence rates for grade 3/4 were 4.6% in the test groups versus 11.8% in the controls (Figure 6.4; Table 6.3). The single study that reported FN (n=89) (Kono et al., 2013) found no significant difference between groups.

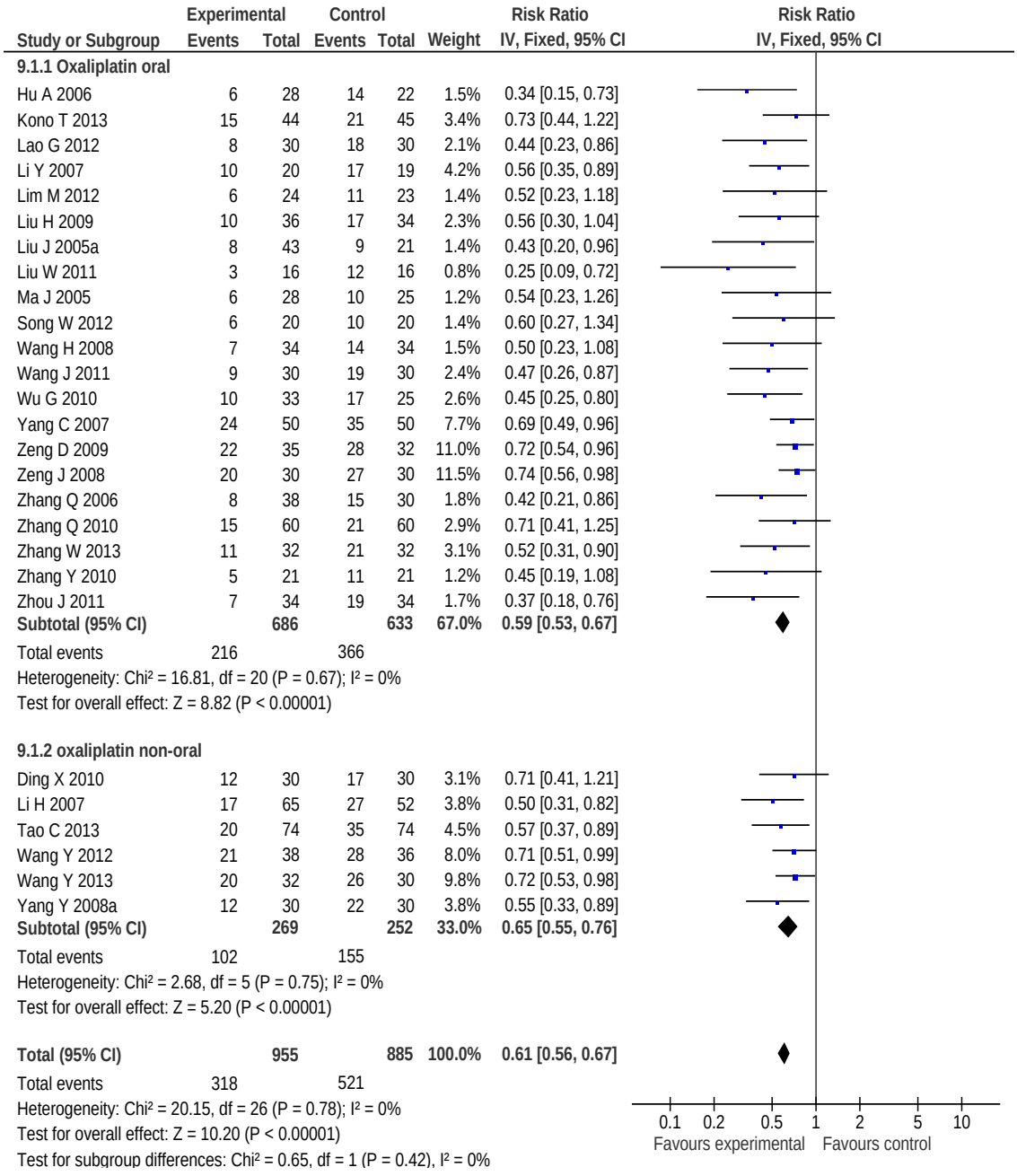


**Figure 6.4: Forest plot of risk ratio for Chinese herbal medicine alleviation of chemotherapy induced neutropenia (included oral and non-oral sub-groups)**

### *6.4.3.3 Granulocyte colony-stimulating factor group*

One study used an injection (Fang & Li, 2008) and one used oral CHM (Deng & Shen, 2010). For the pooled data there was no significant difference between groups in grade 3/4 and all grades neutropenia. No data were available on FN (Table 6.3).

**Table 6.3: Meta-analysis results for chemotherapy induced neutropenia (febrile neutropenia, grades 4, 3, 3/4, 1/2, all grades), neutrophil count**

| Group | No. studies (participant No. T/C) | EoT (between groups) RE | $I^2$ | Incidence T/C (%) |
|---|---|---|---|---|
| **1. Injection group (no G-CSF): 7 studies** | | | | |
| Gr 4 | 1 (65/52) | 0.27 [0.01, 6.44], p = 0.42 | NA | T: 0% (0/65); C: 1.9% (1/52) |
| Gr 3 | 4 (199/186) | 0.62 [0.28, 1.40], p = 0.25 | 0 | T: 4.5% (9/199); C: 7.5% (14/186) |
| Gr 3/4 | 5 (221/207) | 0.47 [0.22, 0.98], p = 0.04* | 0 | T: 4.5% (10/221); C: 11.1% (23/207) |
| Gr 1/2 | 4 (199/186) | 0.58 [0.44, 0.77], p = 0.0001* | 0 | T: 26.1% (52/199); C: 46.2% (86/186) |
| All grades | 6 (269/252) | 0.65 [0.55, 0.76], p < 0.00001* | 0 | T: 37.9% (102/269); C: 61.5% (155/252) |
| **2. Oral administration group (no G-CSF): 21 studies** | | | | |
| FN | 1 (44/45) | 0.20 [0.01, 4.14], p = 0.30 | NA | T: 0% (0/44); C: 4.4% (2/45) |
| Gr 4 | 4 (116/116) | 0.25 [0.05, 1.18], p = 0.08 | 0 | T: 0% (0/116); C: 5.2% (6/116) |
| Gr 3 | 15 (509/462) | 0.37 [0.21, 0.65], p = 0.0005* | 0 | T: 3.1% (16/509); C: 9.5% (44/462) |
| Gr 3/4 | 17 (583/537) | 0.44 [0.29, 0.66], p = 0.0001* | 0 | T: 4.6% (27/583); C: 12.5% (67/537) |
| Gr1/2 | 19 (635/585) | 0.62 [0.54, 0.72], p < 0.00001* | 0 | T: 25.8% (164/635); C: 44.1% (258/585) |
| All grades | 21 (686/632) | 0.59 [0.53, 0.67], p < 0.00001* | 0 | T: 31.5% (216/686); C: 57.8% (366/633) |
| **Total: groups 1 and 2 (no G-CSF): 28 studies** | | | | |
| Gr 4 | 5 (181/168) | 0.26 [0.06, 1.03], p = 0.05 | 0 | T: 0% (0/181); C: 4.2% (7/168) |
| Gr 3 | 19 (708/647) | 0.42 [0.27, 0.67], p = 0.0002* | 0 | T: 3.5% (25/708); C: 9.6% (62/647) |
| Gr 3/4 | 22 (804/743) | 0.45 [0.31, 0.65], p < 0.0001* | 0 | T: 4.6% (37/804); C: 11.8% (88/743) |
| Gr 1/2 | 23 (834/771) | 0.61 [0.54, 0.70], p < 0.00001* | 0 | T: 25.9% (216/834); C: 43.3% (334/771) |
| All grades | 27 (955/885) | 0.61 [0.56, 0.67], p < 0.00001* | 0 | T: 33.3% (318/955); C: 58.9% (521/885) |
| **3. G-CSF group: 2 studies** | | | | |
| Grade 3/4 | 2 (66/63) | 1.00 [0.15, 6.63], p =1.00 | 0 | T: 3.0% (2/66); C: 3.2% (2/63) |
| All grades | 2 (66/63) | 0.91 [0.48, 1.74], p =0.78 | 27 | T: 36.3% (24/66); C: 38.1% (24/63) |
| **4. Neutrophil count: 2 studies** | | | | |
| Total neutrophils (MD) | 2 (122/90) | MD: 1.62 [0.93, 2.32], p < 0.00001* | 66 | NA |

T: treatment group; C control group; EoT: end of treatment; FN: febrile neutropenia; RE: random effect; * significant at p< 0.05

#### *6.4.3.4 Neutrophil count*

In the two studies that presented data as mean neutrophil count (Xu & Wang, 2010; Zeng et al., 2013) there was a significant difference in favour of the test groups (MD 1.62 [0.93, 2.32] $I^2$=66%) (Table 6.3).

### 6.4.4 **Effect of number of cycles of chemotherapy on chemotherapy induced neutropenia incidence**

Grade 3/4 CIN incidence was stratified by number of cycles of chemotherapy (2, 3, 4, 6, 8+) for the oxaliplatin control groups and the test groups (Table 6.4). In the control groups, the mean grade 3/4 CIN incidence was higher at 2 cycles (12.30%) than at 3 cycles (6.92%), but then the incidence increased with increasing number cycles to 19.00% for 8 or more cycles (Figure 6.5). There were significant reductions in grade 3/4 CIN incidence in the test groups for two cycles (RR 0.37 [0.17, 0.80]) and four cycles (RR 0.26 [0.10, 0.66]), with a marginal difference at 6 cycles (RR 0.36 [0.13, 1.02], p=0.05). There were no significant differences at 3 and 8 cycles or more. It was not feasible to calculate accumulated oxaliplatin and 5-FU dose due to variation in data reporting

**Table 6.4: Grades 3/4 chemotherapy induced neutropenia incidence by number of cycles of oxaliplatin-based chemotherapy**

| No. Cycles of chemotherapy | No studies (participants) | Control gps. (Mean% ±SE) | Test gps. (Mean% ±SE) | RR [95%CI] $I^2$ |
|---|---|---|---|---|
| 2 | 7 (380) | 12.30 ± 1.88 | 4.14 ± 1.00 | 0.37 [0.17, 0.80] $I^2$=0* |
| 3 | 4 (350) | 6.92 ± 0.82 | 4.19 ± 1.06 | 0.66 [0.27, 1.64] $I^2$=0 |
| 4 | 5 (391) | 11.28 ± 6.09 | 2.55 ± 0.75 | 0.26 [0.10, 0.66] $I^2$=0* |
| 6 | 3 (219) | 12.75 ± 3.82 | 5.13 ± 0.58 | 0.36 [0.13, 1.02] $I^2$=0, p=0.05 |
| 8+ | 3 (207) | 19.00 ± 8.91 | 10.28 ± 7.12 | 0.61 [0.32, 1.15] $I^2$=0 |

*significant in favour of test groups

Meta-analysis of tumour response rate (tRR)

To test whether the reduction of CIN was associated with reduction of the cytotoxicity of the chemotherapeutic agents, we pooled results of the studies that also reported tRR. Data were available for 6 studies in the injection group, seventeen studies in the oral group, one in the G-CSF group and two in the neutrophil count group. The results showed there were no significant reductions in tRR in the pooled data (Table 6.5)

### 6.4.5 **Sensitivity analyses for selection of herbs for chemotherapy induced neutropenia**

The multi-ingredient CHMs differed in name but their main ingredients were similar. The effects on reduction of CIN of the plant-based HM ingredients used in multiple studies are reported below at the level of the single HM, pair of HMs, and groups of 3 up to 10 HMs. It was not feasible to use this procedure for studies of injection products since these were likely to vary according to manufacturer and administration.

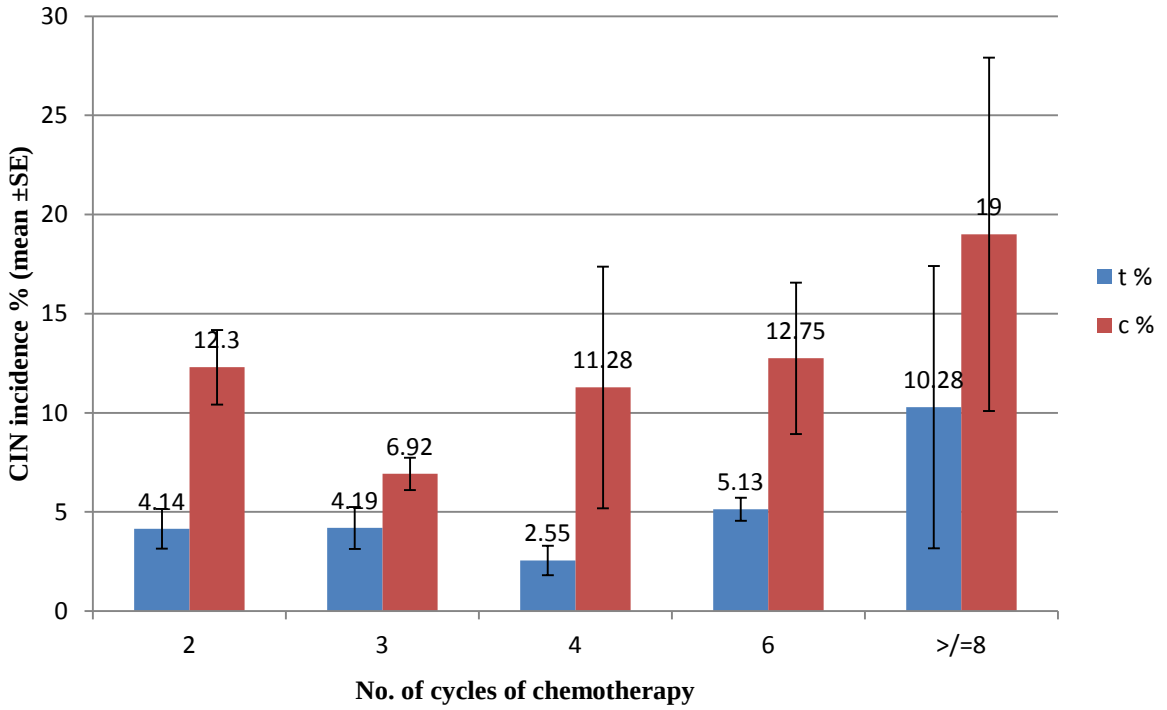


**Figure 6.5: Grade 3/4 chemotherapy induced neutropenia incidence by number of cycles of oxaliplatin based chemotherapy**
t: test groups; c: control groups

All plant-based ingredients (n=85) in the oral HMs were recorded in a spread-sheet. The number of ingredients per intervention averaged 10 and ranged from 1 to 23. Thirty-six HMs were used in two or more interventions. The RR (CIN all grades) of the group of studies that included each particular HM was calculated and the RRs were ranked from low to high. The significant RRs were identified (n=36), and groups with moderate or higher heterogeneity ($I^2$> 30%) were excluded (n= 6) leaving 30 different HMs in the following analyses. Since the aim was to select HMs for further research, only significant RR results that were equal or lower than the total pooled RR of oral interventions (0.59 [0.53, 0.67]) are reported in the text. See Table 6.6 for all significant RR results. For each of the 30 HMs, the full botanical name is given in Table 6.6 together with the Chinese name in pinyin. Therefore, the name is shortened to the genus only in the text. The most frequently used plants were: *Atractylodes* (n=15); *Poria* (n=13); *Coix* (n=13); *Astragalus* (n=12); and *Codonopsis* (n=10).

#### *6.4.5.1 Level 1: Single herbal medicines*

Of the 30 HMs included at level 1, six were not always associated with another HM in the CHM interventions (Table 6.6). Of these, *Poria* (n=13) had the lowest RR (0.50 [0.42, 0.60], $I^2$=0%), followed by *Panax N* (n=4) (RR 0.51 [0.36, 0.72], $I^2$=0%), *Codonopsis* (n=10) (RR 0.54 [0.45, 0.65], $I^2$=0%), *Astragalus* (n=12) (RR 0.55 [0.47, 0.65], $I^2$=0%), *Coix* (n=13) (RR 0.56 [0.48, 0.66], $I^2$=0%),

and *Atractylodes* (n=15) (RR 0.57 [0.50, 0.66], $I^2$=0%). The remaining HMs always appeared in association with at least one other HM.

**Table 6.5: Tumour response rate (tRR) for studies included in chemotherapy induced neutropenia groups**

| Group | No. studies (participant No. T/C) | EoT (between groups) RE | $I^2$ | Incidence T/C (%) |
|---|---|---|---|---|
| **1. Injection group (no G-CSF): 6 studies that reported CIN data for Grade 3/4 and/or all grades** | | | | |
| Reported CIN Gr 3/4 | 4 (191/177) | 1.43 [1.04, 1.96], p = 0.03* | 37 | T: 52.4% (100/191); C: 35.0% (62/177) |
| Reported CIN all grades | 5 (239/222) | 1.38 [1.07, 1.78], p = 0.01* | 27 | T: 51.9% (124/239); C: 36.5% (81/222) |
| **2. Oral administration group (no G-CSF): 17 studies** | | | | |
| Reported CIN Gr 3/4 | 16 (534/482) | 1.21 [1.03, 1.42], p = 0.02* | 0 | T: 40.3% (215/534); C: 33.0% (159/482) |
| Reported CIN all grades | 17 (569/514) | 1.20 [1.03, 1.41], p = 0.02* | 0 | T: 40.6% (231/569); C: 33.5% (172/514) |
| **Total for groups 1 and 2 (no G-CSF): 23 studies** | | | | |
| Reported CIN Gr 3/4 | 20 (725/659) | 1.29 [1.13, 1.48], p = 0.0002* | 0 | T: 43.4% (315/725); C: 33.5% (221/659) |
| Reported CIN all grades | 23 (832/759) | 1.27 [1.13, 1.44], p = 0.0001* | 0 | T: 44.6% (371/832); C:34.9% (265/759) |
| **3. G-CSF group: 1 study** | | | | |
| Reported CIN Gr 3/4 & all grades | 1 (48/45) | 1.16 [0.77, 1.74], p=0.47 | NA | T: 54.2% (26/48); C:46.7% (21/45) |
| **4. Neutrophil count: 2 studies** | | | | |
| Did not report CIN grades | 2 (122/90) | 1.24 [0.92, 1.66], p=0.16 | 0 | T: 52.5% (64/122); C:42.2% (38/90) |

T: treatment group; C control group; EoT: end of treatment; Gr. Grade; RE: random effect.

#### *6.4.5.2 Level 2: Pairs of herbal medicines*

When the 30 HMs that showed significant RR results were paired with other HMs from level 1, thirteen pairs showed RRs that were lower than the total pool for oral HM interventions (Table 6.6).

**Table 6.6: Effects of specific herbal medicines on chemotherapy induced neutropenia (all grades): single herbal medicines and combinations**

| Level | Herbal Medicine (HM) | RR | 95% CI | No. studies (part.) | $I^2$ |
|---|---|---|---|---|---|
| 1 | *Angelica sinensis* (Oliv.) Diels. (*dang gui*) | 0.38 | [0.22,0.64] | 2 (118) | 0 |
| 1 | *Ligusticum chuanxiong* Hort. (*chuan xiong*) | 0.38 | [0.22,0.66] | 2 (114) | 0 |
| 1 | *Glycyrrhiza uralensis* Fisch. (*gan cao*) | 0.40 | [0.27,0.60] | 3 (178) | 0 |
| 1 | *Amomum kravanh* Pierre ex. Gagnep. (*bai dou kou*) | 0.41 | [0.26,0.65] | 2 (126) | 0 |
| 1 | *Pinellia ternata* (Thunb.) Breit. (*ban xia*) | 0.45 | [0.31,0.64] | 3 (186) | 0 |
| 1 | *Smilax glabra* Roxb. (*tu fu ling*) | 0.46 | [0.27,0.80] | 2 (121) | 0 |
| 1 | *Citrus reticulata* Blanco (*chen pi*) | 0.46 | [0.29,0.72] | 2 (120) | 0 |
| 1 | *Eclipta prostrata* L. (*mo han lian*) | 0.47 | [0.29,0.75] | 2 (138) | 0 |
| 1 | *Paeonia lactiflora* alba. (*bai shao*) | 0.47 | [0.34,0.65] | 4 (212) | 0 |
| 1 | *Sparganium stoloniferum* Buch.-Hamil. (*san leng*) | 0.47 | [0.27,0.81] | 2 (132) | 0 |
| 1 | *Spatholobus suberectus* Dunn (*ji xue teng*) | 0.48 | [0.34,0.68] | 3 (192) | 0 |
| 1 | *Actinidia arguta* (Sieb. & Zucc) Planch. ex Miq. (*teng li gen*) | 0.49 | [0.29,0.84] | 2 (108) | 0 |
| 1 | *Smilax china* L. (*ba qia*) | 0.49 | [0.30,0.81] | 2 (113) | 0 |
| 1 | *Poria cocos* (Schw) Wolf (*fu ling*) | 0.50 | [0.42,0.60] | 13 (780) | 0 |

| Level | Herbal Medicine (HM) | RR | 95% CI | No. studies (part.) | $I^2$ |
|---|---|---|---|---|---|
| 1 | *Ligustrum lucidum* Ait. (*nu zhen zi*) | 0.51 | [0.33,0.79] | 2 (130) | 0 |
| 1 | *Curcuma zedoaria* (Berg.) Rosc. or *C. phaeocaulis* Val. (*e zhu*) | 0.51 | [0.38,0.67] | 6 (379) | 0 |
| 1 | *Panax notoginseng* (Burk.) F.H. Chen (*san qi*) | 0.51 | [0.36,0.72] | 4 (245) | 0 |
| 1 | *Lycium barbarum* L. (*gou qi zi*) | 0.52 | [0.38,0.71] | 3 (168) | 0 |
| 1 | *Akebia quinata* (Thunb.) Decne. (*ba yue zha*) | 0.52 | [0.38,0.72] | 5 (264) | 0 |
| 1 | *Dioscorea opposita* Thunb. (*shan yao*) | 0.52 | [0.39,0.71] | 4 (261) | 4 |
| 1 | *Curcuma wenyujin* Y. H. Chen et C. Ling (*yu jin*) | 0.53 | [0.36,0.79] | 2 (91) | 0 |
| 1 | *Vitis quinquangularis* Rehd. (*ye pu tao teng*) | 0.53 | [0.38,0.73] | 4 (218) | 0 |
| 1 | *Codonopsis pilosula* (Franch.). Nannf. (*dang shen*) | 0.54 | [0.45,0.65] | 10 (615) | 0 |
| 1 | *Astragalus membranaceus* (Fisch.) Bge. (*huang qi*) | 0.55 | [0.47,0.65] | 12 (682) | 0 |
| 1 | *Coix lacryma-jobi* L. (*yi ren*) | 0.56 | [0.48,0.66] | 13 (773) | 0 |
| 1 | *Epimedium brevicornum* Maxim (*yin yang huo*) | 0.57 | [0.43,0.77] | 4 (271) | 0 |
| 1 | *Atractylodes macrocephala* Koidz. (*bai zhu*) | 0.57 | [0.50,0.66] | 15 (962) | 0 |
| 1 | *Scutellaria barbata* D. Don. (*ban zhi lian*) | 0.61 | [0.50,0.74] | 6 (356) | 0 |
| 1 | *Hedyotis diffusa* Willd. (*she she cao*) | 0.63 | [0.51,0.79] | 5 (288) | 0 |
| 1 | *Agrimonia pilosa* Ledeb. (*xian he cao*) | 0.65 | [0.48,0.88] | 2 (170) | 0 |
| 2 | *Poria + Coix* | 0.46 | [0.37,0.57] | 9 (523) | 0 |
| 2 | *Poria + Atractylodes* | 0.47 | [0.39,0.57] | 11 (633) | 0 |
| 2 | *Paeonia + Poria* | 0.47 | [0.34,0.65] | 4 (212) | 0 |
| 2 | *Vitis + Atractylodes* | 0.50 | [0.36,0.70] | 4 (218) | 0 |
| 2 | *C. zedoaria + Atractylodes* | 0.51 | [0.38,0.67] | 6 (379) | 0 |
| 2 | *Akebia + Atractylodes* | 0.52 | [0.38,0.72] | 5 (264) | 0 |
| 2 | *Poria + Dioscorea* | 0.52 | [0.39,0.71] | 4 (261) | 4 |
| 2 | *Codonopsis + Atractylodes* | 0.54 | [0.45,0.65] | 10 (615) | 0 |
| 2 | *Coix + Atractylodes* | 0.56 | [0.47,0.67] | 10 (595) | 0 |
| 2 | *Astragalus + Atractylodes* | 0.56 | [0.48,0.67] | 11 (650) | 0 |
| 2 | *Epimedium + Coix* | 0.57 | [0.43,0.77] | 4 (271) | 0 |
| 2 | *Atractylodes + Hedyotis* | 0.63 | [0.51,0.79] | 5 (288) | 0 |
| 2 | *Agrimonia + Atractylodes* | 0.65 | [0.48,0.88] | 2 (170) | 0 |
| 3 | *Poria + Atractylodes + Ligusticum* | 0.38 | [0.22,0.66] | 2 (114) | 0 |
| 3 | *Poria + Coix + Amomum* | 0.41 | [0.26,0.65] | 2 (126) | 0 |
| 3 | *Poria + Coix + Pinellia* | 0.45 | [0.31,0.64] | 3 (186) | 0 |
| 3 | *Poria + Dioscorea + Paeonia* | 0.45 | [0.32,0.64] | 3 (172) | 0 |
| 3 | *Poria + Coix + Atractylodes* | 0.46 | [0.37,0.59] | 8 (465) | 0 |
| 3 | *Poria + Astragalus + Smilax glabra* | 0.46 | [0.27,0.80] | 2 (121) | 0 |
| 3 | *Poria + Atractylodes + Astragalus* | 0.47 | [0.38,0.60] | 8 (479) | 0 |
| 3 | *Poria + Coix + Astragalus* | 0.48 | [0.37,0.61] | 7 (415) | 0 |
| 3 | *Spatholobus + Poria + Atractylodes* | 0.48 | [0.34,0.68] | 3 (192) | 0 |
| 3 | *Poria + Atractylodes + Paeonia* | 0.48 | [0.33,0.71] | 3 (154) | 0 |
| 3 | *Poria + Atractylodes + Actinidia* | 0.49 | [0.29,0.84] | 2 (108) | 0 |
| 3 | *Poria + Atractylodes + Codonopsis* | 0.50 | [0.40,0.62] | 9 (515) | 0 |
| 3 | *Astragalus + Atractylodes + C. zedoarea* | 0.50 | [0.36,0.69] | 5 (315) | 0 |
| 3 | *Astragalus + Atractylodes + Akebia* | 0.51 | [0.35,0.72] | 4 (224) | 0 |
| 3 | *Coix + Atractylodes + Astragalus* | 0.58 | [0.48,0.69] | 9 (545) | 0 |
| 4 | *Poria + Atractylodes+ Angelica + Coix* | 0.38 | [0.22,0.64] | 2 (118) | 0 |
| 4 | *Poria + Atractylodes + Glycyrrhiza + Coix* | 0.40 | [0.27,0.60] | 3 (178) | 0 |
| 4 | *Paeonia + Dioscorea + Poria+ Atractylodes* | 0.45 | [0.29,0.71] | 2 (114) | 0 |
| 4 | *Astragalus + Atractylodes + Coix + Eclipta* | 0.47 | [0.29,0.75] | 2 (138) | 0 |
| 4 | *Astragalus + Atractylodes + Akebia + Vitis* | 0.48 | [0.31,0.74] | 3 (154) | 0 |
| 4 | *Codonopsis + Astragalus + Atractylodes + Poria* | 0.48 | [0.38,0.62] | 7 (411) | 0 |
| 4 | *C. zedoaria + Poria + Atractylodes + Codonopsis* | 0.49 | [0.36,0.67] | 5 (309) | 0 |
| 4 | *Poria + Codonopsis + Atractylodes + Hedyotis* | 0.49 | [0.35,0.70] | 4 (228) | 0 |
| 4 | *Astragalus + Coix + C. zedoaria + Atractylodes* | 0.51 | [0.36,0.73] | 4 (251) | 0 |
| 4 | *Codonopsis + Atractylodes + Poria + Akebia* | 0.52 | [0.34,0.80] | 3 (153) | 0 |
| 4 | *Paeonia + Codonopsis + Atractylodes + Poria* | 0.55 | [0.35,0.86] | 2 (104) | 0 |
| 4 | *Astragalus + Coix + Atractylodes + Scutellaria* | 0.61 | [0.50,0.74] | 6 (356) | 0 |

| Level | Herbal Medicine (HM) | RR | 95% CI | No. studies (part.) | $I^2$ |
|---|---|---|---|---|---|
| 4 | *Astragalus + Atractylodes + Coix + Hedyotis* | 0.61 | [0.46,0.80] | 4 (248) | 0 |
| 5 | *Astragalus + Atractylodes + Poria + Smilax + Coix* | 0.46 | [0.27,0.80] | 2 (121) | 0 |
| 5 | *Codonopsis + Poria + Astragalus + C. zedoaria + Atractylodes* | 0.48 | [0.33,0.70] | 4 (245) | 0 |
| 5 | *Astragalus + Coix + Poria + Codonopsis + Atractylodes* | 0.49 | [0.37,0.63] | 6 (348) | 0 |
| 5 | *Codonopsis + Atractylodes + Poria + C. zedoaria + Vitis* | 0.51 | [0.35,0.73] | 3 (177) | 0 |
| 5 | *Atractylodes + Hedyotis + Akebia + Poria + Codonopsis* | 0.52 | [0.32,0.84] | 2 (100) | 0 |
| 5 | *Astragalus + Coix + Atractylodes + C. zedoaria + Akebia* | 0.52 | [0.35,0.76] | 3 (183) | 0 |
| 5 | *Lycium + Atractylodes + Coix + Astragalus + Scutellaria* | 0.53 | [0.39,0.73] | 3 (168) | 0 |
| 5 | *Atractylodes + Astragalus + Coix + Hedyotis + Scutellaria* | 0.61 | [0.46,0.80] | 4 (248) | 15 |
| 6 | *Codonopsis + Astragalus + Coix + Glycyrrhiza + Atractylodes + Poria* | 0.43 | [0.27,0.68] | 2 (128) | 0 |
| 6 | *Astragalus + Coix + Poria + Pinellia + Spatholobus + Atractylodes* | 0.45 | [0.28,0.72] | 2 (128) | 0 |
| 6 | *Hedyotis + Scutellaria + Coix + Codonopsis + Astragalus + Poria* | 0.47 | [0.32,0.69] | 3 (188) | 0 |
| 6 | *Astragalus + Atractylodes + Poria + Codonopsis + C. zedoaria + Sparganium* | 0.47 | [0.27,0.81] | 2 (132) | 0 |
| 6 | *Codonopsis + Atractylodes + Astragalus + Coix + Poria + Scutellaria* | 0.50 | [0.37,0.68] | 4 (226) | 0 |
| 6 | *Astragalus + Atractylodes + Poria + Codonopsis + Coix + C. zedoaria* | 0.50 | [0.33,0.75] | 3 (181) | 0 |
| 6 | *Codonopsis + Atractylodes + Poria + C.zedoaria + Vitis + Spatholobus* | 0.50 | [0.33,0.75] | 2 (124) | 0 |
| 6 | *C.zedoaria + Astragalus + Atractylodes + Coix + Panax + Scutellaria* | 0.51 | [0.35,0.74] | 3 (198) | 0 |
| 6 | *Codonopsis + Poria + Atractylodes + Coix + Astragalus+ Epimedium* | 0.53 | [0.37,0.83] | 3 (152) | 0 |
| 7 | *Astragalus + Coix + Codonopsis + Poria + Atractylodes + C. wenyujin+ Epimedium* | 0.55 | [0.37,0.83] | 2 (91) | 0 |
| 8 | *Scutellaria + Hedyotis + Codonopsis + Atractylodes + Poria + Astragalus + Coix + Citrus* | 0.46 | [0.29,0.72] | 2 (120) | 0 |
| 8 | *Codonopsis + Poria + Atractylodes + Coix + Astragalus + Lycium+ Scutellaria+ Epimedium* | 0.53 | [0.36,0.76] | 2 (98) | 0 |
| 9 | *Scutellaria + Hedyotis + C.zedoaria + Codonopsis + Atractylodes + Astragalus + Poria + Coix + Panax* | 0.48 | [0.30,0.78] | 2 (128) | 0 |
| 9 | *Ligustrum + Panax + C. zedoaria + Akebia + Scutellaria + Lycium + Coix + Atractylodes + Astragalus* | 0.51 | [0.33,0.79] | 2 (130) | 0 |
| 10 | *Akebia + C. zedoaria + Vitis + Coix + Atractylodes + Astragalus + Codonopsis + Poria + Smilax china + Epimedium* | 0.49 | [0.30,0.81] | 2 (113) | 0 |

RR: risk ratio; N: number; part.: participants; CI: confidence interval; stud.: studies.

Notes: Full botanical names are provided for level 1 with Chinese pin yin names in parentheses. Genus names are used for subsequent levels with abbreviated botanical name being used when clarity is required. RRs are listed in ascending order for each level. Lower RRs are associated with greater reductions in risk. Data with no significant effect or heterogeneity greater than 30% have been excluded.

The most frequent pairs were: *Poria* + *Atractylodes* (n=11) (RR 0.47 [0.39, 0.57], $I^2$=0%), and *Astragalus* + *Atractylodes* (n=11) (RR 0.56 [0.48, 0.67], $I^2$=0%). The lowest RRs were for *Poria* +

*Coix* (n=9) (RR 0.46 [0.37, 0.57], $I^2$=0%), followed by *Poria* + *Atractylodes* (n=11) (RR 0.47 [0.39, 0.57], $I^2$=0%).

#### *6.4.5.3 Level 3: Combinations of three herbal medicines*

The significant pairs from level 2 were combined with other HMs from level 1. Fifteen different triplets showed significant RRs that were lower than the total pool (Table 6.6). The most frequent combinations were: *Poria* + *Atractylodes* + *Codonopsis* (n=9) RR (0.50 [0.40, 0.62], $I^2$=0%), and *Coix* + *Atractylodes* + *Astragalus* (n=9) RR (0.58 [0.48, 0.69], $I^2$=0%). The combination of *Poria* + *Atractylodes* + *Ligusticum* had the lowest RR (0.38 [0.22, 0.66], $I^2$=0%) based on two studies.

#### *6.4.5.4 Level 4: Combinations of four herbal medicines*

The significant combinations from level 3 were combined into groups of four, using the same method. Thirteen combinations were significant, with RR lower or equal to the pool. *Codonopsis* + *Astragalus* + *Atractylodes* + *Poria* (n=7) (RR 0.48 [0.38, 0.62], $I^2$=0%) was the most frequent combination. The lowest RR was for *Poria* + *Atractylodes* + *Coix* + *Angelica* (RR 0.38 [0.22, 0.64], $I^2$=0%) (Table 6.6).

#### *6.4.5.5 Level 5: Combinations of five herbal medicines*

Eight combinations of five HMs showed RRs lower than the total pool. The most common combination was *Astragalus* + *Coix* + *Poria* + *Codonopsis* + *Atractylodes* (n=6) (RR 0.49 [0.37, 0.63], $I^2$=0%). The combination of *Astragalus* + *Atractylodes* + *Poria* + *Coix* + *Smilax* (n=2) had the lowest RR (0.46 [0.27, 0.80], $I^2$=0%) (Table 6.6).

#### *6.4.5.6 Level 6: Combinations of six herbal medicines*

Nine combinations of six HMs showed significant RRs lower than the total pool. The most common combination (n= 4) was *Codonopsis* + *Atractylodes* + *Astragalus* + *Coix* + *Poria* + *Scutellaria* (RR 0.50 [0.37, 0.68], $I^2$=0%). The combination *Codonopsis* + *Astragalus* + *Coix* + *Glycyrrhiza* + *Atractylodes* + *Poria* (n= 2) had the lowest RR (0.43 [0.27, 0.68], $I^2$=0%) (Table 6.6).

#### *6.4.5.7 Level 7: Combinations of seven or more herbal medicines*

There was one combination of seven, two combinations of eight and nine, and one combination of ten HMs that showed RRs that were significant and lower than the total pool, but all combinations were based on two studies only (Table 6.6).

### 6.4.6 **Herbal medicines with consistent results at multiple levels**

To select HMs for further research we identified those that showed reduced RRs at multiple levels. RR results that were significant and lower than the pool total, with heterogeneity less than 30%, were

evident at all eight levels for *Atractylodes*, *Poria*, *Coix*, *Astragalus* and *Codonopsis* when used in various combinations. Therefore, when these five HMs were included in CHM interventions, the data suggested a clinical benefit for CIN based on multiple studies. Also, the RRs for the pools of studies that included these HMs were lower than the total pool for the oral interventions. This was also the most frequent combination of five HMs. In the six studies (320 participants) that used this combination, the incidence of all grades CIN was 26.7% (47/176) compared to 56.4% (97/172) in the control groups. For 3/4 CIN, this combination showed a significant reduction (RR 0.41 [0.17, 0.96] $I^2$=0, 5 studies, 295 participants) with incidences of 4.7% (7/148) in the test groups and 12.2% (18/147) in the control groups.

### 6.4.7 **Potential synergistic effects of herbal medicines**

A number of combinations of HMs showed RRs that were lower than those of the HMs singly. These included two pairs of HMs: *Poria* + *Coix* and *Poria* + *Atractylodes*; four triplets: *Poria* + *Coix* + *Atractylodes*, *Poria* + *Atractylodes* + *Astragalus*, *Poria* + *Coix* + *Astragalus*, and *Poria* + *Atractylodes* + *Codonopsis*; two groups of four HMs: *Codonopsis* + *Astragalus* + *Atractylodes* + *Poria* and *Astragalus* + *Coix* + *Poria* + *Codonopsis* + *Atractylodes*; and the group of five mentioned above: *Atractylodes* + *Poria* + *Coix* + *Astragalus* + *Codonopsis* (Table 6.6).

### 6.4.8 **Summary of the results for chemotherapy induced neutropenia**

The 32 studies included in the meta-analysis were comparable in that they all employed oxaliplatin regimens in the test and control groups; all participants were diagnosed with CRC; all studies assessed CIN incidence using the WHO criteria; all studies assessed performance status; and all required no use of chemotherapy in the month prior to commencement. With regard to these aspects, the included studies were similar to other studies of oxaliplatin regimens for CRC. One difference was these studies typically used fewer cycles of chemotherapy (mostly 2 to 4) than the large international studies (Cassidy et al., 2008; de Gramont et al., 2000; Hind et al., 2008) which may account for the relatively low CIN incidence rates. Number of cycles is an important factor in CIN profile. Sugihara et al (2012) reported 55-88% CIN (all grades, median of 7-12 cycles) for two Asian studies of FOLFOX 4 for CRC. In the present analysis, the 58.9% (all grades) in the pool of 27 studies (1,840 participants) that did not employ prophylaxis with G-CSF was within this range (Table 6.6). In the case of grade 3/4 CIN, Sugihara et al (2012) reported 37-52% and Park et al. (2015) reported 21.9% (median 9.7 cycles), both of which were considerably higher than the 11.8% (88/743) in the control groups of the pooled result for 22 studies (743 participants). A likely reason for this difference is seventeen of the included studies reported CIN at 4 cycles or less of chemotherapy. For studies that employed 8 or more cycles, the CIN incidence for Grade 3/4 (19%) was close to the result of 21.9% CIN reported by Park et al. (2015).

When the effects of number of cycles of chemotherapy on grade 3/4 CIN incidence was stratified by 2, 3, 4, 6, and 8+ cycles, there was a trend towards increased grade 3/4 CIN incidence as the number of cycles increased. In Gascon et al (2016), which included various cancers, there was an increase in grade 4 CIN probability with increase in cycles from one (7%) to six cycles (16%). The results of the present analysis support this relationship between CIN incidence and number of cycles of chemotherapy. Some other factors that are known to impact in CIN/FN incidence that could not be assessed due to data availability included stage of cancer, treatment history and gender (Aapro et al., 2010).

CIN incidence in the two studies that used G-CSF (Deng & Shen, 2010; Fang & Li, 2008) for two cycles was 38% (all grades) and 3.2% for grade 3/4 compared to the pool of studies of 2 cycles which reported 63.1% (all grades) and 12.9% (grade 3/4). This suggests that the G-CSF was effective in reducing CIN incidence and there were no further reductions with the addition of the CHMs.

Overall, the addition of the CHMs to the oxaliplatin regimens reduced all grades CIN by 24% (95% CI 20% to 28%) and grade 3/4 CIN by 6% (95% CI 3% to 8%). Importantly, the addition of the CHM interventions did not reduce tumour response rate (Table 6.6). This result was consistent with Chen et al. (2016). From the safety perspective, none of the studies reported serious adverse events associated with the addition of the CHM intervention to oxaliplatin regimens. Also, the sensitivity analyses based on HM ingredients did not identify any HMs or HM combinations that significantly increased the incidence of CIN in the test groups relative to the controls.

### 6.4.9 **Effects of the five selected HMs in models relevant to chemotherapy induced neutropenia**

When *Atractylodes*, *Poria*, *Coix*, *Astragalus* and *Codonopsis* were included in a CHM intervention, the incidence of CIN in the treatment groups showed greater reductions than found in the pool of 22 studies of oral CHMs. Moreover, in the studies that used all five of these HMs orally, the incidence of CIN was lower in the test groups (28.0% all grades; 5.2% grade 3/4) than in the controls (58.9% all grades; 14.3% grade 3/4) and there was zero heterogeneity in these pools. Based on these analyses it appears that this group of HMs showed the best available clinical evidence for CIN reduction when combined with oxaliplatin regimens (without G-CSF) for CRC. There were insufficient data to assess the effects of any of these HMs when combined with G-CSF.

Significant reductions in neutropenia incidence were also found for other HMs and HM combinations but the data were insufficient for a consistent pattern of results to be apparent. For example, *Panax notoginseng, Ligusticum chuanxiong, Angelica sinensis* and *Glycyrrhiza uralensis* all appeared in sub-groups that showed the greatest reductions in CIN incidence at one level. However, these sub-groups always included *Astragalus*, *Atractylodes*, *Codonopsis*, *Poria* and/or *Coix* which makes assessment of their contributions more difficult. Nevertheless, it is possible that these HMs also contributed to the

results, so these HMs could also be considered for further research. Some have already received research attention. In particular, the combination of *Astragalus* and *Angelica* is frequently used for neutropenia in clinical practice and has been the subject of animal studies (Yang et al., 2007; Yan et al., 2008). Also, *Astragalus*, *Atractylodes*, *Codonopsis*, *Poria*, *Angelica* and *Glycyrrhiza* are all commonly used in China for the treatment of myelosuppression induced by chemotherapy for various cancers (Wang et al., 2014).

To explore the question of how the five main HMs might reduce CIN incidence, PubMed and CNKI were searched for experimental studies in models of myelosuppression induced by cytotoxic drugs or irradiation, haematopoiesis, and immune-regulation. These studies are summarised briefly below.

In murine models of myelosuppression induced by cyclophosphamide, intraperitoneal injections of extracts of *Astragalus membranaceus* injection (AMI) have been reported to promote colony-forming unit-fibroblast (CFU-F) formation that was associated with improved production of interleukin 6 (IL-6) and Granulocyte-macrophage colony-stimulating factor (GM-CSF) by bone marrow stromal cells (BMSC), increasing Bcl-2 protein and mRNA expression in BMSC, thus enhancing survival of BMSC and proliferation of CFU-F (Zhu & Zhu, 2007). Oral administration of an extract of *Astragalus* root increased counts of leucocytes and bone marrow CD34+ cells, mean number of CD20 immunopositive B lymphocytes, and produced significant decreases in the mean area of fat in bone marrow sections (Ismail et al., 2014). A study of the hematopoietic effect of Astragalus polysaccharides (APS) on healthy human marrow *in vitro* found that compared to negative control (APS 0 mg/L), APS significantly promoted colony-forming unit granulo-monocyte formation at 5 mg/L with or without presence of GM-CSF, and improved the formation of colony-forming unit erythrocytes with erythropoietin. At 125 mg/L, APS promoted erythroid burst-forming units only in combination with erythropoietin or phytohemagglutinin (Zhang & Hong, 2000).

Studies of *Shenqifuzheng* injection, which is comprised of bioactive components of *Astragalus* and *Codonopsis* that mainly contain calycosin-7-O-β-glucoside, lobetyolin, and astragaloside IV, have reported increases in peripheral white blood cell and bone marrow cell counts, enhanced T cell and B cell proliferation responses, enhanced splenic NK1 cell activity and peritoneal macrophage phagocytosis, and restoration of the level of interleukin-2 (IL-2) in the serum (Wang et al., 2012; Wang et al., 2014).

A CHM called *Liujunzitang*, which contains *Codonopsis*, *Atractylodes*, *Poria* and *Glycyrrhiza*, was found to increase counts of white blood cells, reticulocytes, and nucleated bone marrow cells in mice with leukopenia induced by cyclophosphamide (Yuan, 2008). In a similar model, the CHM *Shenbaiyin*, which mainly is composed of *Astragalus* and *Coix*, produced a similar result (Li et al., 2006). In mice administered the microtubule inhibitor epothilone B, the co-administration of the CHM

*Sijunzitang* (SJZ), which contains *Atractylodes*, *Poria* and *Glycyrrhiza*, improved immunity and attenuated myelosuppression (Zhang, 2014).

In irradiation induced myelosuppression in mice, SJZ was administered 7 days before irradiation, and continuously for 7 days after the irradiation. The SJZ showed a protective effect on peripheral blood counts of white cells and platelets compared to negative controls (Li et al., 2015). In mice subjected to irradiation, intragastric administration of the saponin astragaloside IV attenuated radiation-induced apoptosis of bone marrow cells, indicating a radio-protective effect (Li et al., 2011). When irradiated mice were injected with an extract of *Astragalus* root and *Angelica sinensis* root with weekly assessments for 21 days, the treatment group showed significant recovery of blood cells (platelets, WBC, RBC), and in the colony growth and histology of bone marrow cells harvested after 21 days, compared to the controls (Yang et al., 2009). A multi-HM formula that contained *Codonopsis* was reported to promote the recovery of bone marrow haematopoietic function in a myelosuppression model in irradiated mice (Liu et al., 2014).

A number of studies have investigated the effects of these five herbs on immune response. In healthy chickens, Astragalus polysaccharides (APS) were injected for seven days and the phagocytosis rate and phagocytic index of neutrophil granulocytes in peripheral blood were measured for 56 days. The results showed both measures increased significantly in the APS group, reached the highest level at 28 days, then slowly decreased. At 56 days, the phagocytosis rate and phagocytic index were still higher than the negative control for APS doses of 12.5mg/mL, 25 mg/mL and 50 mg/mL. (Li & Jin, 2014). A study of the effects of an aqueous extract of APS reported the extract stimulated activity of RAW 264.7 macrophages (Zhao et al., 2011). A polysaccharide derived from *Codonopsis* was reported to reduce tumour growth and activate the immune system in mice inoculated with Hepatoma-22 (H22) cells (Xu et al., 2012). Codonopsis polysaccharides have also been reported to improve immunity in mice with immunosuppression induced by cyclophosphamide (Zhang et al., 2003; Gong et al., 2012). Polysaccharides from *Atractylodes* exhibited immune-enhancing activity *in vitro* in a study of splenic T-lymphocyte proliferation (Sun et al., 2015). A glycoprotein purified from *Poria* was reported to activate RAW 264.7 macrophages *in vitro* (Chang et al., 2009) and a review of the pharmacological properties of *Poria* found that its polysaccharides appear to potentiate immune response by up-regulating immune stimulators and down-regulating immune suppressors (Rios, 2011).

*Coix* has been developed into an anti-tumour drug in China called *Kanglaite* injection which can be combined with chemotherapy. Besides its inhibitory effects on tumour growth, it has been reported to stimulate T cell proliferation in C57BL/6 mice with Lewis lung carcinoma (Pan et al., 2012), rescue the levels of CD4+ T cells in tumour bearing mice, and increase the number of T cells and natural killer cells in the blood of hepatocellular carcinoma patients (Huang et al., 2014).

Although there have been studies of these five HMs or their constituents in experimental models, this does not mean that these HMs acted via the same mechanisms in the clinical studies. Also, it cannot be inferred that the HMs for which there was more experimental evidence were more effective than other short-listed herbs. These issues require further research. It should be noted that the sensitivity analysis procedure used for the selection of the above five herbs did not allow any tests between the groups of HMs included in Table 6.6, so relative ranking is not an index of clinical effectiveness. The purpose of the ranking was shortlisting candidates for further research from amongst a large list of potentially interesting HMs.

### 6.4.10 **Potential synergetic effects**

The HM combinations identified as potentially having synergistic effects in the above sensitivity analyses were various combinations of *Codonopsis*, *Atractylodes*, *Poria*, *Astragalus* and *Coix*. Of these, *Codonopsis*, *Atractylodes* and *Poria* are the main components of the multi-component CHM *Sijunzitang* (Duan et al., 2011). *Sijunzitang* and its modifications, which frequently include addition of *Astragalus*, have been used clinically as adjuncts to chemotherapy for various cancers in China (Huang et al., 2014; Zhang et al., 2013; Gan et al., 2010). Not studies that investigated potential synergistic effects of the components of *Sijunzitang* in models of neutropenia could be located but there have been reports in other models. In AHH-1 human lymphoblasts, the addition of aqueous extracts of *Sijunzitang* improved survival and growth following irradiation, with an effect that was greater than for extracts of any of the four component HMs used singly (Li et al., 2015). A study of normal rat small intestine epithelial IEC-6 cells, which aimed to investigate the effects of the individual ingredients of *Sijunzitang* plus *Astragalus* on promoting healing of small intestine epithelial IEC-6 cells, reported that the combination of *Codonopsis* plus *Atractylodes* and the combination of *Atractylodes* plus *Astragalus*, showed enhanced effects when compared to these HMs used singly (Zhang & Chen, 2002).

A study of rat bone marrow stem cells investigated the effects of extracts of *Astragalus* and *Angelica sinensis*, singly and in combination, on proliferation and vascular endothelial growth factor (VEGF) expression. It reported that the *Astragalus* promoted stem cell proliferation and induced protein expression of VEGF while there was no significant effect for the *Angelica* extract, however, the combination of *Astragalus* plus *Angelica* was better than *Astragalus* extract alone (Shen et al., 2011). In a study of HMs used for neutropenia in cancer treatment in China, *Astragalus* and *Angelica* were the two most frequent (Wang et al., 2014). However, the frequency of *Angelica* was relatively low in the included studies, so it was not possible to determine whether it was consistently associated with reductions in CIN incidence.

Overall, the evidence for potential synergistic effects of these HM combinations was scanty and the effects on neutropenia have received relatively little research attention. However, an *in vivo* study of a

different combination of four HMs found marked differences in tumour response, body weight and/or mortality in a CRC model when certain HMs were omitted from the formulation (Liu & Chang, 2012). Future studies could consider a similar approach to investigating the effects of specific HM combinations in CIN models.

### 6.4.11 Summary of results for chemotherapy induced neutropenia

These meta-analyses suggest that the addition of CHMs to oxaliplatin regimens was associated with reduced CIN incidence. However, there was considerable risk that bias in the conduct of the studies could have influenced results, so strong conclusions could not be drawn. Future clinical studies require adequate protocols, sufficient durations and sample sizes, and should report results for each grade of CIN and for neutrophil counts.

The sensitivity analyses identified a short-list of HMs: *Atractylodes*, *Poria*, *Coix*, *Astragalus* and *Codonopsis*. Experimental studies also indicated anti-myosuppression and/or immnoregulation effects for these five HMs *in vitro* and *in vivo*. These herbs warrant further clinical research as additions to chemotherapy regimens in patients whose CIN is not sufficiently well-controlled by conventional therapies.

## 6.5 Chapter 6 summary and conclusions

In this chapter, the meta-analyses suggest that the combination of orally administered CHMs with oxaliplatin regimens (mainly FOLFOX) significantly increased tRR, and reduced the incidences of CINV and CIN in the treatment of CRC.

The sensitivity analyses showed that the three shortlisted plant-based HMs *Paeonia*, *Curcuma*, and *Sophora* were each associated with consistently higher contributions to the tRR results and each of these HMs have shown anti-cancer effects in multiple experimental studies.

For CINV, there were six shortlisted herbs: *Atractylodes*, *Poria*, *Coix*, *Glycyrrhiza*, *Astragalus* and *Panax ginseng*. These consistently showed greater reductions in all grades of CINV when used in combinations with other HM. In the experimental studies, these HMs have shown effects on vomiting and/or intestinal functioning which may account for their apparent clinical actions but animal models of nausea and vomiting are limited so the experimental support for their actions on CINV was less clear than that found for the herbs shortlisted for tRR.

For CIN, the shortlisted HMs were *Atractylodes*, *Poria*, *Coix*, *Astragalus* and *Codonopsis*. For each of these HM, experimental studies were available that suggested that they may be protective against

myelosuppression or improve immune status but only *Astragalus* showed evidence of effects on the production of blood cells.

It is important to note that the HMs selected above were not the only HMs that showed improved RR for tRR, CINV or CIN when they were included in a CHM intervention. Rather, they are the HMs that showed consistent effects in multiple studies and in multiple combinations. Another caveat on the interpretation of these results is that the short-listed herbs cannot be ranked in order of effectiveness based on the RR, since each RR was based on a different sub-group of studies.

Based on the information in the clinical trial reports it was not possible to determine whether the short-listed HMs were included in order to improve tRR, or reduce CINV or CIN, or for other reasons. This issue is made more complex since CHMs are considered to have multiple effects in traditional medical theory. However, all are clinically used for treating disorders relating to cancer and its treatment with CMT including immunosuppression, nausea, bloating, fatigue, poor appetite, and/or diarrhoea (Bensky et al., 2004).

In experimental studies, these HMs has been reported to exhibit anti-cancer, inhibitory effects on nausea and vomiting (or its animal equivalent), regulation of gastrointestinal motility, gastro-protective effects, attenuation of myelosuppression and/or enhancement of immune response. However, these experimental studies employed a diversity of cell-lines and animal models, the test materials ranged from whole plant extracts to isolated compounds, and there was little evidence of replication. In addition, the evidence from laboratory reports for the synergistic effects of these HM combinations in models relevant to cancer inhibition, vomiting and myelosuppression was scanty. Future studies could investigate the effects of fractionated extracts of these HMs and compounds extracted from these HMs, to systematically assess their effects and safty in anti-cancer, antiemetic, and anti-myelosuppression, singly and in combination, in cell and animal models.

# Chapter 7. Colorectal Cancer Cell-line Study of Matrine from *ku shen* (*Sophora flavescens root)*

## 7.1 Background and rationale

In Chapter 6, the sensitivity analyses showed that the HMs *Sophora* (*ku shen*), *Curcuma* (*e zhu*) and *Paeonia* (*chi shao*) each produced consistently higher contributions to the tumour response rate (tRR) results. Laboratory studies also found that extracts of these herbs or their constituent chemical compounds possessed anti-cancer effects in models of CRC *in vitro* or *in vivo*. Of these three herbs, *ku shen* was selected for further study for the following reasons:

1. The analyses in Chapter 6 found that studies that included *ku shen* in oral CHMs consistently showed better pooled outcomes in the sensitivity analysis of single herbs and herbal combinations for tumour response rate.
2. *Ku shen* products, singly or in combination with other herbal extracts, are used for cancer treatment in China and have been approved by the Chinese State Food and Drug Administration (SFDA) (Sun et al., 2012).
3. In the short list of three herbs for improved tumour response, two herbs (*chi shao* and *e zhu*) can be sourced from multiple species. *Chi shao* can be derived from the roots of *Paeonia lactiflora* and *Paeonia veitchii* (Nanjing University of TCM, 2015), and *e zhu* is from *Curcuma wenyujin*, *C. phaeocaulis* Val., or *C. kwangsiensis* (Nanjing University of TCM, 2015). Whereas, *ku shen* is only derived from the root of *Sophora flavescens* Ait (Nanjing University of TCM, 2015), therefore we can be confident that the *ku shen* mentioned in the different clinical trials always referred to the same species.

Phytopharmacological research has found that crude extracts of *S. flavescens* and its chemical compounds exhibited *in vitro* and *in vivo* pharmacological activity including: antitumour activity, anti-inflammatory activity, anti-nociceptive activity, anti-anaphylaxis and anti-asthma activity, anti-microbial activity (anti-bacterial, anti-viral, insecticidal, and anti-fungal), effects on the cardiovascular system (anti-arrhythmic, anti-myocardial fibrosis, effects on myocardial contractility, vascular relaxing and anti-myocardial hypoxic activity), immune-regulatory activity, neuroprotective effects, and anti-hyperlipidemic effects (He et al., 2015).

### 7.1.1 Chemical compounds in *Sophora flavescens*

To date, more than 200 chemical compounds have been identified from *S. flavescens*, including 47 alkaloids, 124 flavonoids, 9 triterpene glycosides (He et al., 2015), 1 quinone, 47 essential oils, 8 dimethylchromones, 15 amino acids, and 20 fatty acids (Li and Wang, 2014).

The alkaloids and flavonoids are the major bioactive substances derived from this plant and have received the most research attention (He et al., 2015). *S. flavescens* alkaloids are mainly quinolizidine-

type alkaloids, with a small portion of dipiperidine-type alkaloids. The quinolizidine alkaloids can be divided into 4 subtypes: matrine-type, cytosine-type, anagyrine/sparteine-type, and lupinine-type. The matrine-type is the majority and includes matrine, oxymatrine, sophoridine, isomatrine, sophocarpine, sophoramine, and sophoranol (Miao et al., 2001). *S. flavescens* flavonoids are classified into: 14 flavonols, 41 flavanones 13 flavanonols, 27 isoflavones, 3 isoflavanones, 2 homoisoflavones, 7 chalcones, 2 biflavonoids, and 14 pterocarpans. The majority of these flavonoids are isoprenylated or lavandulylated flavonoids (He et al., 2015; Li and Wang, 2014).

The alkaloids form a major portion of the bioactive compounds contained in *S. flavescens*. Of the main alkaloids, the matrine content of extracts of *S. flavescens* roots has been reported to be 0.32% per fraction weight, followed by 0.13% sophocarpine, 0.12% oxymatrine (matrine *N*-oxide) and 0.073% oxysophocarpine (sophocarpine *N*-oxide) (Chen X, 2004). Oxymatrine is metabolised to the more absorbable matrine by intestinal bacteria in the gastrointestinal tract and liver (Wang et al., 2005).

### 7.1.2 **Pharmacokinetics of *S. flavescens* and its principal compounds**

Pharmacokinetics describes the movement of a drug into, through and out of the body. The pharmacokinetics *S. flavescens* alkaloids have been studied in animals and the human body using various mass spectrometric methods.

One study followed a single oral dose of 300 mg oxymatrine (OMT) capsules in six beagle dogs, and the authors quantified oxymatrine (OMT) and its metabolite matrine (MT) in the dog plasma using a liquid-liquid extraction procedure and then used liquid chromatography-electrospray ionization mass spectrometric (LC-ESI-MS) analysis. The results showed that both OMT (about 223 ng/mL) and MT (about 19 ng/mL) were detected at 0.25 h after administration. The time to reach the maximum plasma concentrations ($T_{max}$) following oral administration was 1.3h for OMT and 2.9 h for MT. The elimination half-life ($t_{1/2}$) and the mean residence times of the metabolite MT were longer than those of OMT. The $AUC_{0-t}$ value (AUC = area under the concentration time curve) for the metabolite MT following oral administration of OMT capsules (7.07 mg h/l) was about 1.2 times as large as that of OMT (5.86 mg h/l). These results suggested that most of the MT was from the reduction of OMT and the active MT seemed to play a major role in the pharmacological actions of the orally administered OMT.

Using LC-MS/MS to analyse beagle dog plasma, another study investigated the differences in pharmacokinetic parameters between OMT and MT after oral administration of the pure compounds, and of *S. flavescens* granules (KFG), which contained equivalent amounts of OMT and MT, to determine the possible influence of other constituents of *S. flavescens* on the pharmacokinetic behaviors of OMT and MT. The results indicated that the absorption of OMT and MT after oral

administration of KFG was significantly better than after the oral administration of the pure compounds separately (Wang et al., 2007).

The high-performance liquid chromatography tandem mass spectrometric (HPLC-MS) method has been used for the simultaneous determination of matrine (MT), oxymatrine (OMT) and oxysophocarpine (OSP) in rat plasma after oral administration of *S. flavescens* extract. OMT and OSP were more rapidly absorbed and eliminated from plasma compared to MT. The values of clearance were 4.388 L/h for OSP and 4.198 L/h for OMT, which were much higher than for MT (0.024 L/h). The results suggest that MT plays an important role in the pharmacological actions of orally administered *S. flavescens* extract (Zhang et al., 2008).

In an open-label, randomised, 3×3 crossover human study, of the oral administration of a single dose of matrine (100, 200 and 400mg), the LC/MS/MS method with a lower limit of quantification of 5 ng/mL was used to determine the pharmacokinetic parameters of matrine in human plasma. The pharmacokinetic results for matrine indicated that the increase in mean concentration $C_{max}$ (range 603-2383 ng/mL) and the mean AUC $_{0-t}$ (from 6203 to 20078 ng h/mL) were correlated with the dose increase. The $T_{max}$ and the $T_{1/2}$ had no apparent change as the dose ascended. Therefore, the authors concluded that matrine had linear pharmacokinetic trends in healthy Chinese volunteers (Zhang et al., 2009).

A liquid chromatography tandem mass spectrometry (LC–MS/MS) method was developed for the simultaneous determination of OMT and its metabolite MT in human plasma in a pharmacokinetic study involving intravenous (i.v.) administration of OMT. The result showed that about one fifth of OMT was reduced to MT following i.v. administration of OMT [($AUC_{0-\infty}$) MT/($AUC_{0-\infty}$) OMT = 19.9±5.4%)] (Wu et al., 2006).

Overall, *S. flavescens* alkaloids including MT, OXM, and OSP, appear in plasma after oral administration of pure alkaloids or of *S. flavescens* extract or of an *S. flavescens* formula, in animal and/or human studies. Orally administered OXM can be reduced to the more absorbable MT by intestinal bacteria in the gastrointestinal tract and liver. The absorption of *S. flavescens* alkaloids after oral administration was better in a crude *S. flavescens* extract or a *S. flavescens* formula compared to the pure compounds suggesting that other compounds in the herb may affect the absorption. OMT and OSP were more rapidly absorbed and eliminated in plasma compared to MT. MT plays an important role in the pharmacological action of orally administered *S. flavescens* extract. These studies suggest it is likely that matrine is a major contributor to the therapeutic effects and pharmacological actions of orally administered *S. flavescens* root.

### 7.1.3 **Effects of matrine in cancer**

Matrine has been reported to inhibit proliferation of various cancer cells (Li et al., 2015; Ma et al., 2015; Yang et al., 2015; Li et al., 2016; Zhou et al., 2016); induce cell cycle arrest and differentiation (Liu et al., 2014; Ou & Chen et al., 2014; Xu et al., 2015); accelerate apoptosis (Hu et al., 2015; Yang & Yao, 2015; Zhou et al., 2015); as well as prevent toxicity from chemotherapy or radiotherapy (Rong et al., 2015; Zhou et al., 2015; Wan et al., 2009; Ge et al., 2016). Moreover, products containing matrine and oxymatrine have been approved for use in cancer therapy in China (Sun et al., 2012).

Therefore, matrine was selected as the test compound in the three experiments reported in this chapter.

### 7.1.4 **Aims of the experimental studies**

The experimental studies aimed to test the effects of matrine in CRC cell lines *in vitro*. The experiments involved three arms: negative control groups (vehicle), matrine groups, and positive control oxaliplatin (OXA) group. The experiments were as follows:

Experiment 1: effects of matrine on proliferation in four CRC cell lines;

Experiment 2: effects of matrine on cell morphology; and

Experiment 3: effects of matrine on cell cycle progress and apoptosis.

## 7.2 Methods and materials used in the experiments

See Chapter 3 for more detailed descriptions of the methods used in the assays.

### 7.2.1 **Test compounds**

The two test compounds were:

1. Matrine (PubChem CID 91466); chemical formula $C_{15}H_{24}N_2O$; molecular 248.37 g/mol (Figure 6.1). Purchased from Yuanye Bio-Technology Co., Ltd (Shanghai, China). Purity was >97% confirmed by high-performance liquid chromatography (HPLC); and
2. Oxaliplatin (PubChem CID 9887053); chemical formula $C_8H_{14}N_2O_4Pt$; molecular weight 397.294 g/mol (Figure 6.2). Purchased from Yuanye Bio-Technology Co.

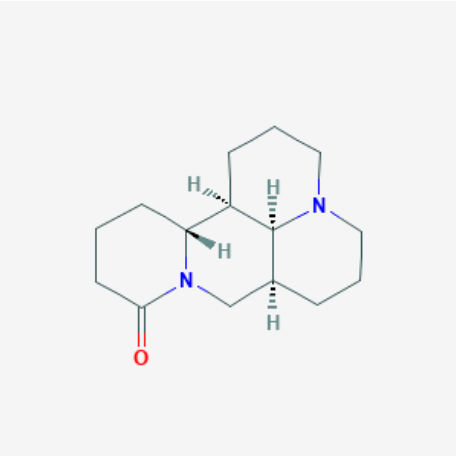


**Figure 7.1: Matrine molecular structure**
Retrieved from PubChem. https://pubchem-ncbi-nlm-nih-gov.ezproxy.lib.rmit.edu.au/compound/91466

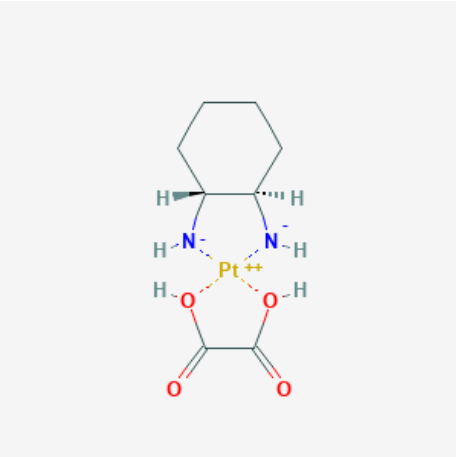


**Figure 7.2: Oxaliplatin molecular structure**
Retrieved from PubChem. https://pubchem.ncbi.nlm.nih.gov/compound/9887053#section=Top

### 7.2.2 **Cell lines**

The four human CRC cell lines were: 1. LS 174T (CL-188™, ATCC); 2. Caco-2 (HTB-37™, ATCC); 3. SW1116 (CCL-233™, ATCC); and 4. RKO (CRL-2577™, ATCC). These were obtained from the Cell Bank of Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China), where they were tested and authenticated. The procedures included cross-species checks, DNA authentication and quarantine.

The LS174T cell line was derived from a Duke's type B adenocarcinoma of the colon that was able to produce high levels of carcinoembryonic antigen (CEA). A morphological study of this cell-line revealed abundant microvilli and intracytoplasmic mucin vacuoles (Tom et al., 1976). The LS174T cell line positively expresses oncogenes c-myc, N-myc, H-ras, N-ras, Myb, and fos, and also expresses p53 mRNA (Trainer et al., 1988).

RKO is a poorly differentiated colon carcinoma cell line (Brattain et al., 1984) that expresses p53 + (wild type) (Smith et al., 1995) and urokinase receptor (u-PAR) (Boyd et al., 1988).

CaCo 2 expresses enterocytic differentiation upon confluency, so it can be a model for the study of the factors involved in the regulation of the differentiation of enterocytes (Jumarie et al., 1991). The cells express retinoic acid binding protein I and retinol binding protein II (Levin MS, 1993); heat stable enterotoxin (Sta, E. coli) (Cohen MB, et al.1993); and epidermal growth factor (EGF) (Basson MD, et al.1994).

The SW1116 cell line is positive for expression of c-myc, K-ras, H-ras, myb, sis and fos oncogenes (Trainer et al., 1988). The cells also express tumour specific nuclear matrix proteins CC-4, CC-5 and CC-6 that scaffold nuclear shape, organise chromatin, and probably are important regulatory proteins as well (Keesee SK, et al.,1994).

### 7.2.3 **Equipment**

- Inverted microscope (Citadel 2000, Thermo Fisher Scientific Inc., USA).
- Benchtop Centrifuges (Thermo Fisher Scientific Inc., USA).
- Biosafety cabinet (ESCO, Esco Airstream®A2, Esco Technologies, Inc., USA).
- CO2 Incubator (Thermo Fisher Scientific Inc., USA).
- Magnetic stirrer (Shanghai Lei magnetic Co Lit, China).
- Flow cytometry (Novo Express™ACEA Biosciences®, Inc., San Diego, California, USA).
- Micropipettor (Eppendorf, Germany).
- Microplate reader (Sunrise™ TECAN, Switzerland).
- Hemacytometer and Electronic balance (Shanghai Precision Instrument & Meter Co., Ltd, China).

### 7.2.4 **Reagents**

- Fetal bovine serum (FBS), Dulbecco's modified Eagle's Medium (DMEM), 0.25% (w/v) trypsin-EDTA and Phosphate Buffer Saline (PBS) (Gibco Invitrogen Life Technologies, Carlsbad, CA, USA).
- Propidium iodide (PI) (Sigma-Aldrich St. Louis, MO, U.S.A.).
- Cell Counting Kit-8 (CCK-8) (Dojindo Laboratories Science and Technology, Inc., Kumamoto, Japan).
- The Annexin V‑FITC Apoptosis Detection kit and DNA content Quantitation Assay for cell cycle analysis (KeyGENBioTECH, Nanjing, China).
- RNase A (50 μg/mL) (KeyGENBioTECH, Nanjing, China).

### 7.2.5 **Preparation of reagents**

1. Culture medium: Fetal bovine serum (FBS) 50 mL was added into Dulbecco's modified Eagle's Medium (DMEM)-high glucose 500 mL, and asepsis was checked for 48 hrs, then stored at 4 deg.C. (expired in one month).

2. Oxaliplatin (100 mg) was dissolved in 5% glucose and saline. The final volume of stock solution was 25.2 mL, and the concentration of stock solution was 4 mg/mL (10 mM). The stock was aliquoted into 500μL sterile EP tubes, and was stored at 4 deg.C in a dark environment. The stock was diluted in medium to requested concentrations prior to each experiment.

3. Matrine (500 mg) was dissolved in 0.9% saline. The final volume of the stock solution was 100 mL, and the concentration of the stock solution was 5 mg/mL (20 mM). The stock solution was aliquoted into 1.5 mL sterile EP tubes, and was stored at -20 deg.C in a dark environment. The stock was diluted in medium to requested concentrations prior to each experiment.

4. Phosphate buffered saline (PBS): the following substances were weighed: KCl, 0.20 g; $KH_2PO_4$, 0.20 g; NaCl, 8.00 g; $Na_2HPO_4 \cdot 12H_2O$, 2.08 g. The substances were put into a flask and stirred using the magnetic stirrer while gradually added into tri-distilled water until completely dissolved. The final volume of the stock solution was 1000 mL. The stock was aliquoted into 500 mL bottles, autoclaved, and stored at 4 deg.C in a dark environment.

### 7.2.6 **Experimental methods and procedures**

The procedures for each of the experiments were detailed in Chapter 3, including methods for cell line culture, maintenance of cell lines, detaching cell lines and sub-culturing, cell viability assays, observation of cellular morphological changes, cell cycle analysis, and apoptosis analysis.

## 7.3 Results of the experiments

The results of each of the four experiments are summarised below.

### 7.3.1 **Effects of matrine on proliferation**

To investigate the effects of matrine and OXA on the proliferation of four CRC cell lines, the viability of cells was measured using the CCK-8 assay. The exposure of the four CRC cell lines to matrine at concentrations of 1, 2, 4, 8, 10mM (100μl/well) for 24, 48 and 72h caused significant growth inhibition in a dose- and time-dependent manner (Figure 7.3a; Table 7.1). The $IC_{50}$ values of matrine on LS 174T, Caco-2 and SW1116, RKO cells were 2.78 mM, 3.92 mM, 1.18 mM and 1.86 mM respectively after 48h treatment.

As the positive control, treatment with OXA at concentrations of 10, 20, 40, 80, 100μM (100μl/well) for 24, 48 and 72h, also inhibited the CRC cells in a dose- and time-dependent manner. After 48h treatment, the $IC_{50}$ values of OXA on LS 174T, Caco-2, SW1116 and RKO cells were 29.83 μM, 10.15 μM, 28.59 μM and 11.67 μM respectively (Figure 7.3b; Table 7.1).

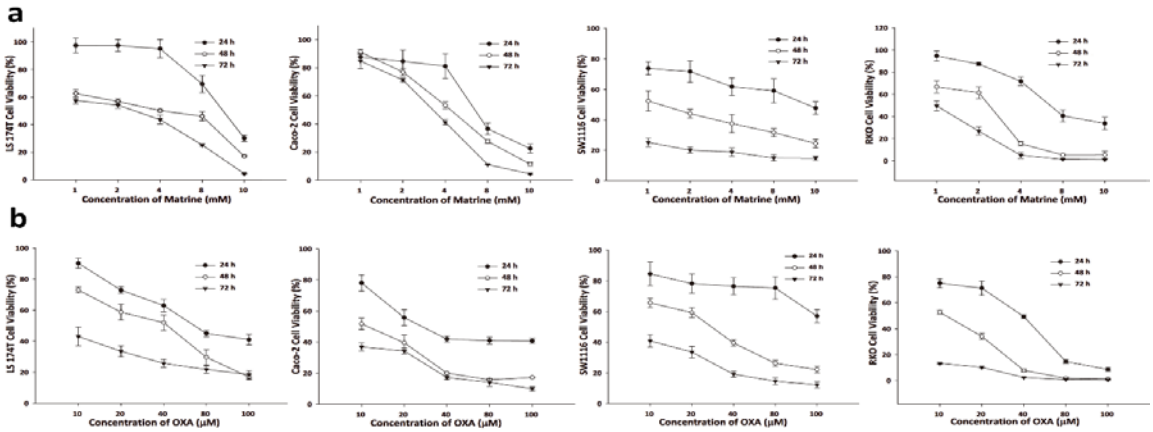


**Figure 7.3: The proliferative inhibition effect of matrine and oxaliplatin on LS 174T, Caco-2, SW1116 and RKO cells**

**a** Matrine and **b** OXA inhibited the growth of CRC cell lines LS 174T, Caco-2, SW1116 and RKO in a dose- and time-dependent manner. Cell viability curves were plotted as viable cell percentage versus control group based on the CCK-8 assay. Data are expressed as mean ± SD of three independent experiments

**Table 7.1: Colorectal cancer cell-line viability (%) under treatment with matrine (MT) and oxaliplatin (OXA) for 24, 48, and 72 hours**

| **Cell-line** | LS174T | | | SW1116 | | | RKO | | | Caco-2 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **End-point** | 24h | 48h | 72h | 24h | 48h | 72h | 24h | 48h | 72h | 24h | 48h | 72h |
| **MT** doses (mM) | CRC cell-line viability: mean± Standard deviation (%) | | | | | | | | | | | |
| 1 | 97.49 ±5.36 | 62.65 ±2.80 | 57.53 ±2.42 | 73.73 ±4.26 | 52.34 ±6.62 | 25.14 ±2.80 | 94.59 ±4.64 | 66.70 ±5.3 | 49.58 ±4.37 | 87.47 ±2.23 | 91.15 ±2.06 | 84.79 ±5.40 |
| 2 | 97.52 ±4.40 | 56.89 ±1.91 | 54.24 ±2.31 | 71.67 ±7.05 | 44.02 ±2.80 | 20.11± 1.89 | 87.35 ±1.54 | 61.52 ±5.38 | 26.87 ±3.60 | 84.61 ±7.96 | 77.04 ±2.60 | 71.30 ±1.30 |
| 4 | 95.12 ±6.76 | 50.26 ±1.08 | 43.67 ±3.21 | 61.74 ±5.69 | 37.51 ±5.97 | 18.79 ±2.65 | 71.84 ±3.81 | 15.48 ±1.97 | 5.02± 2.73 | 81.18 ±8.77 | 53.25 ±2.53 | 41.24 ±1.93 |
| 8 | 69.53 ±6.31 | 46.13 ±3.27 | 25.29 ±0.84 | 59.05 ±7.85 | 31.65 ±2.57 | 14.92 ±2.02 | 40.62 ±5.46 | 5.30± 0.85 | 1.46± 0.2 | 36.49 ±4.11 | 27.62 ±1.25 | 11.11± 0.15 |
| 10 | 30.15 ±2.30 | 17.02 ±0.74 | 4.45± 0.11 | 47.78 ±4.15 | 24.45 ±2.82 | 14.80 ±1.44 | 33.80 ±5.64 | 5.35± 3.31 | 1.09± 0.11 | 22.52 ±3.07 | 11.44± 1.26 | 4.53± 0.39 |
| **OXA** doses (μM) | CRC cell-line viability: mean± Standard deviation (%) | | | | | | | | | | | |
| 10 | 90.29 ±3.15 | 73.03 ±1.90 | 43.09 ±6.09 | 84.49 ±7.70 | 65.61 ±2.98 | 40.89 ±4.05 | 75.14 ±3.51 | 52.72 ±1.32 | 13.30 ±0.63 | 78.10 ±5.26 | 51.72 ±3.68 | 36.86 ±0.55 |
| 20 | 72.91 ±2.16 | 58.74 ±5.02 | 33.51 ±3.50 | 78.18 ±6.27 | 59.31 ±3.21 | 33.56 ±3.72 | 71.29 ±5.50 | 34.07 ±2.39 | 10.26 ±0.88 | 55.78 ±5.16 | 39.57 ±5.01 | 34.24 ±1.88 |
| 40 | 63±4. 02 | 51.98 ±5.01 | 25.75 ±2.63 | 76.50 ±5.53 | 39.46 ±2.04 | 19.13 ±1.91 | 49.40 ±1.45 | 7.84± 1.13 | 2.40± 0.48 | 41.89 ±1.93 | 20.03 ±0.22 | 17.21 ±1.35 |
| 80 | 45.03 ±2.14 | 29.67 ±4.99 | 21.82 ±2.46 | 75.28 ±7.28 | 26.25 ±1.94 | 14.59 ±2.22 | 14.65 ±1.37 | 1.79± 0.68 | 0.81± 0.18 | 40.99 ±2.22 | 15.67 ±0.45 | 14.04 ±2.52 |
| 100 | 41.05 ±3.89 | 16.95 ±1.62 | 18.57 ±2.41 | 57.06 ±4.29 | 22.20 ±2.15 | 12.23 ±2.02 | 8.58± 1.40 | 1.25± 0.46 | 0.49± 0.29 | 40.75 ±1.72 | 17.24 ±0.29 | 9.94± 1.26 |

### 7.3.2 **Effects of matrine on cell morphology**

Morphological features of apoptosis were observed using the Inverted microscope in the four CRC cell lines after treatment with matrine and OXA.

Compared to negative control, cell densities in each of the four cell-lines were decreased in the matrine low dose treatment groups and further decreased in the matrine high dose treatment groups. In each cell line, growth reduction was most evident in the OXA controls. Cells in the matrine and OXA treated groups showed morphological changes consistent with apoptosis including blebbing (bulge or protrusion of the cellular membrane), cell shrinkage, nuclear condensation and fragmentation (Figure 7.4).

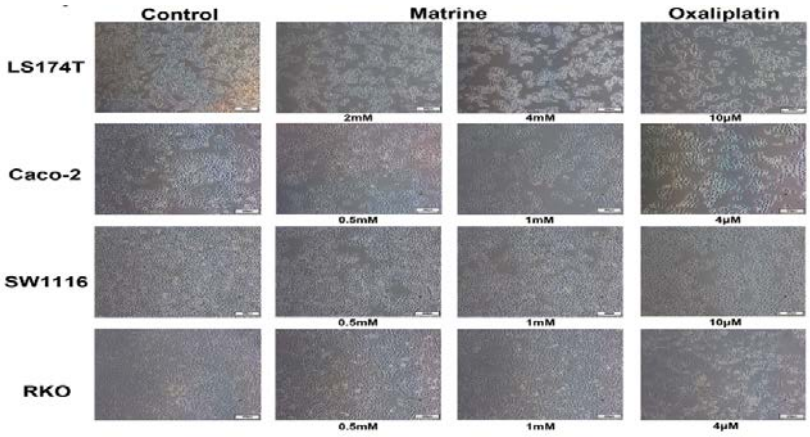


**Figure 7.4: Effects of matrine and oxaliplatin on morphology of LS 174T, Caco-2, SW1116 and RKO cells**

Morphological changes in colorectal cancer cell lines LS 174T, Caco-2, SW1116 and RKO cells treated with matrine (low-dose and high-dose) and OXA for 48h. (Magnification, ×100. Scale bars, 100μM)

### 7.3.3 **Effects of matrine on cell cycle arrest**

The effects of matrine on cell cycle progress in the four CRC cell lines were detected by FCM. As shown in Figure 7.5, matrine increased the population of cells in the G0/G1 phase, and decreased the population of cells in S phase, in all four cell lines. This tendency was more apparent in the high-dose group, which showed statistically significant differences in all cell lines, indicating that matrine induced cell cycle arrest at the G0/G1 phase. OXA increased the population of cells in the G0/G1 phase in SW1116 and RKO cells, but the population of cells in S phase was increased in LS 174T and Caco-2 cells.

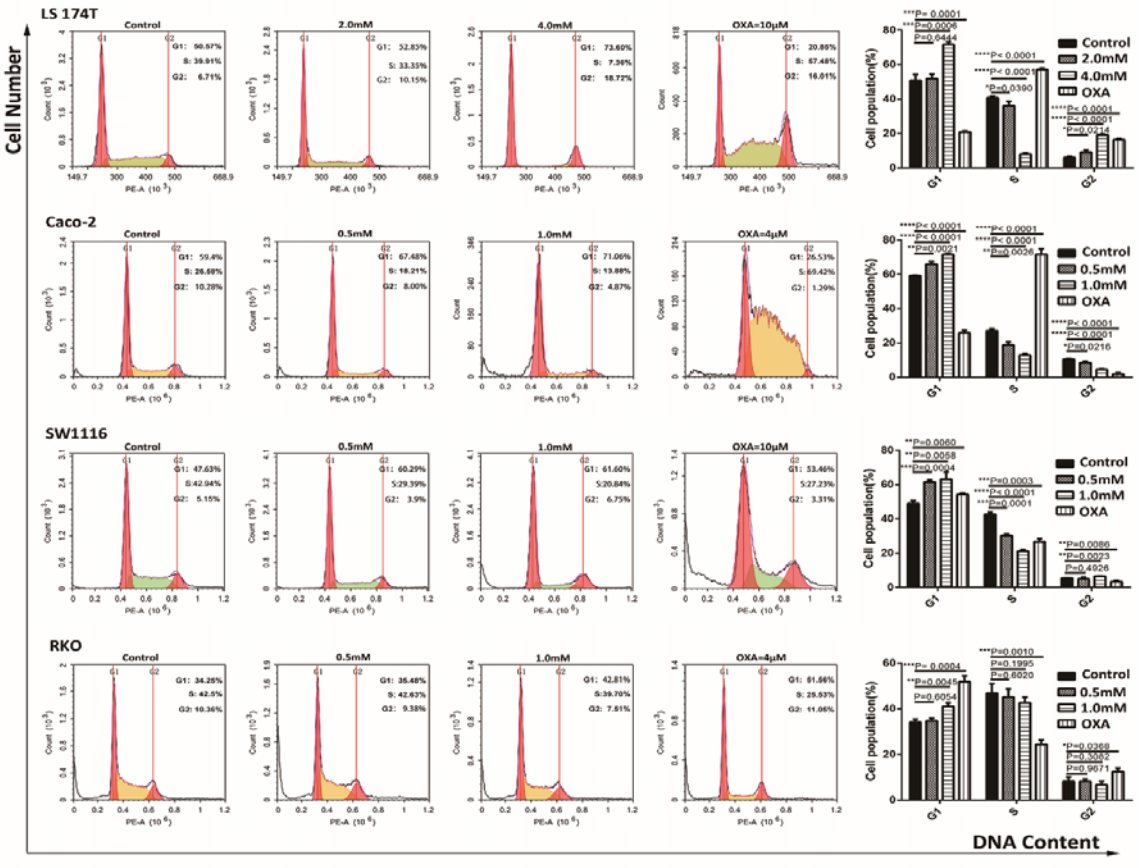


**Figure 7.5: Effects of matrine and oxaliplatin on cell cycle distribution in LS 174T, Caco-2, SW1116 and RKO cells**

The proportion of cells in the G1 phase increased in a dose-dependent manner after treatment with matrine (low-dose and high-dose) for 24h. The percentage of cells in the S phase decreased compared to the negative control. Data are expressed as mean ± SD. Representative profiles are from one of three independent experiments. **P* < 0.05, ***P* < 0.01, or ****P* < 0.001 versus vehicle control

### 7.3.4 **Effects of matrine on apoptosis**

The effects of matrine on apoptosis in the four CRC cell lines were detected by FCM. The Annexin-V-FITC/PI double staining assay shows the percentage of live, dead and apoptotic cells in the scatter plots and representative histograms in Figure 7.6. Matrine induced apoptosis in cells after 24 h treatment. Significant increases in apoptosis rates were evident for matrine 0.5 mM to 1.0 mM in Caco-2 and SW1116, and for matrine 2.0 mM to 4.0 mM in LS 174T cells. In RKO cells, significant increases in apoptosis rates were evident for matrine only at 1.0 mM. In each cell line, the higher the matrine dose, the higher the apoptosis rate. OXA also induced apoptosis in each cell line in same manner.

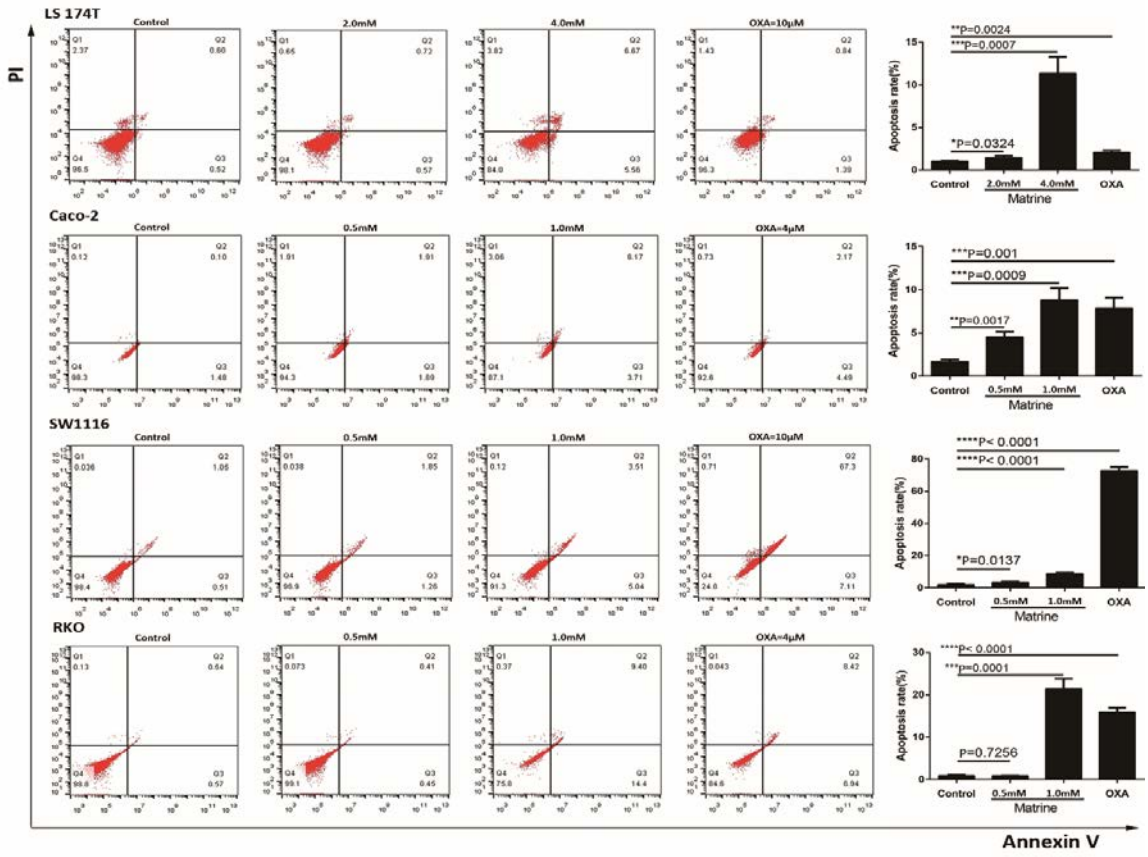


**Figure 7.6: Matrine and oxaliplatin induced apoptosis in LS 174T, Caco-2, SW1116 and RKO cells**

Both matrine (low-dose and high-dose) and OXA induced apoptosis in LS 174T, Caco-2, SW1116 and RKO cells. The apoptosis rates were higher in the higher dose matrine groups. Data are expressed as mean ± SD. Representative profiles are from one of three independent experiments.
*$P < 0.05$, **$P < 0.01$, or ***$P < 0.001$ versus vehicle control

## 7.4 Discussion of the cell-line experiments

Cell proliferation is tightly regulated by pro-proliferation and anti-proliferation molecules in normal cells in order to maintain homeostasis in the number of cells in a tissue. In cancer, the balance is disrupted. Cancer cells proliferate uncontrollably due to alterations in growth ligands, their receptors, and intracellular signal molecules (Fouad and Aanei, 2017). The goal of chemotherapy is to restrict the reproductive potential of cancer cells via various mechanisms including apoptosis, necrosis, mitotic catastrophe, autophagy, cell cycle arrest or senescence (Deloch et al., 2016). However, undesirable side effects including hair loss, nausea, vomiting, anaemia, fatigue, dyspepsia, loss of appetite, immunodeficiency and weight change may damage the quality of life of patients and increase the rates of morbidity and mortality (Nichols and Bae, 2012). Consequently, there is a need for drugs that reduce the proliferation of cancer cells without producing severe adverse effects.

In this study, the cell viability assays (CCK-8) showed that matrine inhibited proliferation dose- and time- dependently in the CRC cell lines LS 174T, Caco-2, RKO, and SW1116 (Figure 7.3a, b). These findings were consistent with previous investigations of matrine in other CRC cell-lines, such as HT-

29 (Chang et al., 2013; Huang et al., 2007; Peng et al., 2005; Ren et al., 2014), SW1116 (Zhu et al., 2009), SW620 (Li et al., 2015; Liang et al., 2008; Wang et al., 2008), SW480 (Xiao 2013, Zhou 2009, Wang 2008, Zou 2016), LoVo (Zhang et al., 2014), and DLD1 (Ren et al., 2014). The $IC_{50}$ results showed the SW1116 cell-line was the most sensitive to matrine, followed by RKO, Caco-2 and LS174T cells.

The cell cycle is a cascade event that consists of five different phases: G0, G1, S, G2, and M phase. Cells in the G0 phase are not in the process of proliferation. The cell cycle initiates in response to mitogenic growth factors stimulating the G1 phase. In this phase, the cell increases supplies of its nutrients, and increases in size. DNA synthesis commences in the S phase, all DNA are replicated and chromosomes double by the end of this phase. In the G2 phase, proteins are synthesised rapidly and there is continuous growth of the cell in readiness for mitosis in the M phase. In the M phase, the cell completes the process of dividing the chromosomes into two identical sets, followed by dividing cell components including nuclei, cytoplasm, organelles and cell membrane into two identical daughter cells (cytokinesis). Initiation of the G1 phase is dependent on tightly-controlled mitogenic growth signalling (Strachan et al., 2015). FCM was used to measure the DNA content of cells at different phases. The results showed matrine induced cell cycle arrest at the G1 phase in all four cell-lines (Figure 7.5). This result was consistent with studies of matrine in other CRC cell-lines including HT-29 cells (Chang et al., 2013; Huang et al., 2007), SW620 cells (Wang et al., 2008), and LoVo cells (Zhang et al., 2014).

Apoptosis induction is an important mechanism of anti-cancer drugs. The Annexin V-FITC/PI double staining assay showed that matrine induced apoptosis in all four CRC cell-lines (Figure 7.6), and the morphological study found that all four cell lines showed apoptotic changes after treatment with matrine and OXA (Figure 7.4). This suggests that the anti-cancer effects of matrine, at the concentrations tested, were via apoptosis rather than necrosis or other process of cell death.

## 7.5 Conclusions from the experimental studies

These studies demonstrated that the dose-dependent, anti-proliferative effects of matrine involved cell cycle arrest at the G1 phase and pro-apoptotic effects in all four CRC cell-lines, each of which has different biological and histological characteristics due to specific oncogenes and mutant genes.

Based on these findings, further experiments could test the validity of the anti-cancer effects of matrine in animal models of CRC. In addition, western blot could be conducted to explore feasible molecular mechanisms of the actions of matrine and docking simulations could be undertaken to identify potential protein-ligand structural interactions. The next chapter will discuss the mechanisms of action of *S. flavescens* and its constituent compounds on cell cycle arrest and apoptosis based on current *in vitro* and *in vivo* studies.

# Chapter 8. The Molecular Mechanisms of Action of *Sophora flavescens* and Its Constituent Compounds

## 8.1 Chapter 8 introduction

The experiments in Chapter 7 have demonstrated that one of the major components of *Sophora flavescens* (*ku shen*) showed pro-apoptotic effects in multiple CRC cell lines. In this chapter, the possible mechanisms for the actions of matrine and other compounds isolated from *S. flavescens* are discussed to identify future directions for research.

## 8.2 Identification of experimental studies

PubMed and CNKI were searched (to December 2016) to locate experimental studies of *S. flavescens*, its extracts and its principal compounds (e.g. matrine and oxymatrine) in CRC cell lines or animal models of CRC. The literature search identified 26 experimental studies that tested a *S. flavescens* extract, total *S. flavescens* alkaloids, total *S. flavescens* flavonoids and 20 different *S. flavescens* compounds. These studies investigated 37 different protein targets and involved 11 different CRC cell lines as well as *in vivo* models. Most of the chemical compounds were classified as alkaloids or flavonoids and most studies investigated the following four quinolizidine type alkaloids (Chen et al., 2004):

- matrine (15 studies);
- oxymatrine (7 studies);
- sophoridine (4 studies);
- sophocarpine (3 studies).

Sixteen flavonoids were studied. Of these, sophoraflavanone G was the most commonly investigated (3 studies) followed by kurarinone (two studies). The other flavonoids were all investigated in one study (Ryu et al., 1997; Table 7.1). The flavonoids can be sub-classed into flavanone-type, isoflavone-type, flavanonol-type, chalcone-type, and pterocarpan-type (He et al., 2015; Table 7.1).

Table 8.1 lists each of the compounds that were investigated in these studies identified from the literature. For each compound the chemical formula, molecular weight and PubChem CID are given. A PubChem CID is given to each unique chemical structure. It is possible for the same compound to have different CIDs since there are different tautomeric forms of the same compound that are recorded from various depositors. Tautomers are structural isomers. For instance, the name ‘kurarinone’ has two CIDs: 5318882 and 11982640, since kurarinone has two structural isomers structurally.

**Table 8.1: *Sophora flavescens* compounds in colorectal cancer studies *in vitro* and *in vivo***

| Compound | Chemical Formula | Molecular weight g/mol | PubChem CID | Chemical class | *Study N |
|---|---|---|---|---|---|
| Matrine | $C_{15}H_{24}N_2O$ | 248.37 | 91466 | Quinolizidine | 1, 2, 4, 5, 7, 10, 11, 13, 18, 19, 22, 23, 24, 25, 26. |
| Oxymatrine | $C_{15}H_{24}N_2O_2$ | 264.369 | 114850 | Quinolizidine | 5, 7, 8, 9, 12, 20, 25. |
| Sophoridine | $C_{15}H_{24}N_2O$ | 246.354 | 165549 | Quinolizidine | 5, 6, 17, 24. |
| Sophocarpine | $C_{15}H_{22}N_2O$ | 246.354 | 115269 | Quinolizidine | 5, 23, 24. |
| Sophoraflavanone G | $C_{25}H28O6$ | 424.48622 | 9910234 | Flavanone | 3, 14, 15. |
| Kurarinone | C26H30O6 | 438.52 | 5318882/ 11982640 | Flavanone | 14,15. |
| 2′-methoxy-kurarinone | C27H32O6 | 452.547 | 11982641 | Flavanone | 15. |
| Kurarinol | C26H32O7 | 456.52808 | 44563198 | Flavanone | 14. |
| Norkurarinol | C25H30O7 | 442.51 | 12146484 | Flavanone | 14 |
| Kushenol B | C30H36O6 | 492.612 | 5318891/ 102004745 | Flavanone | 14 |
| Kushenol E | C25H28O6 | 424.493 | 9979767 | Flavanone | 14 |
| Formononetin | C16H12O4 | 268.268 | 5280378 | Isoflavone | 14 |
| Kosamol A | C30H38O8 | 526.626 | 102506430 | Flavanonol | 14 |
| Kushenol H | C26H30O7 | 454.52 | 21721872 | Flavanonol | 14 |
| Kushenol K | C26H32O8 | 472.534 | 44428630 | Flavanonol | 14 |
| Kushenol L | C25H28O7 | 440.492 | 21721878 | Flavanonol | 14 |
| Kushenol M | C30H36O7 | 508.611 | 180948 | Flavanonol | 14 |
| Kushenol N | C26H30O7 | 454.519 | 102004822/ 10253436/ 381851 | Flavanonol | 14 |
| Kuraridin | C26H30O6 | 438.52 | 9954815 | Chalcone | 14 |
| Trifolirhizin | C22H22O10 | 446.408 | 442827 | Pterocarpan | 14 |

*Study N (1st author (year)): 1. Chang C (2013); 2. Huang J (2007); 3. Kim B (2013); 4. Li N (2015); 5. Liang L (2008); 6. Liang L (2012); 7. Liu L (2008); 8. Lu L (2007); 9. Lu L (2008); 10. Ou X (2014a); 11. Peng Y (2005); 12. Peng Y (2012); 13. Ren H (2014); 14. Ryu S (1997); 15. Sun M (2007); 16. Sun M (2008); 17. Wang Q (2010); 18. Wang X (2008); 19. Wang X (2008a); 20. Xiang Y (2015); 21. Xiao Z (2013); 22. Zhang S (2014); 23. Zhang Y (2008a); 24. Zheng Y (2014); 25. Zhou X (2009); 26. Zou L (2016).

Table 8.2 lists 37 proteins that were reported to have been regulated by these compounds. Due to diversity in the names used, these were standardised to UniProt nomenclature (http://www.uniprot.org/) with the UniProt gene name being adopted as the abbreviation. The tumour-related bio-process was based on the description in UniProt ‘GO - Biological process’. The proteins in Table 8.2 have been associated with the regulation of cell proliferation, cell cycle progression, cell

migration, inflammation and angiogenesis. Each of these proteins is discussed in the following sections.

**Table 8.2: Proteins regulated by *Sophora flavescens* compounds in colorectal cancer studies *in vitro* and *in vivo***

| Target (name used in study) | Uniprot entry | Uniprot gene name | Uniprot protein name | Tumour-related bio-process | *Study N |
|---|---|---|---|---|---|
| Akt | P31749 | AKT1 | RAC-alpha serine/threonine-protein kinase | Regulation of proliferation, cell survival, growth and angiogenesis | 22 |
| Bad | Q92934 | BAD | Bcl2-associated agonist of cell death | BH3 domain only Bcl-2 protein, sensitizer, pro-apoptosis | 22 |
| Bax | Q07812 | BAX | Apoptosis regulator BAX | Multi-domain Bcl-2 proteins, pro-apoptosis | 1, 22 |
| Bcl-2 | P10415 | Bcl-2 | Apoptosis regulator Bcl-2 | Multi-domain Bcl-2 protein, anti-apoptosis | 1, 12, 18, 22 |
| caspase-3 | P42574 | CASP3 | Caspase-3 | Caspase executor, pro-apoptosis. | 1, 6, 18 |
| caspase-7 | P55210 | CASP7 | Caspase-7 | Caspase executor, pro-apoptosis. | 6, 16 |
| caspase-9 | P55211 | CASP9 | Caspase-9 | Caspase activator, pro-apoptosis. | 1, 6, 18, 22 |
| CyclinD1 | P24385 | CCND1 | G1/S-specific cyclin-D1 | Pro-cell cycle regulator | 9, 19, 22 |
| Cyclin E1 | P24864 | CCNE1 | G1/S-specific cyclin-E1 | Pro-cell cycle regulator | 8 |
| CDK2 | P24941 | CDK2 | Cyclin-dependent kinase 2 | Signal transducer, pro-cell cycle regulator | 8 |
| CDK4 | P11802 | CDK4 | Cyclin-dependent kinase 4 | Signal transducer, pro-cell cycle regulator | 9, 12, 20 |
| Cyto C | P99999 | CYCS | Cytochrome c | Caspase activator, pro-apoptosis. | 1 |
| E2F1 | Q01094 | E2F1 | Transcription factor E2F1 | Transcription activator, regulation of G1/S transition of mitotic cell cycle | 9 |
| GSK-3b | P49841 | GSK3B | Glycogen synthase kinase-3 beta | Glucose homeostasis regulator, intracellular signal transduction | 22 |
| IKKβ | O14920 | IKBKB | Inhibitor of nuclear factor kappa-B kinase subunit beta | Signal transducer, Inhibitor of nuclear factor kappa-B kinase | 18 |
| IL-6 | P05231 | IL6 | Interleukin-6 | Cytokine, pro-inflammation | 12, 23 |
| MEK1/2 | Q02750 | MAP2K1 | Dual specificity mitogen-activated protein kinase kinase 1 | Signal transducer of the MAPK/ERK cascade | 26 |
| c-myc | P01106 | MYC | Myc proto-oncogene protein | Transcription factor, positive regulation of cell proliferation. | 12 |
| MMP2 | P08253 | MMP2 | 72 kDa type IV collagenase | Ubiquitinous metalloproteinase, extracellular matrix disassembly, pro- | 13 |

| Target (name used in study) | Uniprot entry | Uniprot gene name | Uniprot protein name | Tumour-related bio-process | *Study N |
|---|---|---|---|---|---|
| | | | | metastasis. | |
| MMP9 | P14780 | MMP9 | Matrix metalloproteinase-9 | Extracellular matrix disassembly, pro-metastasis. | 13 |
| NF-κB1 | P19838 | NFKB1 | Nuclear factor NF-kappa-B p105 subunit | Transcription factor, regulation of inflammation, differentiation, cell growth, tumourigenesis and apoptosis. | 18 |
| OCLN | Q16625 | OCLN | Occludin | Bicellular tight junction assembly, tumour metastasis inhibitor | 12 |
| P21 | P38936 | CDKN1A | Cyclin-dependent kinase inhibitor 2A | Inhibitors in cell cycle progression. | 8, 22 |
| P27 | P46527 | CDKN1B | Cyclin-dependent kinase inhibitor 1 | Inhibitors in cell cycle progression | 8, 22 |
| P16 | P42771 | CDKN2A | Cyclin-dependent kinase inhibitor 1B | Inhibitors in cell cycle progression | 20 |
| P38 | Q16539 | MAPK14 | Mitogen-activated protein kinase 14 | MAP kinase signal transduction, | 13 |
| PARP | P09874 | PARP1 | Poly [ADP-ribose] polymerase 1 | DNA repair gene, negative regulation of apoptosis | 6 |
| PSMD9 | O00233 | PSMD9 | 26S proteasome non-ATPase regulatory subunit 9 | Transcription co-activator | 12 |
| p65 | Q04206 | RELA | Transcription factor p65 | Regulation of inflammation, differentiation, cell growth, tumourigenesis and apoptosis. | 18 |
| RhoA | P61586 | RHOA | Transforming protein RhoA | Regulation of cell migration and adhesion assembly and disassembly | 4 |
| Skp2 | Q13309 | SKP2 | S-phase kinase-associated protein 2 | Pro-cell cycle progression | 9 |
| STAT3 | P40763 | STAT3 | Signal transducer and activator of transcription 3 | Regulation of inflammation, cell cycle progression, apoptosis and angiogenesis. | 3 |
| hTERT | O14746 | TERT | Telomerase reverse transcriptase | Apoptosis suppressor, negative regulation of cellular senescence | 11, 22 |
| TNF-α | P01375 | TNF | Tumour necrosis factor | Cytokine, regulation of inflammation and proliferation. | 12, 23 |
| p53 | P04637 | TP53 | Cellular tumour antigen p53 | Tumour suppressor, induces growth arrest or apoptosis | 9, 12, 17, 18 |
| TUBA1A | Q71U36 | TUBA1A | Tubulin alpha-1A chain | Regulation of G2/M transition of mitotic cell | 12 |

| Target (name used in study) | Uniprot entry | Uniprot gene name | Uniprot protein name | Tumour-related bio-process | *Study N |
|---|---|---|---|---|---|
| | | | | cycle, cell division | |
| VEGF | P15692 | VEGFA | Vascular endothelial growth factor A | Pro-angiogensis | 17 |

***Study N (**1st author (year)**)**: 1. Chang C (2013); 2. Huang J (2007); 3. Kim B (2013); 4. Li N (2015); 5. Liang L (2008); 6. Liang L (2012); 7. Liu L (2008); 8. Lu L (2007); 9. Lu L (2008); 10. Ou X (2014); 11. Peng Y (2005); 12. Peng Y (2012); 13. Ren H (2014); 14. Ryu S (1997); 15. Sun M (2007); 16. Sun M (2008); 17. Wang Q (2010); 18. Wang X (2008); 19. Wang X (2008a); 20. Xiang Y (2015); 21.Xiao Z (2013); 22. Zhang S (2014); 23. Zhang Y (2008); 24. Zheng Y (2014); 25. Zhou X (2009); 26. Zou L (2016).

## 8.3 Effects of *S. flavescens* compounds on proliferation in colorectal cancer cells

The *S. flavescens* compounds and extracts were examined *in vitro* and *in vivo* at different doses and for different treatment durations. The cell inhibitory rates (IR) and apoptosis rates (compared to negative control) are shown in Table 8.3. Of the six cell-lines, the most commonly tested was HT-29. The results showed inhibition of proliferation in time- and dose-dependent manners. In Chapter 7, matrine inhibited the growth of the CRC cell lines LS 174T, Caco-2, SW1116 and RKO in a dose- and time-dependent manner. This result was consistent with other studies.

In SW620 cells, various alkaloids inhibited proliferation in dose-dependent manners. The inhibition rates, from lowest to highest, were: *S. flavescens* total alkaloids (10.2%), oxymatrine (18.8%), sophorcarpine (21.4%), matrine (32.5%) and sophoridine (58.8%) (Liang et al., 2008). Xiang (2015) reported that oxymatrine (8-32 μmol/L) reduced survival of SW620 cells dose- and time-dependently (Xiang, et al., 2015).

Cell morphology studies of HT-29, SW480 and SW620 cells showed they had at least partially undergone apoptotic changes, and compared to negative controls these changes were significant in both a dose-dependent and a time-dependent manner (Huang et al., 2007; Liang et al., 2008; Wang et al., 2008; Zou et al., 2016). In Chapter 7, the morphological changes of apoptosis were observed for matrine, which is consistent with previous studies.

The half inhibitory concentration ($IC_{50}$) expresses the effectiveness of a drug in inhibiting proliferation. A smaller value of $IC_{50}$ indicates more effectiveness. The results for $IC_{50}$ in HT-29 and HC116 indicated oxymatrine was more effective than matrine (Liu et al, 2008) (Table 8.4). In SW620 cells, the $IC_{50}$ values, from largest to smallest were: oxymatrine (4.57 ± 0.47 mM), sophorcarpine (4.43 ± 0.48 mM), matrine (4.28 ± 0.18 mM) and sophoridine (3.82 ± 0.43 mM). Sophoridine appeared to be the most effective in inhibiting proliferation among these alkaloids (Zheng, 2014). This ranking was the same as for apoptotic rate in Liang (2008) (see Table 8.1).

**Table 8.3: Inhibitory rate (IR) and apoptosis rate (AR) in colorectal cancer cells for *S. flavescens* compounds**

| Cell-line | Compound | IR%, (dose, treatment time) | AR %,(dose, treatment time) | Reference |
|---|---|---|---|---|
| HT-29 | Matrine | 17.88 ± 3.51, 45.96 ± 3.95, 59.35 ± 3.36 (4, 8 or 16 mg/mL, 24hs); 42.48 ± 2.78, 58.20 ± 4.33, 68.29 ± 4.79 (4, 8 or 16 mg/mL, 36hs) | 16.53, 34.11, 54.83 ( for 4, 8 or 16 mg/mL, 24hs) | Chang C et al., 2013 |
| HT-29 | Matrine | 86. 69 ± 2. 38 (0.5mg/mL, 48hs) | 1.12 ± 0.45, 2.08 ± 0.76, 2.22 ± 0.64, 2.98 ± 0.03, 27.46 ± 4.39 (0.0625, 0.125, 0.25, 0.5, 1.0 mg/mL, 48hs) | Huang J et al., 2007 |
| HT-29 | Matrine | 33.7, 59.0, 66.7, 69.0 (3, 6, 8, 10μg /L, 24 hs) | 15 .31, 17.99, 26.58 (5 μg /L,24,48,72hs) | Peng Y et al., 2005 |
| HT-29 | Matrine | Reported in figure. IR increased on dose dependent. (0.0-2.0mg/mL, 24h) | nr | Ren H et al., 2014 |
| HT-29 | Kushen ethanol extract | 52.0 (2.5 mg/mL, 24 hs) | nr | Xiao Z et al., 2013 |
| SW620 | Matrine | 25.13 ± 1.14, 28.77 ± 1.08 42.06 ± 1.37 (0.5, 1.0, 1.5 mg/mL, 24hs); 42.46 ± 1.43, 51. 84 ± 1. 58, 71.28 ± 1.65 (0.5, 1.0, 1.5 mg/mL, 48hs) | 9.44 ±2.32, 15. 87 ± 1. 63, 24.33 ± 1.45 (0.5, 1.0, 1.5 mg/mL,48hs) | Li N, 2015 |
| SW620 | Matrine | 32.5 (1.0mg/mL, 24hs) | 5.5 ± 1.0, 12.8 ± 3.0, 33.5 ± 3.3, 46.2 ± 2.5 (1.25mg/mL, 0, 12, 24, 48hs) | Liang L et al., 2008 |
| SW620 | Oxymatrine | 18.8 (1.0mg/mL, 24hs) | nr | Liang L et al., 2008 |
| SW620 | Sophocarpine | 21.4 (1.0mg/mL, 24hs) | nr | Liang L et al., 2008 |
| SW620 | Sophoridine | 58.8 (1.0mg/mL, 24hs) | 5.0 ± 1.0, 14.7 ± 3.0, 37.1 ± 4.0, 56.0 ± 4.5 (1.0mg/mL, 0, 12, 24, 48hs) | Liang L et al., 2008 |
| SW620 | Total alkaloids | 10.2 (1.0mg/mL, 24hs) | nr | Liang L et al., 2008 |
| SW620 | Oxymatrine | *CVR: 42.32 ± 3.37, 32.52 ± 2.29, 27.17 ± 1.24 (8, 16, 32μmol/L, 24hs); 32.21 ± 2.36, 20.23 ± 1.83, 16.18 ± 1.19 (8, 16, 32μmol/L, 48hs); 5-FU: 48.32 ± 4.68, 40.21 ± 2.39 (4mg/L, 24, 48hs). | nr | Xiang Y, 2015 |
| SW620 | Matrine | 38.38 ± 11.72, 37.89±8.58, 59.60 ± 8.80(0.5, 1.0, 1.5 mg/mL, 24 hs); 24.11 ± 5.34, 45.57 ± 16.44, 56.11 ± 10.33 (0.5, 1.0, 1.5 mg/mL, 48hs) | nr | Wang X, 2008a |
| SW480 | Sophoridine | 55.69 ± 1.77 (1.0 mg/mL, 48hs) | 38.7 ± 3.4 (0.8 mg/mL, 48 hs) | Liang L et al., 2012 |
| SW480 | Matrine | 36.08 ± 6.88, 53.86 ± 5.43, 68.44 ± 5.90 (0.5, 1.0, 1.5 mg/mL, 24 hs); 45.77 ± 7.20, 67.68 ± 3.58, 89.46 ± 10.50 (0.5, 1.0, 1.5 mg/mL, 48hs) | 3.77 ± 0.47, 22.73 ± 1.78 (0.5 mg/mL, 24, 48 hs) | Wang X, 2008 |
| SW480 (K-Ras mutation) | Matrine | 0.71 ± 0.01, 1.27 ± 0.01, 1.47 ± 0.02 (0.125 mg/mL, 24, 48, 72hs) | 3.38 ± 0.10, 4.06 ± 0.10, 4.40 ± 0.13, 7.54 ± 0.12, 10.78 ± 0.45 (0.125, 0.25, 0.5, 1 mg/mL, 24 hs | Zou L et al., 2016 |
| SW1116 | Matrine | Reported in figure on dose and time dependent. (0.0-2.0mg/mL, 24, 48, 72, 96h) | nr | Zhou X et al., 2009 |
| LOVO | Oxymatrine | 17.74 ± 7.54, 27.51 ± 8.79, 34.90 ± 5.11(1.0 mg/mL, 6, 24, 48hs); 34.42 ± 9.65, 54.36 ± 6.97, 67.64 ± 7.87 (2.0mg/mL, 6, 24, 48hs) | 4.07 ± 1.77, 6.08 ± 1.59, 7.40 ± 1.38 (0.1 mg/mL, 6, 24, 48hs); 4.27 ± 1.66, 8. 27 ± 2. 77,9.80 ± 1.32 (0.2 mg/mL, 6, 24, 48hs) | Peng Y et al., 2011 |
| LOVO | Matrine | Reported in figure. IR increased on dose and time dependent (0.0-2.0mg/mL, 24, 48, 72h) | 8.6, 16.7, and 24.4 %, (0.4, 0.8, 1.6mg/mL. 48 hs) | Zhang S et al., 2014 |
| DLD1 | Matrine | Reported in figure. IR increased on dose dependent (0.0-2.0mg/mL, 24h) | nr | Ren H et al., 2014 |

CVR: cell viability rate; IR: inhibition rate; AR: apoptosis rate; nr: no report

Zhou (2009) reported that the $IC_{50}$ of matrine was 1.13 mg/mL (48h) in SW1116 cells. However, in this project, the $IC_{50}$ of matrine was 1.18 mM (=0.293 mg/mL, 48h) (see Chapter 7). The reason for this difference in the $IC_{50}$ value on SW1116 cells between the two studies was unclear. There are several factors that could affect to the result, such as human error, accuracy of equipment, purification of the test drug, and variation in the cell-line or contamination of the test cells.

In CaCo-2 cells, kurarinone had the smallest $IC_{50}$ value compared to *ku shen* flavonoids and sophoraflavanone G. (Sun et al., 2007). In a study of 15 flavanoids in HCT15 CRC cells, kushenol B had the lowest $IC_{50}$ value (3.0 ± 0.2 µg/mL, 48 hs) and was closest to the positive control cisplatin (2.2 ± 0.4 µg/mL, 48 hs) (Ryu et al., 2013) (Table 8.4).

**Table 8.4: $IC_{50}$ in colorectal cancer cell-lines of *S. flavescens* compounds**

| **Compound** | **Cell-line ($IC_{50}$ unit, hs)** | **Reference** |
|---|---|---|
| | **CaCo-2** | |
| Kurarinone | 13 µg/mL, 48hs | Sun M et al., 2007 |
| Sophoraflavanone G | 16 µg/mL, 48hs | Sun M et al., 2007 |
| 2'-methoxy-kurarinone | 27 µg/mL, 48hs. | Sun M et al., 2007 |
| Kushen flavonoids (KS-Fs) | 29 µg/mL, 48hs | Sun M et al., 2007 |
| | **DLD1** | |
| Matrine | 1.569 mg/mL, 24hs | Ren H et al., 2014 |
| | **HCT15** ($ED_{50}$ µg/mL,48hs) | |
| Kushenol B | 3.0 ± 0.2 | Ryu S et al., 2013 |
| Kushenol E | 4.6 ± 0.3 | Ryu S et al., 2013 |
| Kuraridin | 5.0 ± 0.1 | Ryu S et al., 2013 |
| Kushenol M | 5.1 ± 0.3 | Ryu S et al., 2013 |
| Sophoroflavanone G | 5.7 ± 0.3 | Ryu S et al., 2013 |
| Kosamol A | 6.5 ± 0.2 | Ryu S et al., 2013 |
| Kushenol L | 8.4 ± 0.3 | Ryu S et al., 2013 |
| Kurarinone | 8.6 ± 0.3 | Ryu S et al., 2013 |
| Kushenol N | 14.5 ± 0.1 | Ryu S et al., 2013 |
| Norkurarinol | 16.5 ± 0.3 | Ryu S et al., 2013 |
| Trifolirhizin | 21.0 ± 0.4 | Ryu S et al., 2013 |
| Kurarinol | 28.7 ± 0.4 | Ryu S et al., 2013 |
| Kushenol H | >50 | Ryu S et al., 2013 |
| Kushenol K | >50 | Ryu S et al., 2013 |
| Formononetin | >50 | Ryu S et al., 2013 |
| Cisplatin (pos. control) | 2.2 ± 0.4 | Ryu S et al., 2013 |
| | **HCT116** | |
| Oxymatrine | 11.61 ± 4.06 µg/mL, 48hs, | Liu L et al., 2008 |
| Matrine | 158.62 ± 19.02 µg/mL, 48hs, | Liu L et al., 2008 |

| Compound | Cell-line ($IC_{50}$ unit, hs) | Reference |
|---|---|---|
| | **HCT116 $p53^{+/+}$** | |
| Matrine | 3.25 ± 0.16 mM, 72hs | Ou X, 2014 |
| | **HT29** | |
| Matrine | 1.569 mg/mL, 24hs | Ren H et al., 2014 |
| Oxymatrine | 17.89 ± 3.04 μg/mL, 48hs | Liu L et al., 2008 |
| Matrine | 28.11 ± 1.02 μg/mL,48hs | Liu L et al., 2008 |
| Kushen flavonoids (KS-Fs) | 21.95 μg/mL, 48hs | Sun M et al., 2008 |
| | **LoVo** | |
| Matrine | 2.62 mg/mL, 24hs | Wang X, 2008 |
| Matrine | 0.738 mg/mL, 48hs | Zhang S et al., 2014 |
| | **SW480** | |
| Sophoridine | 0.78045 mg/mL (median), 48hs | Liang L et al., 2012 |
| Matrine | 0.81 mg/mL, 24hs | Wang X, 2008 |
| | **SW480 (K-Ras mutation)** | |
| Matrine | 0.66, 0.59, 0.45 mg/mL, 24, 48, 72hs | Zou L et al., 2016 |
| | **SW620** | |
| Matrine | 1.01 mg/mL, 24hs | Wang X, 2008a |
| Sophoridine | 3.818 ± 0.425 mM, 48hs | Zheng Y et al. 2014 |
| Matrine | 4.284 ± 0.181 mM, 48hs | Zheng Y et al., 2014 |
| Sophocarpine | 4.431 ± 0.484 mM, 48hs | Zheng Y et al. 2014 |
| Oxymatrine | 4.571 ± 0.467 mM, 48hs | Zheng Y et al. 2014 |
| Kushen flavonoids (KS-Fs) | 18.26 μg/mL, 48hs | Sun M et al., 2008 |
| | **SW1116** | |
| Matrine | 1.74, 1.13, 0.89, 0.65 mg/mL, 24, 48, 72, 96hs | Zhou X et al., 2009 |

IC50: The half maximal inhibitory concentration; $ED_{50}$: value of compound against each cancer cell line, defined as the concentration that caused 50% inhibition of cell proliferation *in vitro*.

Xenograft tumour studies of HT-29 and SW480 cells in mice further supported the anti-cancer effects of matrine and sophoridine. The tumour growth inhibition rates were significantly higher in these cells than the negative controls (Table 8.5). The anti-tumour effect of the high-dose sophoridine was not inferior to the 5-FU positive control (Liang et al., 2012).

Overall, *S. flavescens* alkaloids and flavonoids inhibited cell proliferation in a variety of CRC cell-lines. Among the alkaloids, sophoridine appeared more effective. Sophoridine and matrine also demonstrated inhibition of tumour growth *in vivo*. Of the *S. flavescens* flavonoids, a number have shown anti-tumour effects but direct comparison between specific flavonoids and alkaloids in the same CRC cell lines were not available. However, it is notable that one study in other cancer cell-line studies, found the flavonoids to be more effective than the alkaloids (Sun 2007). Future *in vitro* and *in vivo* studies could focus on selecting the most effective compounds, or combinations of alkaloids and

flavonoids. Such studies should include anti-proliferative assays, toxicity assessment, and pharmacokinetics.

**Table 8.5: Tumour growth inhibition in colorectal cancer studies *in vivo* for *S flavescens* compounds**

| Xenograft tumour | Compound | Tumour growth inhibitory rate %(IR), (dose, treatment time) | Others | Reference |
|---|---|---|---|---|
| HT29 | matrine | Proximally 50% reduction of volume and weight of tumour (intragastric administration (60 mg/kg/day, for 21 days) | No. of lung metastatic sites decreased 42.47% | Ren et al., 2014 |
| SW480 | sophoridine | 62.4 ± 4.3 (25 mg/kg/day, 5 days/wk, ip. 4weeks) | IR compared to 69.0 ± 2.3% for 5-FU (30 mg/kg, 3 days/week) p>0.05 | Liang et al., 2012 |
| SW480 (p53mutant) | sophoridine | 34.07 (16.9 mg/kg/day, ip, 28 days.) | na | Wang et al., 2010 |
| SW480-EGFP | matrine | 59.52 (80 mg/kg/day, ip, 4 weeks) | $ID_{50}$ of matrine to mice was 157.13 mg/kg, 95% (88.08, 280.31) | Wang, 2008 |

Ip: intraperitoneal injection; $ID_{50}$: the infective dose that will cause 50% of exposed individuals to become ill.

### 8.3.1 **Outline of the cell cycle**

The cell cycle is a cascade event that consists of four different phases: Gap1 (G0), Synthesis (S), Gap 2 (G2), and Mitosis (M) phases. Cells in the Gap 0 (G0) phase are not in the process of proliferation. The cell cycle initiates in response to mitogenic growth factors stimulating the G1 phase. In this phase, the cell increases supplies of its nutrients, and increases in size. DNA synthesis commences in the S phase, all DNA are replicated and chromosomes double by the end of this phase. In the G2 phase, proteins are synthesised rapidly and there is continuous growth of the cell in readiness for mitosis in the M phase. In the M phase, the cell completes the process of dividing the chromosomes into two identical sets, followed by dividing cell components including nuclei, cytoplasm, organelles and cell membrane into two identical daughter cells (cytokinesis) (Strachan, 2015).

The progression of the cell cycle in each phase is positively regulated by the combination of cyclin dependent kinases (CDKs) and their subunits – cyclins, as complexes of cyclins/CDKs. CDKs are present throughout the cell cycle, whereas cyclins are synthesised and degraded in response to cellular signals at various cell cycle phases. The newly synthesised cyclin binds to the CDK and activates it. The activated cyclin/CDK complexes phosphorylate target proteins positively or negatively in each phase in order to progress the cell cycle sequentially (Diehl J, 2002). Mutant cyclin D can result in cell cycle changing from mitogenic dependent progression to enter into mitogenic independent progression (Sherr and Roberts, 1999). The development of inhibitors of CDKs attracts researchers interested in the development of novel anti-cancer drugs.

#### *8.3.1.1 Cell cycle checkpoints*

Cell cycle checkpoints allow cells to monitor and regulate cell cycle progression. There are three important checkpoints in the cell cycle:

- G1/S checkpoint: where the cell checks its preparation to fully replicate its DNA;
- G2/M checkpoint: where the cell checks the cell contents (e.g. cytoplasm and phospholipids) to ensure there is sufficient for two daughter cells, and determines when to replicate;
- metaphase (mitotic) checkpoint: where the cell checks the chromosomes are ready before anaphase begins.

During DNA replication and chromosome division, any deficiency could activate a checkpoint that could slow cell cycle progress or induce cell cycle arrest to allow the cell to repair the deficiency by modulating CDK activities. If the damage is irreversible, cell apoptosis will occur (Malumbres and Barbacid, 2009). Cancer cells with dysfunctional checkpoint control are more sensitive to additional genotoxic or microtubular damage. Therefore, components of cell cycle checkpoints are potential targets for new anti-cancer drugs (Shapiro and Harper, 1999).

#### *8.3.1.2 G1 phase*

In a cell in the resting G0 phase, cell cycle is initiated by mitogenic growth factors through extracellular signalling pathways (such as the MAPK signalling pathway). Progression of the cell cycle from the G0 phase into the G1 phase is dependent on mitogenic growth factors up until the late G1 phase which is the G1/S checkpoint, also referred to as the restriction point. Beyond this point, the cell cycle does not require mitogenic growth factors in order to progress (Zetterberg et al., 1995; Diehl J, 2002). In cancer, due to mutation and dysregulation of genes or signalling pathways, cell cycle initiates independently of mitogenic growth factors and the proliferation becomes uncontrollable (Fouad & Aanei, 2017).

#### *8.3.1.3 Cyclin D/CDK4/6 complex and cyclin E/CDK2 complex*

In humans, cyclin D has three homologues: cyclin D1, cyclin D2 and cyclin D3. In the G1 phase, stimulation of mitogenic growth factors triggers cyclin D production and assembly with CDK 4/6 to form the activated cyclin D/CDK4 complex, which then moves to the nucleus (Diehl J, 2002.). The p27 and p21 members of the CDK interacting protein/kinase inhibitory protein (cip/kip) family act as assembly factors by binding to cyclin D and CDK4 in the cytoplasm and promoting movement of the cyclin D complex to the nucleus (Coqueret et al., 2002).

The assembly and nuclear localisation of the cyclin D/CDK4 complex phosphorylates retinoblastoma protein (Rb). This inactivates Rb as a repressor of E2F1, which initiates the subsequent activation of

genes that promote cell cycle progression (Tannoch et al., 2000). The released E2F1 activates the cyclin E/CDK2 complex. The activation of cyclin E/CDK2 complex can inactivate cip/kip proteins and Rbs through phosphorylation and promote a positive feedback loop guarding the cell cycle's progression from the G1 phase to the S phase. This is the restriction point in the G1/S phase. Withdrawal of mitogens before the restriction point will result in the release of the cip/kip proteins that are retained by the cyclin D/CDK4 complex. The cip/kip proteins inhibit cyclin D/CDK4 and cyclin E/CDK2 complexes and induce cell cycle arrest during the G1 phase. Cyclin D acts as a link between the mitogen-dependent and mitogen-independent stages of the G1 phase and is not involved in the rest of the cell cycle. The initiation of phosphorylation of Rb by cyclin D in the G1 phase is maintained by cyclin E in the transition from the G1 phase to the S phase, and by cyclin A and cyclin B in later stages of the cell cycle until the cell exits mitosis. After mitosis, retinoblastoma protein returns to a hypophosphorylated state in readiness for the next G1 phase (Sherr & Roberts, 1999; Diehl, 2002). Overexpression of cyclin D1 and cyclin E is associated with early adenoma development and later carcinoma genetic progression in CRC (Hur et al., 2000).

#### *8.3.1.4 CDK inhibitor families*

Progression from the G1 phase to the S phase is negatively regulated by two CDK inhibitor families: the inhibitor of kinase 4/alternative reading frame (INK4/ARF) family and the cip/kip family. These inhibitor families are known as tumour suppressors because they can inhibit cell cycle progression. The inhibitors of kinase 4 (p16INK4A, p15INKB, p18INK4C and p19INK4D) only bind to CDK4/6 and inhibit catalytic activity in the early G1 phase. The cip/kip family (p21, p27 and p57) can bind to both cyclins and CDKs, and inhibits the catalytic activity of cyclin D and E in the G1/S phases (Sherr & Roberts, 1999). Also, p21 and p27 are required in the cyclin D/CDK4 complex assembly and nuclear localisation under mitogenic stimuli in the early G1 phase (LaBaer et al., 1997; Coqueret et al., 2002).

#### *8.3.1.5 Roles of Skp2, GSK-3β, c-Myc, p53 in G1 phase*

S-phase kinase-associated protein 2 (Skp2) is a member of the F-box protein family. The Skp2/SCF complex (including Skp1, Cullin-1, F-box protein Skp2 and Rbx1) can inhibit cip/kip by ubiquitin-mediated degradation, which activates cyclin E/CDK2 and leads to G1/S transition (Ungermannova et al., 2005; Wang et al., 2005). Overexpression of Skp2 has been found in several cancers. Skp2 is a substrate of Akt, which regulates Skp2 activities in terms of mediating cell cycle progression, cell migration and tumourigenesis. Thus, Skp2 may be an ideal target in future cancer therapy (Chan et al., 2010).

The role of cyclin D as a G0/G1 initiator is also negatively regulated by glycogen synthase kinase-3B (GSK-3β). Phosphorylation of cyclin D by GSK-3β induces export of cyclin D from the nucleus and subsequent ubiquitin-dependent degradation, thereby halting the G1 phase (Sherr & Roberts, 1999).

Inhibition of GSK-3β activity through the Ras/PI3K/Akt pathway results in cyclin D1 nuclear localization and stabilization (Diehl, 2002). In addition, activation of GSK-3β can inhibit Skp2 expression, increasing nuclear import of p27Kip1 which binds to CDK2 and inhibits its activity (Wang et al., 2008).

The MYC family includes three nuclear phosphoproteins: c-MYC, N-MYC and L-MYC. The oncogenic transcription factor c-Myc participates in cell proliferation, transformation and death. Growth factors and mitogenic signals regulate c-Myc expression via the MAPK/ERK pathway. Deregulation and overexpression of c-Myc have been frequently found in both inflammatory bowel disease and CRC (Sipos et al., 2016). The transcription factor c-MYC promotes G1/S progression by directly targeting MYU (c-Myc upregulated IncRNA, LOC100128881, NCBI: NR_036480.1), which interacts with the RNA binding protein hnRNP-K to stabilise CDK6 expression and binds with cyclin D to form cyclinD/CDK complexes, thereby activating G1 progression (Kawasaki et al., 2016). Molecular agents that target c-Myc or other genes acting in the c-Myc pathway are of interest as future CRC treatments (The Cancer Genome Atlas Network, 2013). DNA damage can activate p53 which binds to p21 (WAF1), thereby activating p21 to inhibit cyclin D and induce cell cycle G1 arrest (Li et al., 2015).

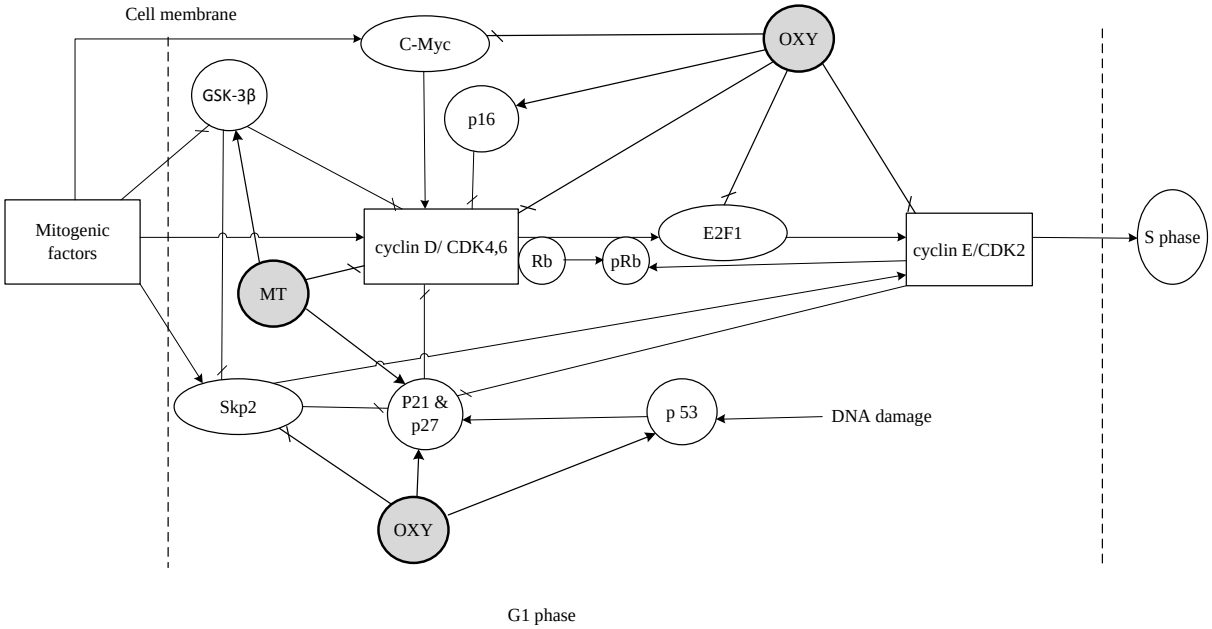


**Figure 8.1: Major proteins involved in the G1 phase of the cell cycle and the actions of S. flavescens compounds**

Proteins: GSK-3β - Glycogen synthase kinase-3 beta; C-Myc - Myc proto-oncogene protein; p16 - Cyclin-dependent kinase inhibitor 1B; E2F1 - Transcription factor E2F1; Rb – Retinoblastoma; p53 - Cellular tumour antigen p53; p21 - Cyclin-dependent kinase inhibitor 2A; p27 - Cyclin-dependent kinase inhibitor 1; Skp2 - S-phase kinase-associated protein 2.

*S. flavescens* compounds: MT – matrine; OXY- oxymatrine. '⊦' : down-regulation; '←': up-regulation

#### *8.3.1.6 Effects of matrine and oxymatrine on cell cycle arrest at G1*

Matrine dose-dependently induced cell cycle arrest at the G1 phase in HT-29 (Chang et al. 2013; Huang et al., 2007), SW480, SW620 (Wang X, 2008) and LoVo cell lines (Zhang et al., 2014). Oxymatrine dose-dependently induced cell cycle arrest at the G1 phase in SW1116 (Lu and Ran, 2007; Lu et al., 2008), SW620 (Xiang et al., 2015) and LoVo (Peng et al., 2012) cell lines.

In SW1116 cells, oxymatrine down-regulated the expression of cyclin D1, CDK4, cyclin E, E2F1 and Skp2 at the mRNA and protein levels. Oxymatrine up-regulated expression of p53, p21 and p27 at the mRNA and protein levels, whereas CDK2 expression was only down-regulated at the mRNA level. These effects were both time dependent and dose dependent (Lu and Ran, 2007; Lu et al., 2008). SW620 cells treated with oxymatrine showed significant up-regulation of p16 and down-regulation of cyclin D and CDK4 (Xiang et al., 2015).

In LoVo cells, oxymatrine suppressed the mRNA expression of CDK4 and c-Myc at a dose of 0.5 g/L at 48 and 96 hours. However, at a dose of 0.25 g/L at 48 hours, oxymatrine only suppressed the c-Myc mRNA expression and had no significant effect on CDK4. It is possible that c-Myc is more sensitive to low-dose oxymatrine in LoVo cells (Peng et al., 2012). Matrine dose-dependently suppressed protein expression of cyclin D1, up-regulated p27 and p21, and increased phosphorylated GSK-3b (activation) in LoVo cells (Zhang et al., 2014). The actions of matrine and oxymatrine on protein targets in G1 phase are shown in Figure 8.1.

In summary, oxymatrine and matrine both induced cell cycle arrest at the G1 phase in multiple CRC cell lines. The mechanism of the induction of cell cycle arrest was suppression of cyclin/CDKs (cyclin D1, CDK4, cyclin E and CDK2) and the promotion of expression of the CDK inhibitors p16, p21 and p27. These compounds also regulate cyclin/CDKs via associated transcription factors and signalling proteins including p53, E2F1, GSK-3B, Skp2 and c-Myc. Therefore, matrine and oxymatrine may be ideal candidates for the development of new anti-cancer drugs that target either CDK2/4 and the components of cell cycle entry or the G1/S checkpoints such as Skp2, E2F1, p16, p21 and p27.

### 8.3.2 **Outline of apoptosis**

Apoptosis is one type of programmed cell death. The balance between pro-apoptotic and anti-apoptotic molecules maintains the homeostasis of an organism. Physically, apoptosis eliminates cells that are damaged or non-functioning, and prevents the accumulation of genetically mutant cells. In cancer, malfunctions of apoptotic mechanisms that favour anti-apoptosis lead to oncogenesis and the development of resistance to antitumour drugs. Morphological changes during apoptosis include cytoplasmic filament aggregation, chromatin condensation, DNA fragmentation, plasma membrane blebbing and packing of cytosolic ingredients that result in formation of the apoptotic body. After

apoptosis, the cell is phagocytised by neighbouring cells. Two classical signalling pathways are involved in apoptotic processes: the intrinsic pathway and the extrinsic pathway. Two families of proteins are important in the regulation of apoptosis: the caspase family and the Bcl-2 family (Kiraz et al., 2016).

#### *8.3.2.1 Intrinsic pathway*

The intrinsic pathway is independent of death ligand or receptor signalling. Cells undergo apoptosis in response to intracellular signalling, which positively or negatively mediates mitochondria-dependent apoptosis events. The positive stimuli (e.g. radiation, toxins, hypoxia, hyperthermia and viral infections) induce apoptosis by directly activating pro-apoptotic factors; the negative stimuli (e.g. deprivation of growth factors, hormones and different cytokines) induce apoptosis by suppressing anti-apoptotic factors. All of these stimuli eventually cause mitochondrial outer membrane permeabilisation (MOMP) and release a set of pro-apoptotic proteins including: cytochrome C, apoptosis-inducing factor (AIF), endonuclease G, Smac/DIABLO (second mitochondria-derived activator of caspase/direct IAP-binding protein with low PI); and the serine protease Omi/HtrA2. The released cytochrome C binds to apoptosis protease activating factor-1 (Apaf-1) and forms the apoptosome complex that leads to activation of caspase-9, which in turn activates caspase 3 and executes cell apoptosis (Kiraz et al., 2016; Plati et al., 2011). The mitochondrial-initiated events (mitochondrial outer membrane permeabilisation and cytochrome C release) are crucial to the intrinsic pathway and are mainly mediated by the Bcl-2 family (Elmore, 2007).

#### *8.3.2.2 Extrinsic pathway*

The extrinsic pathway is initiated by extracellular ligands (e.g. TNF and Fas-L) binding to the extracellular death domain of the transmembrane death receptor (TNFR1, Fas and TRAIL). This results in the transmembrane death receptors binding with the intracellular death domains that interact with death domain-containing adaptor proteins (FADD [FAS-associated via death domain] and TNFR-associated death domain [TRADD]). These adaptor proteins have a death effector domain (DED) that interacts with pro-caspase 8 (which also contains a death effector domain) to form the death-inducing signalling complex (DISC). The death-inducing signalling complex mediates caspase-8 activation, which in turn activates caspases -3, -6 and -7, and leads to cell death (Goldar et al., 2015). Caspase-8 is crucial to the extrinsic pathway. The active caspase-8 also activates BID by truncating BID to form tBID, which translocates to the mitochondria resulting in mitochondria-dependent apoptosis (the intrinsic pathway) (Plati et al., 2011).

#### *8.3.2.3 Caspase family*

The caspase family consists of cysteine aspartic-specific proteases with a N-terminal peptide or pro-domain and two subunits (Cohen G, 1997). The caspase family members are important in the

mediation of apoptotic process. The 14 caspase family members found in mammals are classed into three sub-groups: initiator caspases (caspase-2, -8, -9 and -10), effector caspases (caspase-3, -6, -7 and -14) and cytokine activators (caspase-1, -4, -5, -11, -12 and -13). Caspases are involved in cell death, proliferation, differentiation and inflammation.

In response to apoptotic signals, initiator caspases (caspase-8 or -9) are activated and then activate effector caspases (caspase-3, -6, -7). The activated caspase-3 cleaves the poly (ADP-ribose) polymerase (PARP) protein that promotes DNA repair and leads to the execution of apoptosis. Initiator caspases can be self-activated, whereas effector caspases are activated by initiator caspases through internal cleavage. Caspase-3 is important for the cleavage of PARP and the DNA fragmentation that are hallmarks of the apoptotic process. Caspase-3 can be activated in both the intrinsic and extrinsic pathways (Kiraz et al., 2016).

#### *8.3.2.4 Bcl-2 family*

The Bcl-2 family includes three sub-families: BH3-domain-only proteins (BID, BIM, BAD, PUMA and BIK) that act as sensors of pro-apoptosis signals, pro-apoptosis multi-domain proteins (BAX, BAK and BOK) and anti-apoptosis multi-domain proteins (Bcl-2, Bcl-XL, MCL1, Bcl-W, Bcl-B and A1). The BH3-domain-only proteins are activated under stress stimuli. The activated BH3-only family members directly activate pro-apoptosis multi-domain proteins (e.g. BAX) and neutralise anti-apoptosis multi-domain proteins (e.g. Bcl-2 and Bcl-XL), which results in mitochondria-dependent apoptosis (Plati et al., 2011). The balance of pro- and anti-apoptotic Bcl-2 proteins determines the death or survival of cells. The ratio of Bcl-2 to Bax is an indicator of cell susceptibility to apoptosis, with a decreasing ratio indicating an increase in apoptosis (Khodapasand et al., 2015).

#### *8.3.2.5 Effects S. flavescens compounds on the intrinsic (mitochondrial) pathway*

In HT-29 cells treated with matrine, apoptosis was induced in a dose-dependent manner. There was up-regulation of cleaved caspase-3 and -9, increased cytochrome C translocation to the cytosol, increased Bax expression and decreased expression of Bcl-2, which resulted in a decreased ratio of Bcl-2 to Bax (Chang et al., 2013).

Matrine induced apoptosis in LoVo cells, reduced phosphorylation of Akt (the upstream factor), reduced the ratio of Bcl-2 to Bax and increased phosphorylation of Bad (Zhang et al., 2014). Sophoridine induced apoptosis in SW480 cells up to 67.5±5.9%. Sophoridine time-dependently up-regulated cleaved caspase-3, -7 and -9. The initiator, caspase-9, was cleaved as early as 12 hours after treatment. Cleaved PARP was also up-regulated (Liang et al., 2012).

Among the experimental studies of *S. flavescens* and its compounds, there were no direct tests of the extrinsic pathway. Some studies measured TNF, but these were in relation to inflammation rather than

apoptosis (Zhang et al., 2008). The actions of matrine and sophoridine on protein targets in the intrinsic (mitochondrial) pathway are illustrated in Figure 8.2.

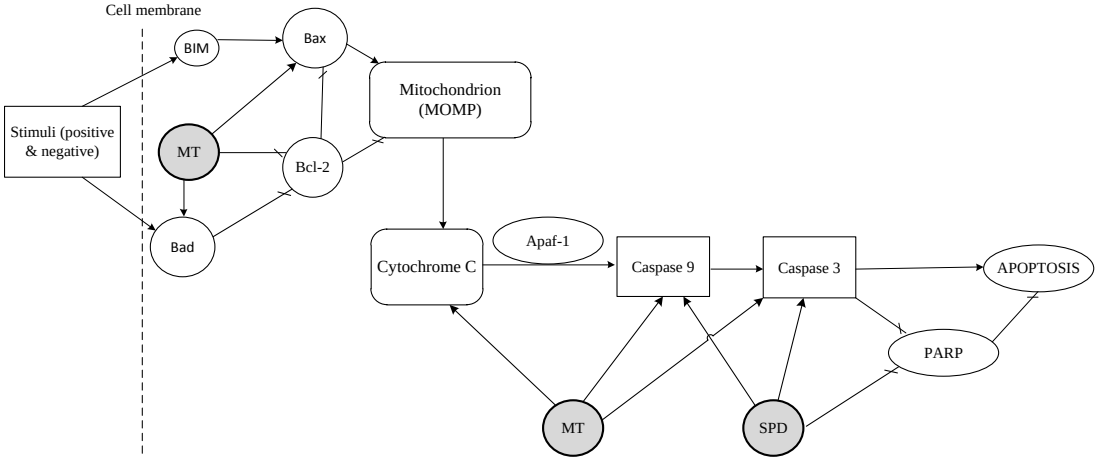


**Figure 8.2: Major proteins involved in the intrinsic (mitochondrial) pathway and the actions of *S. flavescens* compounds**

Proteins: BIM - Bcl-2-like protein 11; Bax - Apoptosis regulator BAX; Bcl-2 - Apoptosis regulator Bcl-2; Bad - Bcl2-associated agonist of cell death; Apaf-1 - Apoptotic protease-activating factor 1; PARP - Poly [ADP-ribose] polymerase 1; MOMP - mitochondrial outer membrane permeabilization.
*S. flavescens* compounds: MT – matrine; SPD – Sophoridine. '⊦' : down-regulation; '←': up-regulation.

## *8.4* Intracellular signalling pathways

Intracellular signalling pathways transmit biological signals from the cell surface to the nucleus. The process of signal transmission responds to extracellular stimuli and involves sequential reactions from the cell surface to a variety of intracellular targets that result in changes in gene expression (Cooper, 2000). The anti-cancer effects of *S. flavescens* compounds appear to involve regulation of key components in the following cellular signalling pathways.

### 8.4.1 **MAPK/ERK pathways**

The mitogen-activated protein kinase (MAPK) signalling pathways are sub-divided into four distinct pathways: extracellular signal-related kinases (ERK1/2), Jun amino terminal kinases (JNK), p38-MAPK and ERK5 (Sun et al., 2015). The MAPK signalling pathways receive extracellular stimuli in response to a broad range of cellular activities including proliferation, differentiation, migration, senescence and apoptosis (MacCorkle & Tan, 2005). The Ras family of GTPases (H-Ras, K-Ras and N-Ras) are involved in signal transduction upstream of the MAPK pathways (Meloche & Pouyssegur, 2007), while Ras oncogenes are involved in many cancers (Fernández-Medarde & Santos, 2011).

The MAPK/ERK pathway is a classical MAPK pathway that is also known as the Ras-Raf-MEK-ERK pathway. MEK refers to the dual-specificity protein kinases MAP2K1/MeK1 and MAP2K2/MeK2. MEK and ERK both play key roles in the MAPK/ERK signalling pathway. Upstream growth factor receptors such as receptor tyrosine kinases (RTK) activate Ras/Raf1 and then further activate MEK/ERK, leading to sequential activation and further transduction of the signal within the

MEK/ERK cascade (Cook et al., 2017). Each of the proteins Raf1, MEk1/2 and ERk1/2 is required for cell proliferation and survival (Liu et al., 2004). In tumours, activated MEK/ERK promotes cell survival by activating pro-survival BcL-2 proteins (BcL-2, BcL-XL and MCL1) and repressing pro-apoptotic proteins (BAD, BIM, BMF and PUMA) (Cook et al., 2017). Mutant Ras is found in about 40% of CRC, and can lead to independent activation of the downstream MEK/ERK and PI3K/AKT pathway without the presence of growth factors (De Roock et al., 2010).

#### *8.4.1.1 Effects of S. flavescens compounds on the MAPK/ERK pathways*

SW480 cells, which have a K-Ras gene mutation, were treated with matrine (0.125, 0.25 and 0.5, 1.0 mg/mL) for 24 hours. The results showed that matrine significantly inhibited the proliferation and migration of the SW480 cells and promoted apoptosis through the down-regulation of MEK1/2 protein expression in the MAPK/ERK pathway (Zou et al., 2016).

### 8.4.2 **PI3K/AKT pathway**

AKTs are serine/threonine-protein kinases, including AKT1, AKT2, and AKT3. Under the stimuli of growth factors, phosphatidylinositide (PI) 3-kinase (PI3K) is activated by receptor tyrosine kinases (RTKs)/Ras. PI3K phosphorylates PIP2, which then converts to PIP3. The activated PIP3 binds to AKT and transports it to the inner face of the plasma membrane where AKT is phosphorylated by phosphatidylinositide (3,4,5) P3-dependent protein kinase-1 (PDK1). Activated AKT phosphorylates downstream target proteins that promote survival and proliferation (Arcaro & Guerreiro, 2007; Cooper, 2000). This pathway is also called the Ras /PI3K/Akt pathway, since Ras is an upstream activator.

In CRC, AKT mediates proliferation and metastasis via the PI3K/AKT pathway (Hu et al., 2017; Li et al., 2017; Sun et al., 2017). High expression (68.3%) of active AKT has been reported in CRC (Zhang et al., 2012), while suppression of AKT pathway activity prolongs non-metastatic CRC patient survival (Zhou et al., 2017).

#### *8.4.2.1 Effects of S. flavescens compounds on the PI3K/AKT pathway*

In LoVo cells, matrine (0.4 and 0.8 mg/mL, 48h) induced apoptosis and suppressed expression of phosphorylated AKT. Matrine also regulated molecules downstream of the P13/AKT signalling pathway including GSK-3, BAD, p21, p27 and cyclin D1, which affect cell proliferation and apoptosis (Zhang et al., 2014).

### 8.4.3 **Cross-talk in MEK/ERK and PI3K/Akt pathways**

The MEK/ERK and PI3K/Akt intracellular signalling pathways are both downstream of Ras. In cancer, these pathways can be activated by genetic alterations in upstream signalling components as

well as by mutations in the components of these two pathways. Cross-talk, feedback loops and point convergence are evident in these two pathways. Over-activity of these pathways is associated with chemotherapeutic drug resistance and the proliferation of cancer. Dual targeting of these two pathways may lead to optimal outcomes in cancer treatment (Mendoza et al., 2011; Ramjaun and Downward, 2007; Saini et al., 2013).

Inhibitors targeting MEK, PI3K and AKT have been used in cancer treatment. For instance, Selumetinib (AZd6244 and Arry-142886) is a MEK inhibitor that has been used in Phase I, II trials for melanoma, liver cancer, pancreatic cancer, CRC, lung cancer and breast cancer. Perifosine (Krx-0401) targets AKT, MEK 1/2, ERK 1/2 and JNK, and has also been used in Phase I, II trials for multiple myeloma, leukemias, non-small cell lung cancer and advanced solid tumours (Chappell et al., 2011).

### 8.4.4 **p38 MAPK pathway**

The p38 protein is a stress-activated MAPK that responds to cell stress and cytokines. In mammals, p38 is activated via the Rac/MEKK3/MKK3 signalling pathway in response to various stimuli. The biological effects of p38 MAPK depend on the stimuli and the type of cell (Bradham & McClay, 2006).

In CRC, activation of p38 MAPK has been shown to be associated with CRC cell proliferation, migration and invasion (Huang et al., 2017). Inhibition of expression of p38 is associated with decreased expression of matrix metalloproteinases (MMPs) that play important roles in the degradation of the extracellular matrix that is a critical barrier in tumour migration and invasion (Wei et al., 2013). In CRC patients, high expression of MMPs were associated with lower DFS and lower histological grade (i.e. higher-grade tumours), and was more common in carcinomas of the descending colon and rectum (Buhmeida et al., 2009). High MMP expression was associated with more invasive tumours and with death (Araújo et al., 2015; Salem et al., 2016).

#### *8.4.4.1 Effects of S. flavescens compounds on the p38 MAPK pathway*

Figure 8.3 illustrates the actions of *S. flavescens* compounds in intracellular signalling pathways. Matrine inhibited p38 activation by reducing the phosphorylation level of p38 in HT29 CRC cells in a dose-dependent manner. The inhibition of p38 activation was associated with suppression of MMP-2 and MMP-9 activity and the CRC cells' migration and invasion. Synergistic reduction of invasion was found when matrine was combined with SB203580 (a p38 inhibitor) *in vitro*.

In an HT29 tumour xenograft mouse, a lung metastatic model was established whereby matrine was found to inhibit the growth and metastasis of HT29 CRC tumours. Matrine inhibited the migration and invasion of the tumours by supressing the p38 signalling pathway (Ren et al., 2014).

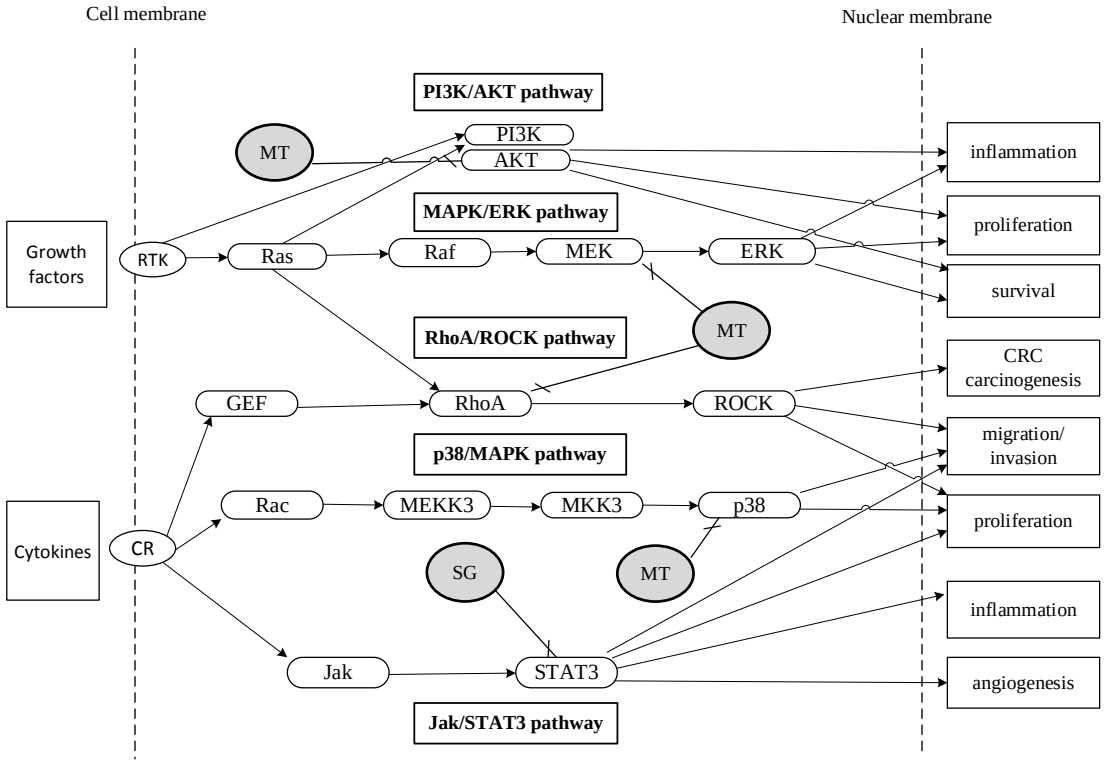


**Figure 8.3: Major proteins involved in intracellular signalling pathways and the actions of *S. flavescens* compounds**

Proteins: RTK - Receptor tyrosine kinases; CR: cytokine receptor; PI3K - Phosphoinositide 3-kinase; AKT: RAC-alpha serine/threonine-protein kinase; Ras - Ras GTPases; Raf - RAF proto-oncogene serine/threonine-protein kinase; MEK - Dual specificity mitogen-activated protein kinase kinase 1; ERK - Extracellular signal-related kinases; GEF - Guanine nucleotide-exchange factor; RhoA - Transforming protein RhoA; ROCK - Rho-associated protein kinase; Rac - Rac GTPase; MEKK3 - Mitogen-activated protein kinase kinase kinase 3; MKK3 - Dual specificity mitogen-activated protein kinase kinase 3; p38 - Mitogen-activated protein kinase 14; Jak - Janus kinase; STAT3 - Signal transducer and activator of transcription 3.
*S. flavescens* compounds: MT – matrine; SG - Sophoroflavanone G. '⊦' : down-regulation; '←': up-regulation.

### 8.4.5 **JAK/STAT3 pathway**

STAT3 is a member of the signal transducer and activator of transcription (STAT) protein family, in which there are seven members in mammals (Darnell, 1997). The JAK/STAT pathway is initially activated by cytokines or interferons binding to membrane receptor-associated Janus kinases (JAK), a non-receptor tyrosine kinase. The activated JAK then recruits and phosphorylates STATs resulting in the translocation of STATs to the nucleus where they mediate transcription of target genes. The JAK/STAT pathway mediates cell proliferation, differentiation, survival and inflammation (Leonard & O'Shea, 1998). The JAK/STAT3 pathway is crucial to CRC development (including cell growth, survival, invasion, and migration) via mediation of the gene expression of Bcl-2 (up-regulation); p16, p21 and p27 (down-regulation); E-cadherin, VEGF and MMPs (up-regulation) (Xiong et al., 2008). Down-regulating JAK/STAT3 signalling can induce CRC apoptosis through mediation of the intrinsic (mitochondrial) pathway (Du et al., 2012). STAT3 is also essential in RhoA/Rho-associated kinase (ROK) -mediated cell proliferation and cell migration (Debidda et al., 2005).

#### *8.4.5.1 Effects of S. flavescens compounds on the JAK/STAT3 pathway*

Sophoraflavanone G is a flavanone-type of *kushen* flavonoid. In CRC HCT-116 cells treated with Sophoraflavanone G (20 μm) for 9 hours *in vitro,* there was a significant reduction in the expression of phosphorylated STAT3 (p-STAT3). In the same study, the authors reported that in other cancer cell lines, sophoraflavonone G inhibited the activity of many proteins in STAT3 upstream signalling pathways, including down regulation of p-JAK1/JAK2/JAK3, p-TYK, p-Src, p-Lyn, p-Akt, p-ERK1/2, and p-NF-κB in MDA-MB-468 breast cancer cells, HDLM-2 Hodgkin's lymphoma cells and L540 lung cancer cells at the same dosage and time frame.Unfortunately, CRC cell lines were not included in these experiments. Nevertheless, the results suggested that sophoraflavonone G inhibits cancer cell proliferation and induces apoptosis by inhibiting activity of the JAK/STAT3 signalling pathway. Further studies in CRC cell lines are needed to test these potential effects (Kim et al., 2013).

### 8.4.6 **RhoA/ROCK pathway**

RhoA is a member of the Rho GTPases, which is a sub-group of the Ras superfamily. Of the 22 members of the Rho family, Rho (A, B and C), Rac (1, 2 and 3) and Cdc42 have received intensive study. Rho GTPases are able to regulate the transcription factors SRF, NF-κB, and STATs in tumourigenesis. The activation of RhoA is mediated by guanine nucleotide-exchange factors. RhoA is associated with cell-cell adhesion disruption and increased migration and metastasis in CRC. Mutant APC, Ras and p53 are highly prevalent in CRC and mediate the cellular events of tumourigenesis, migration and invasion by activating the Rho/ROCK pathway. Rho GTPases and their regulators have been considered promising therapeutic targets in the development of new anti-cancer drugs (Leve & Morgado-Diaz, 2012).

#### *8.4.6.1 Effects of S. flavescens compounds on the RhoA/ROCK pathway*

SW620 cells were treated with matrine which induced dose-dependent apoptosis. The RT-PCR assay showed that the mRNA of RhoA was dose-dependently down-regulated. The increased apoptotic rate was correlated with decreased expression of mRNA of RhoA ($r = -0.86$, $p<0.05$), which suggested that the down-regulation of mRNA of RhoA is associated with the induction of apoptosis in CRC cells (Li & Zhang, 2015). The actions of matrine and sophoroflavanone G on key components of intracellular signalling pathways are shown in Figure 8.3.

### 8.4.7 **Transcription factor p53**

The p53 protein is a tetrameric transcription factor that mediates many cellular functions including DNA damage detection and repair, cell cycle arrest, and inducing apoptosis and senescence in the presence of cellular stress such as DNA damage, hypoxia or oncogene activation (Li et al., 2015; Fischer M, 2017). Activated p53 binds to p21 (WAF1) to induce cell cycle G1 arrest; p53 is also able to induce G2/M arrest by down-regulating CDK1, which is essential for the initiation of mitosis (Li et

al., 2015). The transcription-dependent apoptotic pathway of p53-induced apoptosis activates pro-apoptosis proteins such as PUMA, BID, BAX, TRAILR2, CD95 and Apaf1 in both the intrinsic and extrinsic apoptosis pathways. In addition, p53 can translocate directly to the mitochondria and bind to Bcl-XL and Bcl-2 to form the inhibitory complex that results in cytochrome C release (Kiraz et al., 2016). In cancers, the expression of mutant forms of p53 leads to failure of its tumour suppression functions. In CRC, about 50% of patients have p53 gene mutations, and over-expression of mutant p53 in CRC is associated with a poor survival rate (Lacopetta B, 2003).

#### *8.4.7.1 Effects of oxymatrine and sophoridine on p53*

In a SW480 xenograft tumour model in mice, high expression of p53 in tumour tissues suggests that p53 was the mutant type from the SW480 xenograft, which typically shows elevated mutant p53. Wild-type p53 is hard to detect due to its short life (5 minutes). SW480 tumour-bearing nude mice were treated with sophoridine for 28 days, which significantly down-regulated the expression of p53 at the protein and mRNA levels in the tumour tissues compared with a negative control group. The tumour cells also showed the morphological changes of apoptosis (Wang et al., 2010).

In LoVo cells, oxymatrine (2 mg/mL) induced apoptosis and time-dependently down-regulated p53 protein expression (Peng et al., 2011). Lu (2008) reported that when SW1116 cells were treated with oxymatrine (2, 3 and 4 mg/mL) for 24 and 48 hours, the cell cycle was arrested at the G0/G1 phase, whereas p53 at both the protein and mRNA level was time- and dose-dependently up-regulated (Lu et al., 2008).

These contradictory results may be due to alteration of the p53 gene in the CRC cell lines. We would expect that down-regulation of mutant p53 to be pro-apoptotic, whereas up-regulation of wild-type p53 should promote tumour suppression. In the above two studies, the type of p53 was unclear. To investigate the effect of p53 on pro-apoptosis or anti-apoptosis, a future study could compare the effect of the test substance on apoptosis in cells with and without p53 use the method of gene modification to knockdown p53 in the CRC cells.

### 8.4.8 **NF-κB signalling**

The nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is a protein complex comprising five components: NF-κB1 (p50/p105), NF-κB2 (p52/p100), RelA (p65), c-Rel and RelB. The protein mediates the transcription of DNA, cytokine synthesis and cell survival or death. Two pathways activate NF-κB: the canonical pathway and the non-canonical pathway (Dutta et al., 2006).

In the canonical pathway, extracellular signalling activates the inhibitor of NF-κB kinase β (IKKβ) leading to the phosphorylation and degradation of the inhibitor of NF-κB (IκB) that releases the NF-

κB dimer p50/RelA (comprising components 1 and 3 above). Then, p50/RelA translocates to the nucleus where it binds to DNA and results in alteration of cell functions (Li et al., 1999).

In the non-canonical pathway, upstream kinases, such as NF-κB-inducing kinase, (NIK) activate the inhibitor of NF-κB kinase α (IKKα) leading to the processing of the NF-κB2 precurs or p100/RelB (a dimer of components 2 and 5 above) into a mature p52/RelB subunit that translocates to the nucleus and binds with DNA (Senftleben et al., 2001, Oeckinghaus et al., 2011).

NF-κB is commonly associated with immune cell function and pro-inflammation, but it can also inhibit programmed cell death to maintain the physiological development and homeostasis of the immuno, hepatic and nervous systems. In human tumours, NF-κB derived from tumour cells or infiltrating inflammatory cells is an important factor contributing to tumourgenesis and chemoresistance by promoting the expression of anti-apoptotic genes (Dutta et al., 2006).

NF-κB can regulate some genes in different manners under different circumstances. As well as inhibiting programmed cell death, NF-κB can also induce programmed cell death in response to certain stimuli in certain cells. Under the conditions of ultraviolet light, expression of the Her2/Neu oncogene, treatment with the chemotherapeutic drug doxorubicin, hypoxia/re-oxygenation or hydrogen peroxide stimulation, NF-κB can be activated via an IKK-independent atypical pathway. In this case, phosphorylation and degradation of IκB could occur via the casein kinase II (CK2) or in a tyrosine kinase-dependent manner. This can change the function of NF-κB to tumour suppression and programmed cell death induction (Perkins & Gilmore, 2006).

In CRC, over-expression of NF-κB was found in 70.2% of human CRCs, and was positively correlated with histological grading, depth of invasion, TNM staging and lymph node metastasis of CRC (Zheng et al., 2015). The over-expression of NF-κB may be also associated with the chemo-resistance in CRC therapy (Hassanzadeh, 2011).

#### *8.4.8.1 Effect of matrine on NF-κB*

In SW480 cells, matrine (0.5 mg/mL) time-dependently induced apoptosis at 8, 24, 48 and 72 hours. The RT-PCR assay showed significantly increased mRNA time-dependent expression of caspase-3 and NF-κB1, and time-dependently decreased mRNA expression of Bcl-2. The mRNA expression of p53 and IKKα were not changed. The Western blot assay showed that matrine activated caspase-3, caspase-7 and caspase-9, and time-dependently supressed Bcl-2 expression. Phosphorylated p65 and IKKβ proteins increased time dependently, but phosphorylated IKKα was not expressed. These results suggest that matrine activates NF-κB1/p65 via the canonical pathway, and therefore, matrine may activate the p65/Bcl-2/caspases/apoptosis pathway (Wang, 2008).

The experiment suggested that matrine can induce CRC cell apoptosis while increasing NF-κB expression. Whether NF-κB expression in CRC cells plays a pro-apoptotic role or an anti-apoptotic role needs further investigation. Methods using the NF-κB suppressive agent pyrrolidine dithiocarbamate or using genetic modification to silence NF-κB as a control may be able to clarify the matter.

### 8.4.9 **VEGF and angiogenesis**

Angiogenesis is the growth of new blood vessels from the existing vasculature. This biological process is tightly regulated by angiogenesis inducers and inhibitors to maintain a dynamic balance (Wang et al., 2017). VEGF and VEGF receptors (VEGFRs) are central regulators of this biological process. The VEGF family includes VEGF (or VEGF-A), placenta growth factor (PLGF), VEGF-B, VEGF-C and VEGF-D. The VEGF ligands bind with three endothelial transmembrane tyrosine kinase receptors: VEGFR-1, VEGFR-2 and VEGFR-3 (Tammela et al., 2005). VEGF expression is up-regulated in response to molecular signals from hypoxia-inducible factor 1a (HIF-1a), proliferator-activated receptor gamma coactivator 1a (PGC-1a), growth factors, inflammatory cytokines, oncogenes and hormones. The activation of angiogenesis is primarily via VEGFR-2. The binding of VEGF-A, VEGF-C and VEGF-D to VEGFR-2 activates the downstream phospholipase Cγ/protein kinase C pathway, which in turn activates the c-Raf-MEK-MAP-kinase pathway or the phosphorinositide 3-kinase (PI3K)-AKT pathway and this leads to proliferation and migration of endothelial cells. In addition, VEGF-induced phospholipase C γ/protein kinase pathway activation can activate another downstream pathway involving protein kinase D-dependent phosphorylation and nuclear export of histone deacetylase 7. All three pathways lead to proliferation and migration of endothelial cells (Lohela et al., 2009).

Overexpression of VEGF has been found in most human cancers (Ferrara N, 2004) and has been reported in 61.7% of CRC cases (Zhang et al., 2012). In the tumour hypoxic microenvironment, the overexpression of HIF-1a can increase expression of VEGF via the Wnt/β-catenin/TCF3 (T cell factor 3)/LEF1 (lymphocyte enhancement factor 1)/VEGF pathway in CRC cell lines (Sui et al., 2017). Monoclonal antibodies targeting VEGF (e.g. bevacizumab) or its receptor VEGFR (e.g. aflibercept) in combination with FOLFOX or FOLFIRI are now standard treatments in stage IV CRC patients (Fakih, 2015).

#### *8.4.9.1 Effect of sophoridine on VEGF*

In a SW480 xenograft tumour model, VEGF and p53 were highly expressed in tumour tissues. The tumour-bearing nude mice were treated with sophoridine (16.9 mg/kg, 0.2 mL/head, ip, once a day for 28 days), which significantly down-regulated the expression of VEGF and p53 at the protein and mRNA levels in the tumour tissues compared with a negative control group. The tumour cells also

showed the morphological changes of apoptosis. This suggested that inhibition of the tumour growth was associated with the suppression of VEGF and p53 (Wang et al., 2010).

### 8.4.10 **Telomerase activity and hTERT**

Telomeres are terminal capping structures in chromosomes that stabilise and protect the chromosome from degradation, recombination and fusion with other chromosomes. Telomerase is a ribonucleoprotein that adds TTAGGG repeats to telomeres to compensate for the shortening of telomeres from normal cell division. Telomerase is normally inactive in somatic cells. Human telomerase reverse transcriptase (hTERT) is the catalytic subunit of the telomerase complex. The expression of hTERT is positively correlated with telomerase activity in tumour cells (Pinol-Felis et al., 2017). In CRC, the dysfunctional overactivity of telomeres promotes chromosomal instability in genes such as APC and MSH2 that play key roles in the early steps of CRC carcinogenesis. The activation of telomerase maintains the length of telomeres and this allows cancer cells to proliferate indefinitely. Thus, the activation of telomerase and expression of hTERT is correlated with CRC formation and tumour progression (Bertorelle et al., 2014).

#### *8.4.10.1 Effect of matrine on telomerase activity and hTERT*

In HT-29 cells, matrine induced apoptosis and suppressed telomerase activity (Peng et al., 2005). In SW1116 cells, matrine treatment for 48 hours, showed a dose-dependent reduction in telomerase activity and down-regulation of expression of the mRNA hTERT (Zhu et al., 2009). Thus, matrine-induced CRC cell apoptosis was associated with inhibition of telomerase activity by inhibiting the expression of mRNA hTERT.

### 8.4.11 **Microtubules, OCLN and TUBA1A**

Microtubules are the backbone of the cytoskeleton in cells. The proteins alpha-tubulin and beta-tubulin form heterodimers that constitute the building blocks of microtubules. Tubulin alpha-1A chain (TUBA1A) is a tubulin, for which the microtubules' dynamic equilibrium is the fundamental basis for many biological processes such as cellular motility, cytoplasmic transport and cell division. Disabling the dynamic equilibrium of microtubules may cause chromosome instability (Cirillo et al., 2017). Tubulins have been targets of anti-cancer drugs (such as paclitaxel and docetaxel) for decades. Tubulin inhibitors increase microtubule stabilisation and prevent the dynamic equilibrium of microtubules. These drugs impair the metaphase–anaphase process of mitosis in cell division and lead to cellular death via apoptosis or mitotic catastrophe. Tubulin inhibitors also can indirectly inhibit angiogenesis and enhance immunotherapeutic effects by increasing the immunogenicity of cancer cells (Hardin et al., 2017).

Occludin (OCLN) is a transmembrane tight junction protein that induces cell-cell adhesion when expressed in cells (Furuse et al., 1993). The metastatic progression of cancer is characterised by matrix

degradation, tight junction reductions and vessel formation (Angiolini et al., 2017). The tight junction is an important paracellular permeability barrier that cancer cells must break through during metastatic progression (Martin et al., 2010).

#### *8.4.11.1 Effect of Oxymatrine on OCLN and TUBA1A*

In a LoVo cell-line, oxymatrine (2 mg/mL, 24 h) induced apoptosis. The RT-PCR assay showed that oxymatrine suppressed mRNA expression of TUBA1A and Bcl-2, and promoted the mRNA expression of OCLN. The Western blot assay showed that oxymatrine promoted the protein expression of OCLN and supressed the protein expression of Bcl-2 (Peng, 2011). These results suggest that oxymatrine induced apoptosis in the LoVo cells by suppressing Bcl-2 and TUBA1A, and promoted the expression of OCLN. Further studies are needed to determine any association between increased OCLN expression and reduced cell migration and invasion in animal models.

## 8.5 Outline of tumour-related inflammation

About 15% of cancers are related to infections or chronic inflammation (Kuper et al., 2000). Several sources of evidence have connected inflammation and cancer, as follows:

- Many chronic inflammatory diseases increase the risk of cancer.
- These cancers develop at sites of chronic inflammation.
- Cells involved in the chronic inflammatory process are present at sites of cancers.
- Many inflammatory factors are found in cancers, and suppressing these inflammatory factors can inhibit cancer development.
- Long-term use of non-steroidal anti-inflammatory medicines is associated with a reduction in mortality in some cancers.
- Inflammatory bowel diseases (IBD) have been identified as one of the factors in the pathogenesis of CRC (Balkwill F, 2006).

In the inflammatory microenvironment, cells are under oxidative and nitrosative stress. Excessive reactive oxygen species (ROS) and reactive nitrogen species (RNS) accumulate in cells and these subsequently cause DNA damage and can induce mutations in cancer-related genes. The mutant genes result in inactivation of tumour suppressor genes (such as p53) and activation of oncogenes (such as K-Ras). These pathological changes initiate malignant transformations of the cells. After the initiation of cancer, infiltrating inflammatory cells and tumour cells can produce proinflammatory cytokines such as IL-6, IL-1β, NF-κB and TNF-α, which are important for the survival, proliferation, invasion and metastasis of cancer cells (Zhang et al., 2017).

It has been proposed that TNFα/NF-κB/IL-6/STAT3 axis cellular signalling plays an important role in the progression of inflammation-related cancer (Aggarwal et al., 2006). Targeting these inflammation-

related molecules and pathways are a useful strategy for the prevention and treatment of cancer (Zhang et al., 2017).

### 8.5.1 **Tumour necrosis factor (TNF) α**

Tumour necrosis factor (TNF) α is a hormone-like peptide that is mainly produced by activated macrophages and monocytes in response to various bioactive process such as inflammation, cellular proliferation, apoptosis and morphogenesis. Two types of receptors, TNFR1 and TNFR2, have been identified. TNFR1 is found in most cell types, but TNFR2 is only expressed by immune cells, endothelial cell and nerve cells (Aggarwal et al., 2012). TNFα binding to TNFR1 or TNFR2 leads to promotion of inflammation, cell survival or induction of apoptosis. TNFα binds to TNF receptors (TNFR) on the cell surface and initiates signal transduction. The three main signalling pathways activated are:

1. NF-kB signalling through the recruitment of receptor-interacting serine/threonine-protein kinase (RIP) that enables activation of IKK and leads to the phosphorylation of IκBα and the subsequent activation of NF-kB.
2. MAP/ERK kinase kinase 1 (MEKK1) and MAPK kinase 7 (MKK7), which in turn activate c-Jun N-terminal kinase (JNK) and activator protein 1.
3. death signalling, via TNFR-associated death domain (TRADD) recruiting FAS-associated via death domain (FADD), which in turn activates caspase 8 dependent apoptosis pathways (Aggarwal et al., 2012).

The activation of p38 MAPK through the sequential activation of TNFR1/TRADD/RIP/MAPK kinase 3 (MKK3) is a less well-known pathway that leads to pro-inflammatory effects and cell survival. The activation of TNFR2 can recruit TNFR-associated factor 2 (TRAF2), and cause the same cascades in the TRAF2-dependant pathways (Aggarwal, 2003).

TNFα was initially identified as a tumour suppressor when there is a high-dose and local application. But TNFα can be produced chronically in small amounts in the cancer micro-environment and plays a role in promoting cancer proliferation (Balkwill, 2002, 2009). TNFα also promotes cachexia by inhibiting lipoprotein lipase (Beutler et al., 1985). CRC patients who show high expression of TNF-α have worse prognosis (in terms of cancer cell differentiation, TNM stage, distant metastasis, and disease-free survival) than patients who show low expression of TNF-α (Li et al., 2016).

### 8.5.2 **Interleukin (IL)-6**

Interleukin (IL)-6 is mainly secreted by monocytes and macrophages in the acute inflammatory phase but is also secreted by T cells in the chronic inflammatory phase. In the normal homeostatic condition, the serum level of IL-6 is low. Under inflammatory conditions, the serum level of IL-6 can rise quickly. The activity of IL-6 is involved in acute and chronic inflammatory diseases and infections,

including inflammatory bowel diseases, arthritis and inflammation-associated carcinogenesis (Naugler & Karin, 2008). The IL-6 receptor (IL-6R) is composed of an IL-6 binding α chain (IL-6Rα) and a homodimer of the signal-transducing glycoprotein 130 (gp130). IL-6 binds to its receptor to form an active complex that activates downstream signalling pathways. There are two IL-6 signalling pathways: the classical signalling pathway and the trans-signalling pathway.

In the classical signalling pathway, IL-6 binds to IL-6Rα at the cell surface and, via gp130, transduces the signal to downstream targets. Only cells that express IL-6Rα at the cell surface, such as hepatocytes and some immune cells, respond to this pathway. In the trans-signalling pathway, cells that do not have IL-6Rα at the cell surface can recruit soluble IL-6Rα (sIL-6Rα) that is found in the serum and can bind to IL-6 and interact with gp130 at the cell surface to transduce intracellular signalling. These two signalling pathways can activate downstream pathways such as JAK/STAT, ERK/MAPK and PI3K/AKT (Waldner et al., 2014).

IL-6 trans-signalling and IL-6/STAT3 signalling are associated with the link between bowel inflammatory diseases and CRC development *in vitro* and *in vivo* (Becker et al., 2005; Grivennikov et al., 2009; Bollrath et al., 2009). Tocilizumab, an anti-IL-6R antibody has been developed and used in a pilot study of patients with active Crohn's disease. The result showed the antibody had an effect on the clinical response rate – a decrease of at least 70 points on the Crohn's Disease Activity Index (CDAI), – and was well tolerated. But there has been no report of the use of tocilizumab for cancer treatment (Waldner et al., 2014). Siltuximab, another anti-IL-6 antibody, has been used in phase I and phase II studies. The results showed that siltuximab was well tolerated, but there was no clinical effect on solid tumours including CRC (Angevin et al., 2014).

### 8.5.3 **Effects of *S flavescens* compounds on inflammation *in vitro* and *in vivo***

In a cachexia model in BALB/c mice induced by inoculation of colon 26 carcinoma cells for 12 days, matrine (50 mg/kg) or sophorcarpine (50 mg/kg) were administered i.p. daily for 5 days. Investigators found the mice that were administrated matrine or sophorcarpine showed significantly reduced cachexia symptoms: loss of body weight, loss of epididymal fat weight, loss of gastrocnemius muscle weight and lower dry carcass weight. In both the matrine and sophorcarpine groups, the mice improved food intake compared with a negative control group in which tumour-bearing mice were only administered 0.2 mL saline i.p. daily for 5 days. Matrine and sophorcarpine significantly reduced TNF-α and IL-6 levels in the serum of the tumour-bearing mice compared with the negative controls. However, this dose of matrine and sophorcarpine did not reduce tumour weight in the mice (Zhang et al., 2008). In the same study, RAW 264.7 mouse macrophage cells and primary macrophages were collected from the peritoneal cavities of mice and cultured with lipopolysaccharide (LPS) for 6 hours in the presence or absence of matrine, sophorcarpine, oxymatrine, sophoramine and sophoridine (0.5 mg/mL). The results showed that the production of TNF-α and IL-6 induced by LPS and the mRNA

expression of TNF-α and IL-6 were all significantly reduced in the presence of these *kushen* alkaloids. A parallel MTT assay showed that, at this dosage, these *kushen* alkaloids did not significantly inhibit proliferation of RAW 264.7 cells and primary macrophages (Zhang et al., 2008). Thus, matrine and sophorcarpine were able to suppress the expression of TNF-α and IL-6 in the serum of tumour-bearing mice and in macrophage cells *in vitro*.

Overall, TNFα/NF-kB/IL-6/STAT3 axis cellular signalling has been linked to the development of tumourigenesis, including proliferation, invasion and metastasis, angiogenesis, and chemo-resistance (Aggarwal, 2006). *S. flavescens* compounds such as matrine and sophorcarpine appear to have anti-inflammatory effects by regulating these cytokinases as well as mediating by a number of key genes in several cellular signalling pathways such as AKT, MEK, p38, and STAT3. Therefore, these *S. flavescens* compounds may be good candidates as anti-inflammatory and chemopreventive agents in the future.

### 8.6 Chapter 8 summary and conclusions

*S. flavescens* extracts and its compounds have shown anti-cancer effects in models of CRC *in vitro* and *in vivo*. The anti-cancer effects included inhibition of cell proliferation, suppression of xenograft tumour growth by causing cell cycle arrest at the G1 phase and inducing apoptosis via the intrinsic pathway.

*S. flavescens* extracts and its compounds showed:

- down-regulation of MEK1/2, AKT, RhoA, p38, STAT3, telomerase, cyclin D/CDK4, cyclin E/CDK2, E2F1, Skp2, c-Myc and Bcl-2, and over-expression of p53
- up-regulation of BAX, caspase-3, -7, -9, cytochrome C translocation to the cytosol, Cleaved PARP, p21, p27, p16, GSK-3B.

Among these protein targets, MEK1/2, AKT, RhoA, p38, and STAT3 are key proteins in the following intracellular signalling pathways:

- MEK/ERK and PI3K/AKT
- JAK/STAT3
- p38/MAPK
- RhoA/ROCK.

The inhibition of VEGF, TUBA1A and MMP-2/-9, and the promotion of OCLN, by *S. flavescens* compounds suggest that the anti-cancer effects of *S. flavescens* are also associated with anti-angiogenesis and anti-metastasis. Matrine and sophorcarpine attenuated tumour-related cachexia *in vivo*. This effect was associated with their anti-inflammatory effects by down-regulating the cytokinases TNFα and IL-6 in the tumour microenvironment.

In conclusion, *S. flavescens* and its compounds have demonstrated multiple actions in CRC management including a range of anti-cancer actions, possible chemopreventive actions and improving quality of life. *S. flavescens* and its compounds show potential as candidate natural products for future clinical and experiment research in CRC.

# Chapter 9. General Discussion and Directions for Future Research

## 9.1 Summary of the project

The primary objectives of this research project were to:

- Evaluate the efficacy and safety of HMs in the clinical management of CRC;
- Identify potentially effective HMs and combinations of HMs that warrant further experimental and clinical research;
- Investigate the actions and mechanisms of action of promising HMs in experimental models of CRC; and
- Determine directions for future experimental and/or clinical research.

In order to achieve these objectives, this project involved the following sequence of stages:

- Systematic reviews of randomised controlled trials (RCTs) of HMs for the management of CRC (Chapters 4 and 5)
- Sensitivity analyses to identify individual HMs that showed promise for further research (Chapter 6)
- Experimental studies on a bioactive compound found in the promising herb S. flavescens (Chapter 7)
- Analyses of the possible mechanisms of action of *S. flavescens* and its constituent compounds (Chapter 8)
- Proposals for future research (Chapter 9)

The following were the specific research questions targeted in this study:

1. Can HM interventions, used either singly or in combination with conventional therapies, elevate tumour response rate and/or prolong the survival of CRC patients? (answered in Chapters 4 and 5)
2. Can HM interventions alleviate the adverse events associated with conventional anti-cancer treatments for CRC? (answered in Chapters 4 and 5)
3. Can HM interventions improve the quality of life of CRC patients? (answered in Chapters 4 and 5)
4. How safe are HM interventions for CRC? (answered in Chapters 4 and 5)
5. Which herbs and herbal combinations appear effective for CRC treatment and/or alleviation of adverse events associated with conventional CRC treatments? (answered in Chapter 6)
6. What are the effects of specific herb-derived compounds in CRC cell lines? (answered in Chapter 7)
7. What are the likely mechanisms of action of potentially effective HMs and their constituent compounds? (answered in Chapter 8)
8. What questions could be addressed in future studies and how would a future study be implemented? (answered in Chapter 9)

The main components of each of the above stages and the main results are summarised below.

## 9.2 Systematic reviews and meta-analysis of randomised controlled trials

Comprehensive searches were conducted of English language and Chinese language biomedical databases, and systematic reviews of randomised controlled trials (RCTs) of HMs were performed using the Cochrane collaboration method (see Chapters 4 and 5, and published paper). Meta-analyses of outcome measure data were conducted to determine whether any HMs showed evidence of potential effects in the management of CRC, including the management of adverse events associated with chemotherapy.

The results of the meta-analyses identified a range of potential beneficial effects on tumour response, survival, quality of life, immunity, and the alleviation of adverse events (AEs) induced by chemotherapy. Most of the evidence was for systemic CHMs combined with systemic chemotherapy for which data from 75 RCTs of were available (Chapter 4). A sub-group analysis of CHMs combined with FOFOX4 for ACRC that included 14 RCTs (Chapter 5) found results which reflected the outcomes of the broader meta-analyses.

The meta-analyses found significant improvements in tumour response rate in the groups of participants who received CHM combined with chemotherapy, without important statistical heterogeneity. Most of the data were for oxaliplatin-based chemotherapies in current use.

However, the results for survival outcomes were less clear, were based on considerably fewer studies and tended to greater heterogeneity. Nevertheless, there was an improvement in one-year survival in the palliative setting for ACRC, without heterogeneity, based on 9 RCTs (Research question 1).

With regard to alleviation of adverse events associated with conventional anti-cancer treatments, most data were for chemotherapy-related adverse events. In the groups that combined systemic CHM with chemotherapy, there were reductions in nausea and vomiting (35 studies), diarrhoea (22 studies), neutropenia (38 studies), thrombocytopenia (18 studies), neurotoxicity (26 studies) and other outcomes for which less data were available. For the oxaliplatin-based chemotherapies, the meta-analysis results showed significant reductions without important heterogeneity for each of the above outcomes (Research question 2).

For quality of life, most data were for KPS which found significant improvements in KPS in the CHM plus chemotherapy groups, but there was substantial heterogeneity in this result (Research question 3).

The meta-analyses did not locate any serious safety concerns associated with combining CHMs with chemotherapy regimens. Conversely, the incidences of impairment in liver function and kidney function tended to be lower in the CHM plus chemotherapy groups (Research question 4).

## 9.3 Sensitivity analyses to identify individual HMs for further research

Based on the results of the meta-analyses, a short list of outcomes was selected and further sensitivity analyses of the outcome data from the RCTs. The outcomes selected were: tumour response rate (tRR) since this is directly related to the anti-cancer effects of herbs; chemotherapy-induced nausea and vomiting (CINV) since this is a common clinical issue that has a major impact on quality of life; and chemotherapy-induced neutropenia (CIN) since this is a serious adverse event that can lead to reduction or cessation of adjuvant and palliative chemotherapy. For each outcome, the intervention groups comprised oxaliplatin-based chemotherapies combined with orally administered CHMs. There was no important heterogeneity in the total meta-analysis for any of these three outcomes.

The approach to sensitivity analysis was novel and developed in order to provide a rational basis for shortlisting herbs for further research. The rationale was that, if a particular herb was effective (or ineffective) in improving the particular outcome, this would be reflected in the pooled result of all the studies that employed this herb as a component of a multi-herb CHM intervention. Also, the reliability of a particular herbs' effect on the outcome could be estimated based on its showing a consistent effect when used in combination with a variety of other herbs in multiple studies. Since this approach required, low statistical heterogeneity in the meta-analysis pool combined with a sufficiently large data set to enable multi-level sensitivity analysis, it could only be applied to a limited number of outcome measures and to herbs that were frequently used as ingredients in the CHM interventions.

The sensitivity analyses for tRR identified *Sophora* (*ku shen*), *Paeonia* (*chi shao*), and *Curcuma* (*e zhu*) as the herbs that were most consistently associated with tRRs that were elevated above that for the total meta-analysis pool, without heterogeneity (Research question 5). In addition, each of these plants and/or their constituent phytochemicals has been reported to have demonstrated anti-tumour properties in multiple experiment models of cancer.

For CINV, the following six herbs were similarly shortlisted: *Atractylodes (bai zhu), Poria (fu ling), Coix (yi yi ren), Glycyrrhiza (gan cao), Astragalus* (*huang qi*) and *Panax ginseng (ren shen)* (Research question 5). There was some experimental evidence for each of these herbs for effects relevant to gastro-intestinal dysfunctions but the relevance to CINV was less compelling compared to that for tRR.

The sensitivity analyses for CIN, identified *Atractylodes* (*bai zhu*), *Poria* (*fu ling*), *Coix* (*yi yi ren*), *Astragalus* (*huang qi*) and *Codonopsis* (*dang shen*) as the herbs which showed the most consistent evidence for reduction in CIN incidence when they were ingredients in the CHM interventions (Research question 5). Experimental studies suggested that each of these herbs may be protective against myelosuppression or improve immune status but only *Astragalus* showed evidence of effects on the production of blood cells.

The procedures used in these sensitivity analyses provided a rational method for shortlisting individual herbs used in multi-ingredient formulations for further research. A large proportion of clinical trials of CHM use such formulas and this leads to questions regarding the meaningfulness of the meta-analyses of pooled results of multi-ingredient formulas that contiain some but not all ingredents in common. It was notable that each of the herbs identified in the short-listing process showed evidence of bioactivity relevant to the outcome.

However, the approach could only provide a relative ranking of the herbs and could not provide comparisons between herbs, so the meaningfulness of differences in ranking was difficult to determine. Also, the procedure could only be undertaken for the most frequently used herbs so less frequently used herbs were excluded from the short lists.

## 9.4 Experimental studies on a bioactive compound found in *S. flavescens*

Of the outcomes investigated in the sensitivity analyses, tRR was selected as most relevant for further research. Of the three short-listed herbs *Sophora* (*ku shen*) was selected as the most promising. This herb is exclusively derived from the plant *Sophora flavescens* which contains a number of bioactive compounds. Based on a review of previous studies of the compounds contained in *S. flavescens*, the alkaloid matrine, which is a major constituent, was selected for the experimental studies.

A series of four experiments were conducted to assess the effects of matrine in four different human CRC cell-lines. The results indicated that matrine inhibited proliferation in these cells cell-lines in a dose-dependent manner. The morphological appearance of cells under microscopy indicated that cell death was likely to be due to apoptosis rather than necrosis. This observation was confirmed by further experiments using flow cytometry (FCM) that showed that the proportions of apoptotic cells increased with concentration of matrine. Moreover, cell cycle analysis showed that matrine increased the population of cells in the G0/G1 phase. Oxaliplatin, which was used as positive control in these experiments, showed a similar pattern of effects in these experiments although oxaliplatin is considerably more toxic than matrine, so the dosage was lower than for matrine.

Overall, the experiments indicated that matrine, one of the principal compounds in *Sophora* inhibited the growth of these human cancer cells. This may, at least partially explain the effect of this herb on tRR (Research question 6). These experiments showed similar results to previous experiments but in multiple and additional cell lines. They formed a basis for ongoing studies in collaboration with the team led by Prof. Mo of Sun Yat Sen University in China. Further studies of the anti-cancer effects matrine and its molecular mechanisms are currently underway.

## 9.5 Possible mechanisms of action of *S. flavescens* and its constituent compounds

In addition to matrine, the alkaloids oxymatrine, sophorcarpine and sophoridine, and the flavonoids kurarinone, sophoraflavanone G and kushenol B have all shown antiproliferative effects in CRC cell lines.

A considerable number of studies have investigated the effects of matrine and/or other compounds from *S. flavescens* on proteins involved in the regulation of cell cycle. Analysis of these studies suggested that they may induce apoptosis via actions on the following four main signalling pathways:

- MEK/ERK and PI3K/AKT,
- JAK/STAT3,
- p38/MAPK, and
- RhoA/ROCK.

In addition, the anticancer effects of *S. flavescens* may be via other mechanisms including actions on:

- Transcription factor p53
- NF-κB signalling
- VEGF and angiogenesis
- Telomerase activity and hTERT
- Microtubules, OCLN and TUBA1A.

Moreover, a number of *S. flavescens* compounds can exert anti-inflammatory effects via down-regulation of TNFα and IL-6 in the tumour microenvironment (Research question 7).

## 9.6 Limitations of the current project

Several limitations to the studies included in this project are acknowledged.

The systematic review findings (Chapters 4 and 5) that HMs, notably CHMs, improved the outcomes of CRC treatment, alone or in combination with chemotherapy, cannot be considered as high level evidence due to the generally poor reporting of methodological aspects of RCT design in the original published reports. In addition, the majority of studies were not placebo controlled and this lack of blinding is likely to have included the outcomes. Consequently, the risk of bias in the studies was often high. These issues have been discussed in more detail in Chapters 4 and 5 and in the published papers (Chen et al. 2014; 2016a; 2016b; 2016c; 2017).

The main approach taken in the meta-analyses to mitigating the effect of bias was to group studies according to the similarity in their design, the type of participants, and the type of chemotherapy. For the more objective outcomes such as tRR and survival, this approach had the effect of reducing heterogeneity in the results. Since multiple studies conducted by different researchers showed similar results for these outcomes and the studies reflected the integrative methods used in hospitals, the

likelihood of the overall direction of effect found in the meta-analyses being solely due to bias seem low, although there was certainly a risk that the magnitude of the effect was inflated. In addition, there was asymmetry in the funnel plots for a number of meta-analysis pools suggesting that there may have selective reporting of outcomes and/or studies with negative results were not published. This issue was addressed, at least partially, by sensitivity analyses that removed outliers. These analyses generally showed that the outliers had inflated the effect sizes but had not changes the overall direction of the results.

The method of sensitivity analysis used to identify shortlisted HMs (Chapter 6) needed relatively large numbers of RCTs in the pooling to allow multiple levels of analyses in order to show a consistent effect of any particular HM. For example, in selecting the HM shortlist for tRR, *Coptis* (*huang lian*) and *Sanguisorba* (*di yu*) appeared the most promising at level 1 based on their individual tRRs but these HMs were infrequent overall, and were consequently eliminated at later levels of the analysis. Hence, this approach represented a compromise between apparent effect on tRR in the first analysis and consistent effects on tRR in multiple analyses. It remains to be seen whether the addition of the dimension of consistency improved selection. Unfortunately, no objective measure of this was possible since the procedure was based on ranking and no direct comparisons between herbs were possible. Even with sufficient data this method cannot assess the relative effectiveness of HMs quantitatively, since the pooled results are from multi-ingredient formulas rather than directly from individual HMs. Also, it was unlikely that small differences in ranking were meaningful since there was no objective way of determining how much change in tRR was important. Literature searches for experimental studies tended to validate the short-listed herbs as worthy of further research, but literature searches were not conducted for the herbs that had been eliminated so it is likely that some of these were also suitable candidates. It should be noted that this project aimed to reduce the short-listed herbs to a single herb to enable a more focussed experimental study, but a broader study could consider a much larger short-list of herbs for screening in assays. In such cases, the above limitations would be less significant.

Future studies could assess this approach to herb selection based on pooled clinical trial data to determine its discrimminatory power. It could be combined into other approaches such as systems pharmacology (Luo et al., 2014), systems biology (Margineanu, 2012), or virtual molecular docking (Kirkpatrick, 2004).

In the experimental studies (Chapter 7), only one the compounds found in *S. flavescens* was tested. Constituent components found in the other short-listed HMs are also potential candidates in CRC management: paeonol and paeoniflorin from the roots of *Paeonia lactiflora* and *P. veitchii*; and beta-elemene and curcumin from the rhizomes *Curcuma wenyujin* and *C. kwangsiensis* have all shown anti-proliferation effects in CRC cell studies (see Chapter 6).

This compound matrine was only studied in cell-lines, so it remains unclear whether matrine would have similar effects in an animal model, let alone in humans with CRC. Therefore, the findings of these experiments can only be considered as preliminary. In addition, other *S. flavescens* compounds could be tested singly and in combination in the same cell-lines to determine which are more or less promising for further research into their effects on apoptosis. In addition, future experiments should assess the effects of these compounds on specific proteins and genes involved in the signalling pathways involved in cell cycle regulation.

The analyses of the pathways potentially involved in the actions of matrine and other compounds found in *S. flavescens* (Chapter 8) were based on data provided by published experimental studies. These studies mainly reported on the up-regulation or down-regulation of the expression of particular proteins in response to the compound. Therefore, it was not possible to determine whether the compound had a direct effect on the particular protein or whether the effect was indirect. Also, within each pathway only a small number of the proteins have been included in the available studies, so the effects of these compounds on the majority of the proteins remains unknown. Consequently, the study of effects of *S. flavescens* on the signalling pathways in Chapter 8 could only summarise the current state of the published evidence and it is likely that as more studies are conducted these proposals will require modification.

Overall, this project presents the best evidence available at the time, with the caveat that the quality of the evidence was variable and there were substantial gaps in the available evidence. Hence, it is possible that the findings of these studies will change as further studies become available.

## 9.7 Implications for clinical practice

The meta-analyses conducted in this study suggest that orally administered CHMs combined with oxaliplatin-based chemotherapies reduced the incidence of chemotherapy-induced nausea and vomiting and chemotherapy-induced neutropenia. In these analyses, the sample sizes were relatively large and there was no evidence that the addition of the CHMs to the chemotherapy adversely affected the tumour response rate. To the contrary, meta-analyses that focussed on tumour response found that the addition of certain CHMs appeared to improve tumour response when combined with oxaliplatin-based chemotherapies in CRC and when combined with FOLFOX4 in advanced CRC.

Of the individual plants included in the CHMs, when the roots of *Sophora flavescens* (*ku shen*) were included in a CHM formula and combined with oxaliplatin-based chemotherapy, tumour response rate appeared to be increased. Of the constituent chemicals found in this plant, the alkaloid matrine has been reported to show anti-proliferative activity. Moreover, the *in-vitro* experiments in CRC cell lines suggested that matrine induced apoptosis. This, at least partially, provides a plausible mechanism for the results of the clinical studies.

From the clinical perspective of the integrated management of CRC, these results suggest that clinicians could consider patient requests to combine CHMs with oxaliplatin-based chemotherapies since the addition of the CHM does not appear to reduce the efficacy of the chemotherapy and may even confer additional benefits.

For practitioners of CHM, this research provides some evidence-based guidance on the ingredients of herbal formulae for the integrative management of the above adverse events associated with oxaliplatin-based chemotherapy for CRC.

## 9.8 Proposals for future experimental research

Matrine is perhaps the most studied of compounds found in *S. flavescens*, but its mechanisms of action in cancer still require further research. Although it appears to be clear that it exerts a pro-apoptotic effect on CRC cells, it remains unclear whether this is via direct actions on specific proteins or whether it exerts up-stream actions which lead to changes in cell cycle. Since it may be impractical to conduct assays on a large number of proteins within four main signalling pathways identified in Chapter 8, an alternative approach would be to conduct computer-based virtual molecular docking studies (Kirkpatrick, 2004) on multiple proteins to determine likely ligand-receptor interactions followed by specific ligand binding assays. As mentioned earlier, other compounds found in *S. flavescens* require further testing both singly and in combinations, so virtual molecular docking may be an efficient approach to selecting short lists of compounds and target proteins for use in future assays. Promising pathways related to proliferation and tumour growth in CRC include: WNT signalling, MAPK signalling, TGF-β signalling, and p53 signalling (The Cancer Genome Atlas Network, 2012). Future studies could investigate the effects of *Sophora* alkaloids and flavonoids on the protein components of these pathways.

In addition to cell-line experiments, testing in *in vivo* models is required. Such models include xenografts of CRC cell lines in nude mice and more recent orthotopic models in which the tumour is located in the mouse colon. Such models enable more realistic estimations of dose-response effects as well as measures of protein and gene expression (Mittal, 2015).

Besides its effects on cell cycle, the analysis in Chapter 8 suggest that matrine and other compounds may affect cancer progression via effects on mutant p53, down regulation of angiogenesis via VEGF signalling and/or tight junction proteins. In addition, the possible roles of matrine and other compounds in regulating the inflammatory microenvironment suggest it may be able to play a role in the prevention of cancer or its recurrence.

Another avenue for further research is to follow-up the results of the meta-analyses and sensitivity analyses for neutropenia and to investigate the possible mechanisms of action of certain HMs and their constituent compounds on the hemopoietic system.

## 9.9 Conclusions

This series of studies suggests that in randomised controlled clinical trials of CRC a number of herbal medicines have shown beneficial effects on tumour response when combined with conventional chemotherapies. In addition, the addition of certain herbal medicines to chemotherapies, notably oxaliplatin-based chemotherapies, have shown reductions in chemotherapy-related adverse events including nausea and vomiting and neutropenia. For each of these outcomes, there are supportive experimental studies which have demonstrated relevant bioactivities in cell and animal models for specific compounds contained in the herbs used frequently in the clinical trials. Furthermore, a series of experiments of the alkaloid matrine (derived from *S. flavescens*) showed that it produced apoptotic effects in CRC cell lines which may at least partially explain the effects of the herb *ku shen* on tumour response rate in the clinical trials. Moreover, by investigating the research evidence on matrine and other compounds derived from *S. flavescens*, the likely mechanisms of action of this herb as an anti-cancer agent were elucidated and directions for future experimental research were identified.

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# Appendix A. TNM Staging System

## Table A. Comparison between TNM Staging System and the 6th and 7th Editions of the AJCC Cancer Staging Manual

| TNM stage | AJCC-6 | AJCC-7 |
|---|---|---|
| I | T1N0M0 | T1N0M0 |
| | T2N0M0 | T2N0M0 |
| IIA | T3N0M0 | T3N0M0 |
| IIB | T4N0M0 | T4aN0M0 |
| IIC | NA | T4bN0M0* |
| IIIA | T1N1M0 | T1N1/1cM0 |
| | T2N1M0 | T2N1/1cM0 |
| | NA | T1N2aM0[ı] |
| IIIB | T3N1M0 | T3N1M0 |
| | T4N1M0 | T4bN1M0 |
| | NA | T1N2bM0[ı] |
| | NA | T2N2a-bM0[ı] |
| | NA | T3N2aM0[ı] |
| IIIC | T1N2M0 | T4aN2aM0 |
| | T2N2M0 | T3N2bM0 |
| | T3N2M0 | T4aN2bM0 |
| | T4N2M0 | T4bN2M0 |
| | NA | T4bN1M0* |
| IVA | Any T any N M1 | Any T any N M1a |
| IVB | NA | Any T any N M1b* |

AJCC, American Joint Committee on Cancer; AJCC-6, AJCC Cancer Staging Manual, 6th edition; AJCC-7, AJCC Cancer Staging Manual, 7th edition; NA, not applicable. T4a: Tumor invades the surface of the visceral peritoneum; T4b: Tumor directly invades or is histologically adherent to other organs or structures; N1a: metastasis in 1 regional node; N1b: metastasis in 2–3 nodes; N1c: T1-2 lesions that lack regional lymph node metastasis but exhibit tumor deposit(s); N2a: metastasis in 4–6 nodes; N2b: metastasis in 7 or more nodes; M1a: single metastatic site; M1b: multiple metastatic sites (Gao et al., 2013). *staging upgrades; ı staging downgrades (Hari et al., 2013).

# Appendix B: Search Strategies

Searches were conducted of the major international biomedical databases: PubMed, Cochrane CENTRAL, CINAHL, ScienceDirect and PsycINFO; and the major Chinese language databases: China Academic Journals (CNKI) and Chinese Sci &Tech Journals (CQVIP).

Search terms were divided into three groups:

1). Disorder: colorectal cancer and related terms;

2). Intervention: Chinese medicine, herbal medicine and related terms;

3). Study type: controlled trial, randomised and related terms.

Individual terms were linked by the Boolean operator “OR”. Then, the three groups of terms were combined using the “AND” operator to limit the retrieved articles to those that were related to clinical trials, colorectal cancer and HM.

No limits were imposed. Publication dates were from the inceptions of the respective databases to the present. The search strategies for each of the electronic databases are detailed below.

## B1. PubMed search strategy

The PubMed website was accessed through the RMIT library. The ‘advanced search’ interface was selected with no limits on fields. The search proceeded as below:

A. Search to identify RCTs: This part follows the Cochrane Handbook (Higgins and Green, 2006), appendix 5b.3.

#1. (randomized controlled trial [pt] OR controlled clinical trial [pt] OR randomized controlled trials [mh] OR random allocation [mh] OR double-blind method [mh] OR single-blind method [mh]) NOT (animals [mh] NOT humans [mh])

#2. (randomized controlled trial [pt] OR controlled clinical trial [pt] OR randomized controlled trials [mh] OR random allocation [mh] OR double-blind method [mh] OR single-blind method [mh] OR mhclinical trial [pt] OR clinical trials [mh] OR ("clinical trial" [tw]) OR ((singl* [tw] OR doubl* [tw] OR trebl* [tw] OR tripl* [tw]) AND (mask* [tw] OR blind* [tw])) OR (placebos [mh] OR placebo* [tw] OR random* [tw] OR research design [mh:noexp]) NOT (animals [mh] NOT humans [mh])

#3. (randomized controlled trial [pt] OR controlled clinical trial [pt] OR randomized controlled trials [mh] OR random allocation [mh] OR double-blind method [mh] OR single-blind method [mh] OR clinical trial [pt] OR clinical trials [mh] OR ("clinical trial" [tw]) OR ((singl* [tw] OR doubl* [tw] OR trebl* [tw] OR tripl* [tw]) AND (mask* [tw] OR blind* [tw])) OR ( placebos [mh] OR placebo* [tw] OR random* [tw] OR research design [mh:noexp] OR follow-up studies [mh] OR prospective studies [mh] OR control* [tw] OR prospectiv* [tw] OR volunteer* [tw]) NOT (animals [mh] NOT humans [mh])

B. Search to identify studied disease.

#4. "colorectal neoplasms"[MeSH Terms] OR ("colorectal"[All Fields] AND "neoplasms"[All Fields]) OR "colorectal neoplasms"[All Fields] OR ("colorectal"[All Fields] AND "carcinoma"[All Fields]) OR "colorectal carcinoma"[All Fields] OR ("colorectal"[All Fields] AND "cancer"[All Fields]) OR "colorectal cancer"[All Fields]

#5. "colonic neoplasms"[MeSH Terms] OR ("colonic"[All Fields] AND "neoplasms"[All Fields]) OR "colonic neoplasms"[All Fields] OR ("colonic"[All Fields] AND "carcinoma"[All Fields]) OR "colonic carcinoma"[All Fields] OR ("colonic"[All Fields] AND "cancer"[All Fields]) OR "colonic cancer"[All Fields]

#6. "rectal neoplasms"[MeSH Terms] OR ("rectal"[All Fields] AND "neoplasms"[All Fields]) OR "rectal neoplasms"[All Fields] OR ("rectal"[All Fields] AND "carcinoma"[All Fields]) OR "rectal carcinoma"[All Fields] OR ("rectal"[All Fields] AND "cancer"[All Fields]) OR "rectal cancer"[All Fields]

#7. "intestinal neoplasms"[MeSH Terms] OR ("intestinal"[All Fields] AND "neoplasms"[All Fields]) OR "intestinal neoplasms"[All Fields] OR ("bowel"[All Fields] AND "cancer"[All Fields]) OR "bowel cancer"[All Fields]

#8. ("intestine, large"[MeSH Terms] OR ("intestine"[All Fields] AND "large"[All Fields]) OR "large intestine"[All Fields] OR ("large"[All Fields] AND "intestinal"[All Fields]) OR "large intestinal"[All Fields]) AND ("neoplasms"[MeSH Terms] OR "neoplasms"[All Fields] OR "cancer"[All Fields])

#9. "sigmoid neoplasms"[MeSH Terms] OR ("sigmoid"[All Fields] AND "neoplasms"[All Fields]) OR "sigmoid neoplasms"[All Fields] OR ("sigmoid"[All Fields] AND "cancer"[All Fields]) OR "sigmoid cancer"[All Fields]

C. Search to identify evaluated intervention (i.e. herbal medicine):

#10. "medicine, east asian traditional"[MeSH Terms] OR ("medicine"[All Fields] AND "east"[All Fields] AND "asian"[All Fields] AND "traditional"[All Fields]) OR "east asian traditional medicine"[All Fields] OR ("medicine"[All Fields] AND "east"[All Fields] AND "asian"[All Fields] AND "traditional"[All Fields]) OR "medicine, east asian traditional"[All Fields]

#11. "herbal medicine"[MeSH Terms] OR ("herbal"[All Fields] AND "medicine"[All Fields]) OR "herbal medicine"[All Fields]

#12. "medicine, chinese traditional"[MeSH Terms] OR ("medicine"[All Fields] AND "chinese"[All Fields] AND "traditional"[All Fields]) OR "chinese traditional medicine"[All Fields] OR ("traditional"[All Fields] AND "chinese"[All Fields] AND "medicine"[All Fields]) OR "traditional chinese medicine"[All Fields]

#13. "complementary therapies"[MeSH Terms] OR ("complementary"[All Fields] AND "therapies"[All Fields]) OR "complementary therapies"[All Fields] OR ("complementary"[All Fields] AND "medicine"[All Fields]) OR "complementary medicine"[All Fields]

#14. ("Medicine, East Asian Traditional "[MeSH Terms] OR ("East Asian"[All Fields] AND "continental"[All Fields] AND "ancestry"[All Fields] AND "group"[All Fields]) OR "asian continental ancestry group"[All Fields] OR "chinese"[All Fields]) AND ("herbal medicine"[MeSH Terms] OR ("herbal"[All Fields] AND "medicine"[All Fields]) OR "herbal medicine"[All Fields])

D. combination of the above three searches

#15. #3 AND (#4or #5 or #6 or #7 or #8 or #9) AND (#10 or #11 or # 12 or #13 or #14)

The resultant citations were downloaded to an Endnote file.

## B2. CENTRAL search strategy

The Cochrane Library website was accessed through RMIT library. The Cochrane Central Register of Controlled Trials (Trials; CENTRAL) database was selected. The advanced search model was used. The search strategy was as follows:

A. The search strategy to identify colorectal cancer was based on that used in the review by Wu et al (2005) as follows:

#1. COLONIC-NEOPLASMS*: ME

#2. RECTAL-NEOPLASMS*: ME

#3. (#1 or #2)

#4. ((((COLORECT* near CANCER) or NEOPLASM*) OR CARCINOM*) OR ADENOM*)

#5. ((((COLO* near CANCER) or NEOPLASM*) OR CARCINOM*) OR ADENOM*)

#6. ((((RECT* near CANCER) or NEOPLASM*) OR CARCINOM*) OR ADENOM*)

#7. ((#4 or #5) or #6)

#8. (#3 or #7)

B. Search strategy to identify herbal medicine:

#9. Chinese traditional medicine*: ME

#10. (herb* or drug*)

#11. ((herb* or medic*) near tradition*)

#12. (#10 or #11)

#13. (#9 or #12)

C. Combination of the above two searches

#14. (#8 and #13) with a restriction to clinical trials.

The resultant citations were downloaded to an Endnote file.

### B3. ScienceDirect search strategy

The ScienceDirect (Elsevier) website was accessed through RMIT library. The advanced search interface was selected with 'Explore all sources'. The search strategy was as follows:

#1. Chinese herbal medicine or herbal drug or traditional medicine

#2. plant medicine or phytotherapy

#3. #1 or # 2

#4. random* or controlled trials or clinical trials

#5. blind* or placebo*

#6. #4 or #5

#7. colorectal cancer or colorectal tumour or colorectal carcinoma

#8. large bowel cancer

#9. rectal cancer or rectal tumour or rectal carcinoma

#10. colon cancer or colon tumour or colon carcinoma

#11. #7 or #8 or #9 or #10

#12. #3 and #6 and #11

The resultant citations were downloaded to an Endnote file.

## B4. CINAHL search strategy

CINAHL with the Full Text (EBSCO host) interface was accessed through RMIT library. The advanced search model with Boolean/Phrase modes was selected. No limits were placed on results. Searches included both Mesh terms (MH) and text terms (TX). The search strategy was as below:

#1. colorectal neoplasms. MH

#2. colonic cancer or rectal cancer or colorectal cancer or large bowel cancer. TX

#3. #1 or #2

#4. medicine, herbal MH or medicine, chinese traditional MH or functional food MH

#5. chinese herbal medicine or herbal drug or traditional medicine. TX

#6. #4 or #5

#7. clinical trial MH

#8. randomised controlled trial or blinding or placebo. TX

#9. #7 or #8

#10. #3 and #6 and #9

The resultant citations were downloaded to an Endnote file.

## B5. PsycINFO (ProQuest) search strategy

The PsycINFO database via ProQuest interface was accessed through RMIT library, and 'advanced search' was selected. No limit was placed on the settings. The following the search strategy as used:

#1. colorectal cancer or colorectal neoplasms or colorectal tumour or colonic cancer or colonic neoplasms or colonic tumour or rectal cancer or rectal neoplasms or rectal tumour

#2. Chinese herbal medicine or traditional medicine or herbal drug or plant medicine or phytotherapy or complementary medicine or complementary therapy

#3. random* or randomised trial or randomised controlled trial or blind* or single blind or double blind or placebo*

#4. #1 and #2 and #3

The resultant citations were downloaded to an Endnote file.

## B6. CNKI search strategy

CNKI was accessed via *Google* at www.CNKI.net and a log-in was used. Advanced search was selected. No limit was placed on the settings. The following is an English translation of the search strategy that was used:

#1. Search terms: (((( (key word=Chinese-English explode (colon cancer) or key word= Chinese-English explode(rectal cancer))) or (key word=Chinese-English explode(large bowel cancer) or key word=Chinese-English explode( colorectal cancer))) and (key word=Chinese-English explode (integrated medicine) or key word=Chinese-English explode (Chinese medicine))) or (key word=Chinese-English explode(Chinese herbal medicine) and key word=Chinese-English explode(clinical trials))) (precisely match).

#2. Search model: multi-sources search.

The resultant citations were downloaded to an Endnote file, and see below for the Chinese terms:

检 索条件：(((( (关键词=中英文扩展(结肠癌) 或者 关键词=中英文扩展(直肠癌))) 或者 (关键词=中英文扩展(大肠癌) 或者 关键词=中英文扩展(结直肠肿瘤))) 并且 (关键词=中英文扩展(中西医结合) 或者 关键词=中英文扩展(中医))) 或者 (关键词=中英文扩展(中药) 并且 关键词=中英文扩展(临床研究))) (精确匹配)

检索方式：跨库检索; 检索到:878条记录 数据库：中国学术期刊网络出版总库,中国重要会议论文全文数据库,中国专利数据库,国家标准全文数据库,中国行业标准全文数据库,国外标准数据库,国家科技成果数据库,德国SPRINGER公司期刊数据库,TAYLOR期刊数据库,Earthscan期刊数据库;

## B7. CQVIP search strategy

CQVIP was accessed via *Google* at www.CQVIP.com and a log-in was used. Advanced search was selected. No limit was placed on the settings. The following is an English translation of the search strategy that was used:

(Keyword C = large bowel cancer + rectalcancer + colon cancer)* (Keyword C = Chinese medicine + integrated medicine + kampo medicine + Chinese herbal medicine) - (Keyword C = animal study)

The resultant citations were downloaded to an Endnote file, and see below for the Chinese terms:

(Keyword_C=大肠癌+直肠癌+结肠癌)*(Keyword_C=中医+中西医结合+汉方医+中药)-(Keyword_C=动物实验)

# Appendix C. Outcome Measurements

## C1. WHO definitions of objective response in solid tumours (Miller et al., 1981)

**Measurable disease**:

- Complete response (CR) - The disappearance of all known disease, determined by two observations not less than four weeks apart.
- Partial response (PR) - 50% or more decrease in total tumour load of the lesions that have been measured to determine the effect of therapy by two observations not less than four weeks apart. Bidimensional: single lesion, greater than or equal to 50% decrease in tumour area (multiplication of longest diameter by the greatest perpendicular diameter); multiple lesions, a 50% decrease in the sum of the products of the perpendicular diameters of the multiple lesions. Unidimensional: greater than or equal to 50% decrease in linear tumour measurement. In addition there can be no appearance of new lesions or progression of any lesion.
- No change (NC) - A 50% decrease in total tumour size cannot be established nor has a 25% increase in the size of one or more measurable lesions been demonstrated.
- Progressive disease (PD) - 25% or more increase in the size of one or more measurable lesions or the appearance of new lesions.

## C2. RECIST guideline (version 1.1) for solid tumours (Eisenhauer et al., 2009)

**Response criteria**

This section provides the definitions of the criteria used to determine objective tumour response for target lesions.

- Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
- Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.
- Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).
- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study

## Table C1. WHO recommendations for grading of acute and subacute toxicity (Miller et al., 1981)

TABLE 1. Recommendations for Grading of Acute and Subacute Toxicity

| | Grade 0 | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
|---|---|---|---|---|---|
| Hematologic (Adults) | | | | | |
| Hemoglobin (g/100 ml) | ≥11.0 | 9.5–10.9 | 8.0–9.4 | 6.5–7.9 | <6.5 |
| Leukocytes 1000/cmm | ≥4.0 | 3.0–3.9 | 2.0–2.9 | 1.0–1.9 | <1.0 |
| Granulocytes 1000/cmm | ≥2.0 | 1.5–1.9 | 1.0–1.4 | 0.5–0.9 | <0.5 |
| Platelets 1000/cmm | ≥100 | 75–99 | 50–74 | 25–49 | <25 |
| Hemorrhage | none | petechiae | mild blood loss | gross blood loss | debilitating blood loss |
| Gastrointestinal | | | | | |
| Bilirubin | ≤1.25 × N* | 1.26–2.5 × N | 2.6–5 × N | 5.1–10 × N | >10 × N |
| SGOT/SGPT | ≤1.25 × N | 1.26–2.5 × N | 2.6–5 × N | 5.1–10 × N | >10 × N |
| Alkaline phosphatase | ≤1.25 × N | 1.26–2.5 × N | 2.6–5 × N | 5.1–10 × N | >10 × N |
| Oral | none | soreness/erythema | erythema, ulcers, can eat solids | ulcers, requires liquid diet only | alimentation not possible |
| Nausea/vomiting | none | nausea | transient vomiting | vomiting requiring therapy | intractable vomiting |
| Diarrhea | none | transient <2 days | tolerable but >2 days | intolerable requiring therapy | hemorrhagic dehydration |
| Renal, bladder | | | | | |
| BUN or blood urea | ≤1.25 × N | 1.26–2.5 × N | 2.6–5 × N | 5–10 × N | >10 × N |
| Creatinine | ≤1.25 × N | 1.26–2.5 × N | 2.6–5 × N | 5–10 × N | >10 × N |
| Proteinuria | none | 1+, <0.3 g/100 ml | 2–3+, 0.3–1.0 g/100 ml | 4+, >1.0 g/100 ml | nephrotic syndrome |
| Hematuria | none | microscopic | gross | gross + clots | obstructive uropathy |
| Pulmonary | none | mild symptoms | exertional dyspnea | dyspnea at rest | complete bed rest required |
| Fever-Drug | none | fever <38 C | fever 38 C–40 C | fever >40 C | fever with hypotension |
| Allergic | none | edema | bronchospasm, no parenteral therapy needed | bronchospasm, parenteral therapy required | anaphylaxis |
| Cutaneous | none | erythema | dry desquamation, vesiculation, pruritus | moist desquamation, ulceration | exfoliative dermatitis, necrosis requiring surgical intervention |
| Hair | none | minimal hair loss | moderate, patchy alopecia | complete alopecia but reversible | nonreversible alopecia |
| Infection (specify site) | none | minor infection | moderate infection | major infection | major infection with hypotension |
| Cardiac | | | | | |
| Rhythm | none | sinus tachycardia >110 at rest | unifocal PVC atrial arrythmia | multifocal PVC | ventricular tachycardia |
| Function | none | asymptomatic, but abnormal cardiac sign | transient symptomatic dysfunction, no therapy required | symptomatic dysfunction responsive to therapy | symptomatic dysfunction nonresponsive to therapy |
| Pericarditis | none | asymptomatic effusion | symptomatic, no tap required | tamponade, tap required | tamponade, surgery required |

TABLE 1. (*Continued*)

| | Grade 0 | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
|---|---|---|---|---|---|
| Neurotoxicity | | | | | |
| State of consciousness | alert | transient lethargy | somnolence <50% of waking hours | somnolent > 50% of waking hours | coma |
| Peripheral | none | paresthesias and/or decreased tendon reflexes | severe paresthesias and/or mild weakness | intolerable paresthesias and/or marked motor loss | paralysis |
| Constipation† | none | mild | moderate | abdominal distention | distention and vomiting |
| Pain‡ | none | mild | moderate | severe | intractable |

* N = upper limit of normal.
† Constipation does not include constipation resulting from narcotics.
‡ Pain—only treatment-related pain is considered, not disease-related pain. The use of narcotics may be helpful in grading pain, depending upon the tolerance level of the patient.

From: Miller et al., 1981

**Table C2. Karnofsky Performance Status Scale (KPS) (Yates et al., 1980)**

TABLE 1. Karnofsky Performance Status Scale

| | |
|---|---|
| 100 | Normal, no complaints, no evidence of disease |
| 90 | Able to carry on normal activity, minor signs or symptoms of disease |
| 80 | Normal activity with effort, some signs or symptoms of disease |
| 70 | Cares for self. Unable to carry on normal activity or to do active work |
| 60 | Requires occasional assistance, but is able to care for most of his needs |
| 50 | Requires considerable assistance and frequent medical care |
| 40 | Disabled, requires special care and assistance |
| 30 | Severely disabled, hospitalization is indicated although death not imminent |
| 20 | Hospitalization necessary, very sick, active supportive treatment necessary |
| 10 | Moribund, fatal processes progressing rapidly |
| 0 | Dead |

From: Yates et al., 1980

# Appendix D. Risk of Bias Assessment

## Table D. Risk of bias domains and judgment criteria

| Bias | Authors' judgement criteria |
|---|---|
| Random sequence generation (selection bias) | Low risk: used computer generated random numbers, or random number table, or coin tossing or similar.<br>Unclear risk: insufficient information or not reported.<br>High risk: used other inadequate methods e.g. date of birth, date of admission, number of record. |
| Allocation concealment (selection bias) | Low risk: central allocation, sequentially numbered, opaque, sealed envelopes.<br>Unclear risk: insufficient information or not reported.<br>High risk: open random allocation or similar. |
| Blinding of participants and personnel (performance bias) | Low risk: blinding or no blinding, but the outcome is unlikely influenced by lack of blinding.<br>Unclear risk: insufficient information or not reported.<br>High risk: no blinding and the outcome could be influenced by lack of blinding. |
| Incomplete outcome data (attrition bias) | Low risk: no missing outcome data or missing data is addressed properly.<br>Unclear risk: insufficient information or not reported.<br>High risk: missing data and is not addressed properly. |
| Selective reporting (reporting bias) | Low risk: study protocol is available or outcomes are reported in non-selected way.<br>Unclear risk: insufficient information or not reported.<br>High risk: selectively reported outcomes or incompletely reported. |
| Other bias | Low risk: baseline data is balanced and appears to be free of other potential bias.<br>Unclear risk: insufficient information or not reported.<br>High risk: imbalanced baseline or other potential bias. |

Adapted from: Higgins and Green, 2011

# Appendix E. Characteristics of Studies Included in Chapter 4 and Risk of Bias summary

## Table E1. Characteristics of Group 1 studies: herbal medicine (HM) vs. chemotherapy or no treatment or placebo (11 studies)

| First author (year); Sample size T/C; Trial duration. | Gender (M) T/C; Age (mean) T/C. | Stage T/C; KPS (mean) T/C. | Test treatment: HM, dosage & administration | Control treatment: Dosage & administration | Outcome measures; HM adverse events |
|---|---|---|---|---|---|
| Gu C (2009);<br>24/23;<br>01/2006 to 01/2009. | 14/12;<br>55.12 ± 13.18/55.16 ± 10.63. | Dukes A: 13,<br>B: 25, C: 9 (all);<br>NS. | Surgery + Xuebijing injection, 100 mL Xuebijing injection diluted with 250 mL 0.9% Nacl injection fluid ID, twice per day, day1-5 post surgery. | Surgery + no specified treatment. | T-cell subset: CD4+, CD8+, ratio of CD4+/CD8+;<br>NS. |
| Hou A (2009);<br>15/15;<br>01/2005 to 12/2006. | 14/13;<br>40-59: 9/7<br>60-79: 16/18. | TNM IIIb: 11/9, IV:14/16;<br>≥50 (all). | Fuzhengxiaoai decoction-I, one decoction daily, for 4 wks. | Best supportive care. For 4 wks. | tRR, mOS, AEs, KPS, TCM symptoms; Mild vomiting. |
| Li H (2000);<br>16/17;<br>5/1997 to 12/1998. | 9/10;<br>54.65/51.23. | Dukes B, C (all);<br>NS. | Chang'ai Kangfu decoction (CAKF), one decoction daily, for 3 months. | 5- FU 500mg/ $m^2$ + MMC 2mg/$m^2$, ID, per course, total for 5-FU 5g, MMC 20mg. | T-cell subset: CD3+, CD4+, CD4+/CD8+; NK cells;<br>NS. |
| Schink M (2007);<br>11/11;<br>08/2002 to 09/2004. | 7/5;<br>72 ± 8.2/69 ± 10.4; | Dukes B: 6/7, C:1/3, D: 4/1;<br>76 ± 20.2/80 ± 15.5. | Surgery+ Iscador® M special injection, 1 mL 'Iscador® M special 5 mg' in 250 mL saline ID during the operation. | Surgery + no specified treatment. | NK (nature killer) cell activity, HLA-DR (human leucocyte antigen-DR);<br>NO. |
| Shen H (2003);<br>51/50;<br>01/1997 to 06/1998. | 29/32;<br>52.6/54.2. | Dukes B: 27/26, C: 24/24;<br>NS. | Surgery + Changbi'an capsule,4 capsules/day, for 3 months. | Surgery + no specified treatment. | 3-year OS;<br>NO. |
| Torisu M (1990);<br>56/55;<br>Over 13 years. | NS;<br>59.3/58.4. | Dukes C: 56/55;<br>NS. | Surgery + PSK (YunZhi powder *Coriolus vesiocolor* spores), 3g daily for 2 months after surgery; then 2g daily for 24 months; 1g daily thereafter. | Surgery + placebo (identical powder). 3g daily for 2 months after surgery; then 2g daily for 24 months; 1g daily thereafter. | mTTP; mOS;<br>Immune assay;<br>Pigmentation of nail, cough when taking the PSK, mild diarroea, constipation. |

| First author (year); Sample size T/C; Trial duration. | Gender (M) T/C; Age (mean) T/C. | Stage T/C; KPS (mean) T/C. | Test treatment: HM, dosage & administration | Control treatment: Dosage & administration | Outcome measures; HM adverse events |
|---|---|---|---|---|---|
| Wang H (2000); 34/42; 07/1995 to 07/1999 | 20/23; 55/51. | TNM III:19/17, IV: 15/25; NS. | Mutmihui Glycoside Pill; 6 pills (1.8g) each time, 3 times/day, for 4 mths. | 5-FU/LV: LV200mg/$m^2$/day, dayl-5, ID, 5-FU 500mg/$m^2$/day, dayl-5, ID. Once mth, for 4 mths. | tRR, CEA, KPS, 1-, 2-, 3-yrs OS; NS. |
| Xion S (2003); 60/60; NS. | 39/36; 57.2/54.5. | TNM III: 39/37, IV: 21/23; ≥50 (all). | Changfukang capsule, 10g/time, tid, for 12 wks. | 5-FU/LV: 5-FU: 10mg/kg/day, CF 2mg/kg/day, ID, 5 days/wks, 3wks/cycle, for cycles. | tRR, KPS, TCM symptoms, CEA; Mild nausea, diarrhoea. |
| Yang Y (2005); 55/55; 03/1998 to 03/2000. | 34/33; 37-68/35-71. | TNM III: 38/36, IV: 17/19; ≥50 (all). | Changfukang capsule, 4 capsules/time, tid, for 12 wks. | 5-FU 0.75g / day, ID, 5 days / wks, 3 wks / cycle, for 3 cycles. | tRR, KPS, TCM symptoms, T-cell subset, CEA; NS. |
| Yang Y (2007); 23/21; 08/2005 to 10/2006. | 14/14; 55.24 ± 29.38/52.4 ± 26.72. | TNM II:15/13, III: 8/8; ≥70. | (After surgery + chemotherapy) Quxie Capsule, 2 capsules bid, for 6 months. | (After surgery + chemotherapy) placebo (not specified). | TTP, KPS, AEs, T-cell subset; Mild diarrhoea. |
| Zhang B (2008); 30/30; NS. | 38 (all); 57 (all). | TNM IV (Liver metastasis, all); ≥70. | Javanica oil emulsion, 100 mL/m2 TACE, repeated every 4-6 wks, average 2.5 times. | OX, 5-FU, HCPT, PDD, combination of selected 2-3 drugs TACE, repeated every 4-6 wks, average 2.5 times. | KPS, TACE AEs; NS. |

T: treatment group; C: control group; M: male; N: number; NS: not stated; ID: intravenous drip; tRR: tumour response rate; OS: overall survival; mOS: median OS; TTP: time to progress; AEs: adverse events; RCT: randomized control trial; KPS: Karnofsky performance scoring; wks: weeks; TACE: transcatheter arterial chemoembolisation; OX: oxaliplatin; HCPT: Hydroxycamptothecine; 5-FU: 5-Fluorouracil; LV: leucovorin; MMC: Mitomycin; PDD: Cisplatin; Tid: three times a day; Bid: twice a day; TCM: traditional Chinese medicine; CEA: carcinoembryonic antigen.

**Table E2. Characteristics of group 2 studies: herbal medicine plus chemotherapy vs. chemotherapy (79 studies)**

| First author (year); Trial duration. | Sample size T/C; Gender (M) T/C; Age T/C | TNM (T/C); KPS or ECOG | HM Intervention; dosage; duration | Oxaliplatin (Ox.) regimen; dose, cycles(T/C) | Outcomes; HM AEs. |
|---|---|---|---|---|---|
| Cao B (2011); 01/2005-12/2007. | 60/60; 32/33; 55.2 ± 13.3/58.8 ± 13.7 | IV (all); ECOG: 0-1. | Yiqizhuyu decoction (YZD); one decoction per day orally administered; up to 48 weeks. | FOLFOX4; up to 24 cycles (all). | tRR, mOS, TTP, AEs (neurotoxicity, gastrointestinal events, and hematologic events); NO. |
| Cao C (2005); NS. | 33/29;20/19; 52/49 (med) | IV (all); NS. | Shengmai injection; 40 mL, ID, day 1–7; NS. | FOLFOX: Ox.130 mg/m², ID, day 1, LV 200 mg /m², ID, day 1-5, 5-FU 750 mg /m², ID, day1-5, cycle/21 days; cycle NS. | tRR, mOS, 1-year OS, AEs (gastrointestinal events, and hematologic events); NS. |
| Cazacu M (2003); 1997–2000. | 29/21; NS; NS. | Dukes C: 18/16, Dukes D: 11/5; NS. | Isorel (MistletoeViscum albu) 5mg/kg, ID, 3 days/week; for whole postoperative period. | 5-FU+ LV (either the Mayo or de Gramont protocol); 6 cycles (all). | mOS, AEs (gastrointestinal events, and hematologic events); NS. |
| Chen X (2005); 2002-2004. | 47/46; 31/32; 54/53 (med) | II: 11/12, III: 21/20, IV:15/14; KPS ≥60 | Composite salviae dripping pill; 25mg/pill, 10 pills/day; for 3 cycles or more. | FOLFOX: Ox. 130 mg/m² 2hours ID, LV 200mg/m², 2 hours ID, 5-FU 500mg, bolus, then 3000mg/m² ID for 48 hours. 3weeks /cycle; for 3 cycles (all). | tRR, KPS, BW, AEs (neutropenia, neurotoxicity, stomatitis), IR (CD4+/CD8+); NS. |
| Deng D (2010); 05/2009-01/2010. | 18/18; 9/9; 54.17±10.04/53.56±11.10. | IV(all); KPS ≥60. | Yiqixiaoji decoction; one decoction per day orally administered; for up to 6 weeks. | XELOX: Ox. 130 mg/m², 2hours ID, day 1; Xel. 850 mg/ m², bid, for 14 days; 21 days/cycle; for 2 cycles. | KPS, AEs (neutropenia, thrombocytopenia, anaemia), CEA; NS. |
| Ding X (2010); 9/2007-8/2008. | 30/30; 18/20; 64.5/63 (med) | ACRC; KPS ≥70 | Co-Kushen injection; 20 mL, ID, day 1-7, 14 day/cycle; for 8 cycles. | FOLFOX4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg /m², ID, day 1-2, 5-FU 400 mg /m², bolus, 600 mg /m², ID, 22 hours, day1-2; 8/8 cycles. | mOS, TTP, AEs (neutropenia, nausea and vomiting, neurotoxicity, IR (CD3+, CD4+, CD4+/CD8+, NK cells); |

| First author (year); Trial duration. | Sample size T/C; Gender (M) T/C; Age T/C | TNM (T/C); KPS or ECOG | HM Intervention; dosage; duration | Oxaliplatin (Ox.) regimen; dose, cycles(T/C) | Outcomes; HM AEs. |
|---|---|---|---|---|---|
| | | | | | NS. |
| Dong J (2011); 01/04/2008-01/03/2010. | 20/20; 14/13; 54/51(mean). | II: 12/15, III: 8/5; KPS ≥60. | Yiqijianpi decoction; one decoction per day orally administered; for up to 6 weeks. | Xel. 1250 mg/ m²/day, for 14 days; 21 days/cycle; for 2 cycles (all). | KPS, AEs (myelosupression, gastrointestinal reactions, liver and kidney impairment), IR (CD3, CD4/CD8, NKcells); NS |
| Fang M (2008); 05/2002-06/2007. | 48/45; 30/28; 59.5 ± 11.3/56.4 ± 10.3 | IV (all); KPS ≥70. | Javanica oil emulsion injection; 30 mL, ID, day 1-14 / cycles; for two cycles | FOLFOX4; 2 cycles (all). | tRR, KPS, AEs (alopecia, neutropenia, thrombocytopenia, anaemia, nausea and vomiting, diarrhoea, neurotoxicity, liver impairment, stomatitis), CEA; NS. |
| Guo Z (1999); 1991-1996. | 38/31; 22/17; 51.4 ± 6.4/50.8 ± 5.4. | B: 8/8, C: 15/11, D: 15/12 (dukes); ≥60. | Fuzheng Yiai decoction, one decoction per day; for 16 weeks. | MeF(V): 5-FU + MeCCNu + Vincristine: 5-FU 500 mg, ID, day 1-5, MeCCNu 200 mg, oral, day1, Vincristine 1mg, ID, day1; 4 cycles. | 1-, 3-, 5-year OS, KPS, AEs (alopecia, leukopenia, thrombocytopenia, liver impairment), CEA, IR (CD3+, CD4+/CD8+, NKcells); NS. |
| Hu A (2006); 01/2001-12/2005. | 28/22; 18/14; 49.3 ± 4.5/48.5 ± 4.3 | IV (all); KPS ≥50. | Treatment with 4 different CHM decoctions according to syndrome differentiation; one decoction per day; for more than 30 days. | FOLFOX: Ox.130 mg/m², ID, day 1, LV 200 mg /m², ID, day 1-2, 5-FU 2400 mg /m², ID, 46 hours, cycle/21 days; for 2 cycles (all). | tRR, KPS, AEs (neutropenia, anaemia, nausea and vomiting, diarrhoea, neurotoxicity, liver impairment); NS. |
| Huang Z (2002); | 30/30; 22/20; 46/47(mean). | Dukes B: 5/11, C: 13/9, D: | Pinxiao capsule; 8 capsules, tid; for 12 weeks. | 5-FU+LV+ MeCCNu; 5-FU 500 mg/ m², ID, day 1-5, LV 100 mg, ID, day1-5, MeCCNu 150 mg /m², day 1, | tRR, KPS, AEs (alopecia n, neutropenia, nausea |

| First author (year); Trial duration. | Sample size T/C; Gender (M) T/C; Age T/C | TNM (T/C); KPS or ECOG | HM Intervention; dosage; duration | Oxaliplatin (Ox.) regimen; dose, cycles(T/C) | Outcomes; HM AEs. |
|---|---|---|---|---|---|
| 06/1997-08/2001. | | 15/10; KPS ≥60. | | 4 weeks/cycle; for 2 cycles. | and vomiting, diarrhoea); NS. |
| Huang Z (2005); 10/2000-11/2002. | 31/30; 21/21; 51/52 (mean). | Dukes B: 5/9, C: 12/11, D: 14/10; KPS ≥60. | Jianpixiaoji formula, one decoction per day orally administered; for up to 12 weeks. | 5-FU + LV + MeCCNu: 5-FU 500mg/ m², ID, day 1-5, LV 100 mg, ID, day1-5, MeCCNu 150 mg /m², day 1, 4 weeks/cycle; for 2 cycles. | tRR, KPS, AEs (alopecia n, neutropenia, nausea and vomiting, diarrohea); NS. |
| Huang Z (2008); 05/2004-12/2007. | 31/30; 20/22; 51/52.2(mean). | Dukes B: 4/8, C: 12/12, D: 15/10; KPS ≥50. | Bazhen decoction, one decoction per day orally administered; for up to 16 weeks | 5-FU + LV + MeCCNu: 5-FU 500 mg/ m², ID, day 1-5, LV 200 mg, ID, day1-5, MeCCNu 150 mg /m², day 1, 1 wk/cycle, 4 cycles/course; for 1 course. (all) | tRR, KPS, AEs (alopecia n, neutropenia, nausea and vomiting, diarrhoea); NS. |
| Jian X (2005); 06/1997-06/2001. | 28/29; 31 (all); 55.64 (mean, all). | Dukes B: 37, C: 17, D: 3 (all); KPS ≥60. | Treatment according to syndrome differentiation using Shenlingbaizhu formula, Shiquandabu wan, Fuzhilizhong tang, Sishen wan, Dabuyin wan, and Zhibaibawei wan. one decoction per day orally administered; for 9 wks | 5-FU + LV (no details); 3 wks/cycle; for 3 cycles (all) | 1-,2-, 3-year OS, QoL (1990 Chinese version), AEs (no classified); NS. |
| Jiang G (2013); 01/2010-06/2012. | 32/31; 18/18; 53. 2 ± 12. 4/53. 1 ± 12.8. | ACRC; NS. | Unnamed multi-CHM formula; one decoction per day; for 8 weeks. | FOLFOX; Ox.135 mg/m², ID, day 1, LV 200 mg /m², ID, day 1-2, 5-FU 400 mg bolus, day 1-2, 2400-3600 mg /m², ID for 48 hours, 14 days/cycle; for 4 cycles (all). | tRR, QoL (FACT-C); NS. |
| Kono T (2013); 01/05/2009 -31/03/2010. | 27/23; NS; 67/61 (mean). | NS;ECOG 0–1 | TJ-107 Goshajinkigan; or placebo was administered orally, tid, before each meal (7.5 g/day); for 26 weeks | FOLFOX4, or mFOLFOX6; Ox.85 mg/m², ID, day 1, LV 200 mg /m², ID, 5-FU 400 mg bolus, follow 2400 mg /m², ID for 46 hours, 14 days/cycle; for 8 cycles or more (all). | tRR, AEs (neutropenia, thrombocytopenia, anaemia, nausea and vomiting, diarrhoea, constipation, neurotoxicity, liver impairment, stomatitis); |

| First author (year); Trial duration. | Sample size T/C; Gender (M) T/C; Age T/C | TNM (T/C); KPS or ECOG | HM Intervention; dosage; duration | Oxaliplatin (Ox.) regimen; dose, cycles(T/C) | Outcomes; HM AEs. |
|---|---|---|---|---|---|
| | | | | | NO. |
| Lao G (2012); 12/2008-12/2010. | 30/30; 21/23; 35.1 ± 20.2/36.7 ± 20.1. | II: 5/7, III: 15/14, IV:10/9; KPS ≥60 | Jianpijiedu decoction; one decoction per day, 21 days /cycle; for two cycles. | FOLFOX; Ox.130 mg/m², ID, day 1, LV 200 mg /m², ID, day 1, 5-FU 500 mg bolus day 1, 2400 mg /m², ID, 48 hours, day 1-2, 21 days /cycle; 2 cycles (all) . | tRR, TTP, AEs (neutropenia, thrombocytopenia, nausea and vomiting, neurotoxicity); NS. |
| Li H (2000); 05/1997-12/1998. | 15/17; 8/10; 56.31/51.23. | II&III: 15/17 (all); NS. | Chang'ai Kangfu decoction; one decoction per day; for 12 weeks. | 5-FU + MMC; 5-FU 500 mg/ $m^2$, ID, 1-2/week, 5g/cycle; MMC 2mg/ $m^2$, ID, 1-2/week, 20mg/cycle; NS. | IR (CD3+, CD4+/CD8+, NK); NS. |
| Li H (2007); 10/2002-02/2006. | 65/52; 43/36; 58/59 (med). | III: 27/19, IV: 38/33; KPS ≥60 | Aidi injection; 60 mL, ID, day 1-10, 14days/cycle; for 11weeks. | FOLFOX4; 5.5/5.5 cycles (mean). | tRR, med. OS, 1-year OS, KPS, AEs (neutropenia, thrombocytopenia, anaemia, nausea and vomiting, diarrhoea, constipation, neurotoxicity, liver and kidney impairments, stomatitis); NS. |
| Li Y (1999); 1991-1995. | 60/36; 38/23; 54.2/53.4(mean) | III: 16/9, IV: 44/27; NS. | HM decoction 100 mL enama, once day; for 8 weeks. | 5-FU 1000mg + Dexamethasone 5 mg (intrperitoneal infusion) & MMC 6-8 mg (ID), once week, 5 cycles/course; for 2 courses. | tRR, 1- , 2-, 3-year OS. |
| Li Y (2007); 01/2005-04/2006. | 20/18; 22 (all); 72.2 (med, all) | III: 15, IV: 23 (all); KPS ≥60 | Wenshenjianpi decoction; one decoction per day; for median 10-12 weeks | FOLOFOX4; 6/5.5 cycles (med.). | tRR, KPS, AEs (neutropenia, thrombocytopenia, anaemia, nausea and vomiting, diarrhoea, neurotoxicity, liver impairment); NS. |

| First author (year); Trial duration. | Sample size T/C; Gender (M) T/C; Age T/C | TNM (T/C); KPS or ECOG | HM Intervention; dosage; duration | Oxaliplatin (Ox.) regimen; dose, cycles(T/C) | Outcomes; HM AEs. |
|---|---|---|---|---|---|
| Liang Q (2009); 10/2005-10/2007. | 76/76; 50/51; 53/52 (med) | III: 45/46, IV: 31/30; KPS ≥60 | Shenqifuzheng injection; 250 mL/day, ID, 3 weeks/cycle; for 2 cycles | FOLFOX: Ox. 130 mg/m² 2 hours, ID, day 1, LV 200 mg/m², 2 hours, ID, day1, 5-FU 500 mg, bolus, day1, then 3000 mg/m², ID, for 48 hours. 3weeks /cycle; for 2 cycles (all). | tRR, med OS, KPS, BW, AEs (neutropenia), IR (CD4+/CD8+); NS. |
| Lim M (2012); NS. | 24/23; 17/14; 56.89 ± 14.77/ 55.37 ± 16.01 | III: 15/16, IV: 9/7; KPS ≥70 | Pianzaihuang capsule; two capsules, bid; 14 days/cycle; 8-10 cycles. | FOLFOX4; 8-10/8-10 cycles (all). | tRR, KPS, AEs (neutropenia, nausea and vomiting, neurotoxicity, liver impairment, stomatitis); NS. |
| Liu H (2009); 01/2004-12/2007. | 36/34; 16/18; 50.2 (mean) | ACRC (all); KPS ≥60 | Kang'aifangyi pian; one decoction per day, 21 days / cycle; for 3 cycles. | FOLFOX; Ox.130 mg/m², ID, day 1, LV 200 mg /m², ID, day 1-5, 5-FU 300 mg /m², ID, day 1-5, 21 days/cycle; 3 cycles (all). | tRR, KPS, AEs (neutropenia, thrombocytopenia, anaemia, nausea and vomiting, diarrhoea, neurotoxicity, liver and kidney impairments, stomatitis), IR (CD4+/CD8+, NKcells); NS. |
| Liu H (2013); 01/2008-01/2009. | 30/30; 12/13; 53.83 ± 8.52/ 53.63 ± 8.14. | II: 7/5, III: 23/25; NS. | CHM formula; one decoction administered orally per day, for 30 days; CHM enema formulae 100-150 mL, enema, once every second day; for 14 times. | 5-FU + LV; 5-FU 600mg/ m², ID, day 1-5, LV 200mg/m², ID, day1-5; for 4 cycles (all). | KPS, AEs (nausea and vomiting, stomatitis), IR (CD4+, CD8+, CD4+/CD8+, NK cells); NS. |
| Liu J (2000); 1989-1996. | 96/58; 74/44; 46/44 (mean). | II: 12/7, III: 36/23, IV: 48/28; NS. | Pi-shen feng decoction administered orally per day; for 6 weeks. | 5-FU (750mg) + MMC (6mg) + ADM (50mg, week 1 and week 4), ID, once week, 4 weeks/cycle; NS. | AEs (neutropenia, thrombocytopenia, nausea and vomiting, diarrhoea), 1-, 3-, 5-year OS; NS. |

| First author (year); Trial duration. | Sample size T/C; Gender (M) T/C; Age T/C | TNM (T/C); KPS or ECOG | HM Intervention; dosage; duration | Oxaliplatin (Ox.) regimen; dose, cycles(T/C) | Outcomes; HM AEs. |
|---|---|---|---|---|---|
| Liu J (2005b); 2002-2003. | 52/26; 24/12; 63.11 ± 11.89/62.78 ± 11.04 | IV (all); KPS ≥50. | Jianpihuoxue formula; one decoction per day, 30 days/ cycle; for 3 cycles. | FOLFOX; Ox.150 mg/m², ID, day 1, LV 200 mg /m², ID, day 1-5, 5-FU 500 mg /m², ID, day 1-5, 30 days/ cycle; 3 cycles (all). | tRR, IR (CD3+, CD4+, CD8+, CD4+/CD8+, NKcells); NS. |
| Liu J (2005a); 2002-2003. | 43/21; 23/10; 61.52 ± 10.12 /60.11 ± 9.78 | IV (all); KPS ≥50. | Jianpihuoxue formula; one decoction per day, 30 days/ cycle; 3 cycles. | FOLFOX; Ox.150 mg/m², ID, day 1, LV 200 mg /m², ID, day 1-5, 5-FU 500 mg /m², ID, day 1-5, 30 days/ cycle; 3 cycles (all). | tRR, AEs (alopecia neutropenia, thrombocytopenia, anaemia, nausea and vomiting, diarrhoea, neurotoxicity, liver and kidney impairments); NS. |
| Liu W (2011); 05/2003-05/2010. | 16/16; 11/10; 51/52 (mean). | IV (all); KPS 40-60. | Yierkang capsule: 4-6 capsules, bid; 5-25 months. | FOLFOX; Ox.130 mg/m², ID, day 1, LV 100 mg /m², ID, day 1-5, 5-FU 400 mg /m², ID, day 1-5, 21 days/ cycle; 6 cycles (all). | Med. OS, KPS, AEs (neutropenia, thrombocytopenia, nausea and vomiting, diarrhoea, neurotoxicity); NS. |
| Lu Q (2010); 05/2007-12/2007. | 20/20; 13/11; 51.00 ± 11.84/54.15 + 12.83 | III: 14/13; IV: 6/7; KPS≥60. | Fuzheng kang'ai formula; one decoction per day; for 6 weeks. | FOLFOX; Ox.200 mg/m², ID, day 1, LV 300 mg/m², ID, day 1-5, 5-FU 120 mg/m², ID, day 1-5, 21 days/ cycle; 2 cycles (all). | KPS, IR (CD3+, CD4+, CD8+, CD4+/CD8+, NK cells); NS. |
| Ma J (2005); 02/1999-02/2003. | 28/25; 15/13; 58.1/57.5 (mean) | II: 7/4, III: 21/21; KPS ≥60. | Jianpi xiaoliu decoction; one decoction per day, 90 days/ cycle; 2 cycles. | FOLFOX; Ox.130 mg/m², ID, day 1, LV 200 mg /m², ID, day 1-5, 5-FU 375 mg /m², ID, day 1-5, 21 days / cycle; 6/6 cycles (all). | 1-, 2-, 3-year OS, KPS, AEs (neutropenia, thrombocytopenia, anaemia, nausea and vomiting, diarrhoea, neurotoxicity, stomatitis); NS. |

| First author (year); Trial duration. | Sample size T/C; Gender (M) T/C; Age T/C | TNM (T/C); KPS or ECOG | HM Intervention; dosage; duration | Oxaliplatin (Ox.) regimen; dose, cycles(T/C) | Outcomes; HM AEs. |
|---|---|---|---|---|---|
| Ma M (2010); 06/2006-06/2009. | 20/20; NS; median 61 (all). | Dukes A: 8, B: 10, C: 15, D: 7 (all); NS. | Aidi injection; 40 mL, ID, per day, 21days/cycle; for 2 cycles. | FOLFOX; Ox.135 mg/m², ID, day 1, LV 200 mg /m², ID, day 1-5, 5-FU 400 mg /m², ID, day 1-5, 21 days / cycle; for 2 cycles (all). | KPS, CD4+/CD25+ Treg; NS. |
| Mao X (2005); 03/2002-03/2004. | 46/33; 48 (all); 45 (mean, all). | NS; KPS≥60. | Decoction of Costus (aucklandia) and Amomum with Six Noble Ingredients, once day; Shen ma injection 50 mL/day, ID; for 4 weeks. | LV 200 mg /m², ID, day 1-5, 5-FU 600 mg /m², ID, day 1-5; for 4 weeks | KPS, BW, AEs (nausea and vomiting, myelosupression, liver impairment); NS. |
| Meng Z (2003); NS. | 20/19; 24 (all); 56.7 ± 13.6 (all) | Dukes D (all); KPS ≥70. | Sijunzhi decoction; one decoction per day; for 4 weeks. | TACE; 5-FU 1000mg, Cisplatin 60 mg, Pirarubicin 60-80 mg; 2.7 time procedure /per patient (mean) | tRR, mOS,1-, 2-, 3-year OS, QoL (EORTC QLQ-C30 V3.0); NS. |
| Pan M (2003); 1994-1997. | 43/40; 28/24; 47.5/47 (mean). | I: 6/5, II: 22/20, III: 15/15; NS. | Yiqitiaofu Decoction; one decoction per day; for 6 weeks. | LV+5-FU; LV 100 mg, ID, day1-5, 5-FU 500 mg, ID, day1-5, 21days/cycle; for 2 cycles. | 1-, 3-, 5-yr OS, 5-year DFS, KPS; NS. |
| Qian Y (2009); 01/2004-04/2008. | 40/30; 48 (all); 54 (mean, all). | Dukes B: 21, C: 49 (all); KPS ≥70. | Jianpi CHM; one decoction per day; for 616 days (mean) | FOLFOX: Ox.100 mg/m², ID, day 1, LV 200 mg /m², ID, day 1, 5-FU 400 mg /m² bolus, and 2400 mg, ID 46 hours, day 1, 14 days / cycle; for 6 cycles (all). | DFS, KPS; NS. |
| Qin Y (2011); 01/2001-08/2007. | 36/37; NS; NS. | ACRC; NS | Fuzhengguban decoction; one decoction per day, 21 days/ cycle, 2 cycles. | FOLFOX: Ox.135 mg/m², ID, day 1, LV 200 mg /m², ID, day 1-2, 5-FU 2400 mg /m², ID, for 48 hours, 21 days/ cycle, 2 cycles (all). | tRR, mOS,1-,2-, 3-year OS, KPS, AEs (%), IR (CD4/CD8, NK cells); NS. |
| Qiu Z (2011); 01/2005-08/2009. | 22/21;14/13; 56.9/52.7 (med) | IV (all); KPS ≥60. | Kang'ai injection; 40 mL, ID, day 1–10, 14 days/cycle; for four cycles | FOLFOX4; for 4 cycles (all). | tRR, KPS, AEs (neutropenia, nausea and vomiting); NS. |
| Song W (2012); 10/2008-07/2010 | 20/20; 12/13; 56.4 ± 9.1 /48.3 ± 8.2 | ACRC; KPS ≥70. | Xiaoliuhuajichang fang II; one decoction per day, 21 days/cycle; 2 cycles. | FOLFOX; Ox.135 mg/m², ID, day 1, LV 200mg /m², ID, day 1-2, 5-FU 2400 mg /m², ID, for 48 hours, 21 days/ cycle; 2 cycles (all). | tRR, KPS, AEs (neutropenia, nausea and vomiting, diarrhoea, neurotoxicity, liver |

| First author (year); Trial duration. | Sample size T/C; Gender (M) T/C; Age T/C | TNM (T/C); KPS or ECOG | HM Intervention; dosage; duration | Oxaliplatin (Ox.) regimen; dose, cycles(T/C) | Outcomes; HM AEs. |
|---|---|---|---|---|---|
| | | | | | impairment); IR (CD3+, CD4+, CD8+, NK cells); NS. |
| Tang X (2009); 04/2002-12/2007. | 30/30; 16/14; 46.1/45.5 (mean) | NS; KPS ≥60. | Yiqijianpi CHM; one decoction per day; for 8 weeks. | FOLFOX4; for 4 cycles (all). | Thrombocytopenia; NS. |
| Tao C (2013); 02/2008-02/2010. | 74/74; 51/50; 60.1 ± 7.9 /60.4 ± 8.9. | ACRC; KPS 65.6 ± 12.3/66.7 ± 14.5 | Co-kushen injection; 15 mL per day, ID, started 14 days before chemotherapy, 5 weeks/cycle; for 1 cycle. | FOLFOX; Ox.135 mg/m², ID, day 1, LV 200 mg/m², ID, 2 hours, day 1-5, 5-FU 500 mg/m² ,ID, 8-10 hours, day1-5, 3 weeks/cycle; for 1 cycle (all). | tRR, AEs (neutropenia, thrombocytopenia, anaemia, nausea and vomiting, neurotoxicity, stomatitis); NS. |
| Wan H (2013); 01/2008-12/2011. | 30/30; 35(all);51.7 (mean, all). | III: 40(all), IV: 20(all); KPS ≥60. | Kushen injection 40 mL, ID, and Huangqi injection 20 mL, ID, per-day, 10 days/cycle; for 6 cycles. | FOLFOX; Ox.100 mg/m², ID, day1, LV 200 mg/m², ID, 5-FU 15-20 mg/kg, ID, day 1-5, 21 days/ cycle; for 6 cycles (all). | tRR, 2-year OS, KPS; NS. |
| Wang C (1999); 01/1992-12/1997. | 46/40; 35/23; 49/53 (mean). | I: 4/5, II: 18/14, III: 13/12, IV: 11/9; NS. | HMs decoction 70 mL enema; for 7 days before operation. | 5-FU 20mg/kg enema; for 7 days before operation. | IR (CD3+, CD4+, CD8+, NK cells), 1-, 3-, 5-year OS; NS |
| Wang D (2012); 01/2006-05/2011. | 49/49; 58(all); 54.5 (mean, all). | ACRC; KPS ≥60. | Unnamed multi-TM formula; one decoction per day; duration NS. | FOLFOX; Ox.120 mg/m², ID, day 1, LV 200 mg /m², ID, day 1-5, 5-FU 300 mg /m², ID, day 1-5, 21 days/ cycle; cycle NS. | tRR, KPS; NS. |
| Wang H (2000); 07/1995-071999. | 56/42; 34/23;53/51 (mean) | III: 25/17, IV: 31/25; NS. | Mutmihui GlycosidePil; 1.8 g each time, tid; for 4 months. | LV+5-FU; CF 200 mg/m$^2$/day, dayl-5, ID, 5-FU 500 mg/m$^2$/day, dayl-5, ID. Once month; for 4 months. | tRR, CEA, KPS, 1-, 2-, 3-year OS; NS. |
| Wang H | 34/34; 20/22; | IV: 34/34; | Yiqiguoxiebuchang decoction; one | FOLFOX: Ox.85 mg/m², ID, day 1, LV 200 mg/m², | tRR, AEs (alopecia, |

| First author (year); Trial duration. | Sample size T/C; Gender (M) T/C; Age T/C | TNM (T/C); KPS or ECOG | HM Intervention; dosage; duration | Oxaliplatin (Ox.) regimen; dose, cycles(T/C) | Outcomes; HM AEs. |
|---|---|---|---|---|---|
| (2008); 06/2001-12/2006. | 52.58 ± 8.12/51.11 ± 7.72 | KPS ≥50. | decoction per day; for 3 months. | ID, day 1-2, 5-FU 500 mg bolus day 1, 5-FU 2500 mg/m², ID, for 48 hours, 21 days/ cycle; 4 cycles (all). | neutropenia, thrombocytopenia, anaemia, nausea and vomiting, diarrhoea, neurotoxicity, liver and kidney impairments); NS. |
| Wang J (2011); 03/2007-02/2O11. | 30/30; 18/21; 52.3 ± 6.2/ 56.7 ± 7.8. | ACRC; KPS ≥60. | Yichangning decoction; one decoction per day; for 2 months. | FOLFOX4; 21 days /cycle; for two cycles (all). | tRR, KPS, AEs (neutropenia, thrombocytopenia, anaemia, nausea and vomiting, diarrhoea, neurotoxicity, liver impairment, stomatitis); NS. |
| Wang Y (2012); 03/2007-03/2010. | 38/36; 26/25; 52 (mean all). | ACRC; KPS ≥70. | Aidi injection; 80 mL, ID, per day, 10 days/cycle; for 4 cycles | FOLFOX4; for 4 cycles (all). | tRR, KPS, AEs (neutropenia, gastrointestinal reactions, neurotoxicity); NS. |
| Wang Y (2013); 08/2008-08/2012. | 32/30; 20/19; NS. | ACRC; KPS ≥70. | Xiaoaiping injection; 60 mL, ID, per day, 14 days/cycle; for two cycles. | XELOX; no details; for two cycles (all). | tRR, KPS, AEs (neutropenia, gastrointestinal reactions, neurotoxicity); NS. |
| Wang Z (2007); 09/2003-07/2005. | 34/33; 47 (all); 55 (mean all). | ACRC; KPS ≥60. | Delisheng injection; 40-60 mL, ID per day, 15 days/cycle; for 3 cycles. | FOLFOX; Ox. 130 mg/m², 2 hours ID, day 1 ,LV 200 mg/m², 2 hours ID, day1-5,5-FU 500 mg/m², ID day1-5, 3 weeks/cycle; for 3 cycles (all). | mOS, tRR, AEs (myelosupression, gastrointestinal events); NS. |
| Wu G (2010); 10/2004- | 33/25; 23/17; 55.4 ±13.6 /52.8 | I: 5/3, II:10/8, III: 15/11, IV: | Fupiyiwei decoction; one decoction per day; for 24 weeks. | FOLFOX4; 12/12 cycles (all). | tRR, KPS, AEs (neutropenia, |

| First author (year); Trial duration. | Sample size T/C; Gender (M) T/C; Age T/C | TNM (T/C); KPS or ECOG | HM Intervention; dosage; duration | Oxaliplatin (Ox.) regimen; dose, cycles(T/C) | Outcomes; HM AEs. |
|---|---|---|---|---|---|
| 05/2008. | ± 15.2. | 3/3; KPS ≥60. | | | thrombocytopenia, anaemia, nausea and vomiting, diarrhoea, neurotoxicity), IR (CD3+, CD4+, CD8+, NK cells); NS. |
| Wu Y (2009); 2007-2009. | 35/35; 42 (all); 53.6 (mean, all) | Dukes B: 19, C: 43, D: 8 (all); KPS ≥60 | Fuzhenghuayujiedu formula; one decoction per day; for 12 weeks. | HCPT: 10 mg, ID, day1-5, 4 weeks/cycle; for 3 cycles. | tRR, 1-year OS, AEs; NS. |
| Xiao Y (2008); 01/2004-05/2006. | 27/24; 15/13; 50/45 (mean). | Dukes A: 8/5, B: 11/10, C: 8 /9; KPS ≥60 | Huangqidangshenmogu decoction; one decoction per day; for 8 weeks. | 5-FU + LV + Cisplatin; Cisplatin. 30 mg/m², ID, day 1-3, LV 150 mg/m², ID, day1-5, 5-FU 500 mg/m², ID day1-5, 4 weeks/cycle; for 2 cycles(all). | tRR, KPS, AEs (neutropenia, thrombocytopenia, anaemia); NS. |
| Xiao Z (1998); 1989-1997. | 50/25; 28/13; 58/56 (mean). | II: 10/6, III: 34/14, IV: 8/5; NS. | Fuyue decoction 15 mL tid, 3 months/cycle; for 3 cycles in first year, for 2 cycles in second years, for one cycles in third years. | 5-FU 500 mg + MMC 4 mg, ID, twice wk, 10 wks/cycle; for 3 cycles in first year, for 2 cycles in second years, for one cycles in third years. | AEs (nausea and vomiting), 5-year OS; NS. |
| Xu Y (2006); 07/2001-06/2004. | 32/20; 21/12; 53/51 (med) | IV: 32/20; KPS ≥60. | Fuzhenghuayujiedusanjie formula; one decoction per day; for 6 months. | FLOFLX; Ox.100 mg/m², ID, day 1, 8, 5-FU 500 mg bolus, 250 mg /m², ID, day 1-15, or plus HCPT 10 mg/ m², ID, day 1-5, 30 days/ cycle; for 6 cycles(all). | tRR, 1-year OS, KPS; NS. |
| Xu Y (2010); 08/2004-11/2008. | 61/60; 38/37; 53/52 (mean) | ACRC; KPS ≥70. | Jiangniling formula; one decoction per day, 14 days/cycle; for 8-10 cycles | FOLFOX4; 11.1/7.8 (mean) cycles. | tRR, TTP, KPS, AEs (anaemia, nausea and vomiting, diarrhoea); NS. |
| Yang C (2007); NS. | 50/50; 29/27; 51.36 ± 10.58 /53.48 ± 9.35. | ACRC; KPS ≥60. | Jianpikangfu pill; 6g, tid; for 4 weeks. | FOLFOX; Ox.135 mg/m², ID, day 1, LV 100mg /m², ID, day 1-5, 5-FU 425 mg /m², ID day 1-5; for 4 weeks (all). | tRR, KPS, AEs (neutropenia), CEA; NS. |
| Yang Y (2008); | 18/19; 10/8; 63.05 ± | IV (all); KPS: 64.71 ± | Quxie capsule; 5 mg/kg, bid; for 12 weeks. | FOLFOX (no details) | OS, TTP, KPS; mild diarrhoea. |

| First author (year); Trial duration. | Sample size T/C; Gender (M) T/C; Age T/C | TNM (T/C); KPS or ECOG | HM Intervention; dosage; duration | Oxaliplatin (Ox.) regimen; dose, cycles(T/C) | Outcomes; HM AEs. |
|---|---|---|---|---|---|
| 03/2003-11/2003. | 11.17/62.35 ± 11.42 | 6.24/64.44 ± 5.11 | | | |
| Yang Y (2008a); 01/2007-11/2007. | 30/30; 16/19; 51.07 ± 10.44 /51.33 ± 10.95. | ACRC; KPS ≥60. | Kang'ainjection; 50 mL, ID, day1-20, 30 days/cycle; for 2 cycles. | FOLFOX4; for 4 cycles (all). | tRR, QoL (zhang yan questionnaire), BW, AEs (neutropenia, thrombocytopenia, anaemia, nausea and vomiting, diarrhoea, neurotoxicity, stomatitis, liver impairment); NS. |
| Yang Z (2005); 2002-2004. | 30/30; 18/20; 29-70 /28-69 | III (all); KPS: 82.21 ± 8.68/83.53 ± 8.56 | Xuesaitong injection, 500 mg, ID; Huangqi injection, 60 mL, ID; Shenmai injection, 50 mL, ID & HM decoction, one decoction per day, day1-5, 21 days/cycle; for 2 cycles. | FOLFOX; Ox.200 mg/m², ID, day 1, LV 200 mg /m², ID, day 1-5, 5-FU 500 mg /m², ID day 1-5; 2/2 cycles. | KPS, AEs (nausea and vomiting, diarrhoea), NK cells; NS. |
| Yin X (2011); 04/2009-01/2012. | 32/32; 17/16; 62.00 ± 5.23 /61.70 ± 6.23 | ACRC; NS. | Jianpi formula; one decoction per-day, 21 days/cycle; for 2 cycles. | XELOX; Ox.130 mg/m², ID, day 1, Xel 250 mg/ m², day 1-14, bid, 21 days/cycle; 2 cycles (all). | tRR, KPS, IR (CD3+, CD4+, CD8+, NK cells); NS. |
| You J (2010); 01/2007-11/2009. | 30/30; 14/13; Range: 30-75/31-74. | III: 8/9, IV: 22/21; KPS ≥50. | WD-3 decoction; 50 mL, tid, 4 weeks/cycle; for 4 cycles. | FOLFOX; Ox.125 mg/m², ID, day 1, LV 100 mg /m², ID, day 1-5, 5-FU 500 mg /m², ID day 1-5, 4 weeks/cycle; for 4 cycles (all). | tRR, KPS, IR (CD3+, CD4+, CD4+/CD8+); NS. |
| Zeng B (2008); 02/2000-02/2003. | 25/25; 16/17; 58.3 ± 12.4/56.2 ± l1.5. | III (all); KPS ≥70. | Kangeifangyi tablet; 10 tablets, tid; for 12 weeks. | FOLFOX4; for 6 cycles. | 1-, 2-,3-yr OS, IR (CD3+, CD4+, CD8+, CD4+/CD8+, NK cells), CEA; NS. |
| Zeng C (2013); 04/2009- | 30/30; 39/19; 54.3 ± 6.3/53.2 ± 6.6 | III: 20/12, IV: 41/18; KPS ≥60. | Fuzhengxiaoji decoction; one decoction per day, 14 days/cycle; for 4-6 cycles. | FOLFOX; Ox.85 mg/m², ID, day 1, LV 200 mg /m², ID, day 1-2, 5-FU 360-500 mg /m² bolus, 600 mg /m², ID, for 22 hours, day 1-2, 14 days/cycle; for 4-6 | tRR, KPS, AEs (neutropenia, anaemia), IR (CD4+, CD8+, |

| First author (year); Trial duration. | Sample size T/C; Gender (M) T/C; Age T/C | TNM (T/C); KPS or ECOG | HM Intervention; dosage; duration | Oxaliplatin (Ox.) regimen; dose, cycles(T/C) | Outcomes; HM AEs. |
|---|---|---|---|---|---|
| 01/2012. | | | | cycles (all) | CD4+/CD8+); NS. |
| Zeng D (2009); 02/2007-12/2008. | 35/32; 25/21; 50<: 4/5, 51-69: 28/25, >70: 3/2. | IV (all); KPS≥70. | Ginsenoside Rg3 capsules; 2 capsules, bid; for 8 weeks. | FOLFOX4; for 4 cycles (all). | tRR, KPS, BW, AEs (neutropenia, neurotoxicity), IR (CD3+, CD4+, CD8+, CD4+/CD8+, NK cells); NS. |
| Zeng J (2008); NS. | 30/30; 19/18; 48/60 (med). | ACRC; KPS ≥60. | Multi-TM formula; one decoction per day; for 4weeks. | FOLFOX4; 2/2 cycles (all). | tRR, KPS, AEs (neutropenia, neurotoxicity); NS. |
| Zeng J (2010); 01/2004-01/2008. | 54/50; 40/38; 54 ± 2.4/51 ± 2.3. | II: 30/28, III: 24/28; NS. | Xiaoliu decoction; one decoction per day; for 8 weeks. | IHPC; 5-FU 1500mg + Cisplatin 80 mg, once per week; for 3 weeks. | 1-, 3-yr OS, KPS, AEs (neutropenia, thrombocytopenia, nausea and vomiting, diarrhoea, neurotoxicity), IR (CD4+/CD8+, NK cells); NS. |
| Zhang H (2008); 03/2003-11/2006. | 31/29; 28/23; 52.35/53.40 (mean) | Advance; KPS ≥60. | 3 CHM decoctions based on syndrome differentiation; one decoction per day; Started on one week before chemotherapy till one week after chemotherapy completed. | FOLFOX4; 4/4 cycles (all). | tRR, KPS, AEs (myelosuppressio, nausea and vomiting); NS. |
| Zhang J (2004); 05/2000-01/2004. | 53/50; 65 (all); 31-75. | Dukes B: 23, C: 80 (all). | Aidi injection; 50 mL, ID, day 1-10; for 6 cycles. | LV+5-FU+HCPT; LV 200 mg /m², ID, day 1-5, 5-FU 500 mg /m², ID, day 1-5, HCPT 6 mg /m², ID, day1-7, 21day/cycle; for 6 cycles. | 1-, 2-, 3-yr OS, KPS, AEs (neutropenia, nausea and vomiting, liver impairment); NO. |
| Zhang Q (2006); | 38/30; 35 (all); 54.8 (mean, all). | ACRC; KPS: 76.5 ± | Yiqihuoxue formula; one decoction per day, 21 days/cycle; for 3 cycles. | FOLFOX; Ox.125 mg/m², ID, day 1, LV 200 mg /m², ID, day 1-2, 5-FU 500 mg /m² bolus, day 1-2, 2000 | tRR, 1 yr OS, KPS, AEs (neutropenia, |

| First author (year); Trial duration. | Sample size T/C; Gender (M) T/C; Age T/C | TNM (T/C); KPS or ECOG | HM Intervention; dosage; duration | Oxaliplatin (Ox.) regimen; dose, cycles(T/C) | Outcomes; HM AEs. |
|---|---|---|---|---|---|
| 04/2002-07/2004. | | 5.8/73.5 ± 6.0 | | mg /m² ID for 72 hours, 21 days/cycle; for 3 cycles (all). | thrombocytopenia, anaemia, nausea and vomiting, diarrhoea, neurotoxicity), IR (CD4+/CD8+, NK cells); NS. |
| Zhang Q (2010); 02/2005-10/2007. | 60/60; 35/33; 56.2 (mean, all) | ACRC; KPS ≥60. | Gubenxiaoliu capsule; 4 capsules, bid; for 8 weeks. | FOLFOX4; 4/4 cycles (all). | tRR, 1-year OS, KPS, AEs (neutropenia, thrombocytopenia, anaemia, nausea and vomiting, diarrhoea, neurotoxicity), IR (CD4+/CD8+, NK cells); NS. |
| Zhang S (2007); 10/2005-09/2006. | 50/46; 35/31; 56.14 ± 7.75/53.83 ± 9.83. | NS; NS. | Yiqijianpijiedu CHM; one decoction per day; for 16 days. | FOLFOX4; one cycle. | QoL (EORTC QLQ-C30, Self-reported Anxiety Scales (SAS) and Depression Scales (SDS); NS. |
| Zhang W (2013); 10/2007-08/2009. | 32/32; 15/16;56.8 ± 10.1/46.4 ± 9.2 | Dukes B: 23/22, C: 9/10; KPS: ≥60. | Xiaoliuhuaji decoction I; one decoction per day; for 20 weeks. | FOLFOX; Ox.135 mg/m², ID, day 1, LV 200 mg /m², ID, day 1-2, 5-FU 2400 mg /m², ID day 1, for 48 hours, 21 days /cycle; for 6 cycles (all). | DFS, KPS, AEs (neutropenia, nausea and vomiting, diarrhoea, neurotoxicity, liver impairment), IR (CD3+, CD4+, CD8+, NK cells); NS. |
| Zhang Y (2010); 01/2005-12/2008. | 21/20; NS; NS; | ACRC; KPS: ≥60. | Jianpijiedu decoction; one decoction per day; for 4weeks. | FOLFOX 4; 2/2 cycles (all). | tRR, med OS,1-year OS, TTP, KPS, BW, AEs (alopecia, neutropenia, thrombocytopenia, |

| First author (year); Trial duration. | Sample size T/C; Gender (M) T/C; Age T/C | TNM (T/C); KPS or ECOG | HM Intervention; dosage; duration | Oxaliplatin (Ox.) regimen; dose, cycles(T/C) | Outcomes; HM AEs. |
|---|---|---|---|---|---|
| | | | | | anaemia, nausea and vomiting, neurotoxicity, liver impairment); NS. |
| Zhang Y (2010a); 07/2008-03/2009. | 20/20; 12/11; 48.5 ± 12.8/ 47.6 ± 11.9). | NS; NS. | Shenqi fuzheng injection; 250 mL, ID, day1-5; duration NS. | FOLFOX; Ox.135 mg/m², ID, day 1, LV 150 mg /m², ID, day 1-5, 5-FU 500 mg /m², ID day 1-5, duration NS. | KPS, AEs (myelosuppression, gastrointestinal events, liver and kidney impairment), CD4+/CD8+; NS. |
| Zheng X (2005); NS. | 75/67; 56/60; NS. | Dukes A: 10/12, B: 28/25, C: 37/30; KPS: ≥60. | Co-Kushen injection; 50 mL, ID, day 1-5; for 4 weeks. | CF+5-FU+HCPT; LV 200 mg /m², ID, day 1-5, 5-FU 500 mg /m², ID, day 1-5, HCPT 8 mg /m², day1-5, ID, 5-day/cycle; for 4 cycles. | KPS, AEs (neutropenia, liver impairment); NS. |
| Zheng Y (2011); 01/2009-01/2011. | 32/30; 20/18; 62.9 ± 7.86/63.1 ± 7.12. | ACRC; KPS: ≥70. | Shenqi san (powder); 5g, tid; for 24 weeks. | 5-FU+LV; LV 200 mg /m², ID, day 1-5, 5-FU 500 mg /m², ID, day 1-5, 30 days/cycle; for 6 cycles. | KPS, AEs (neutropenia, thrombocytopenia, anaemia, nausea and vomiting, diarrhoea, liver and kidney impairment), CEA; NS. |
| Zhou J (2011); 08/2007-08/ 2009 | 34/34; 22/20; 51.2/52.5 | II: 14/13, III: 16/11, IV: 6/5; KPS: ≥60. | Fuzhengjianpi decoction; one decoction per day; for 8 weeks. | FOLFOX; Ox.130 mg/m², ID, day 1, LV 100 mg /m², ID, day 1-5, 5-FU 500 mg /m², ID, day 1-5, 28 days/cycle; for 2 cycles (all) | tRR, KPS, AEs (neutropenia, thrombocytopenia, anaemia, gastrointestinal reactions), IR (CD3+, CD4+, CD8+, CD4+/CD8+, NK cells); NS. |
| Zou B (2007); | 32/27; 29/22; | ACRC; KPS: | Gubenkang'ai decoction; one | FOLFOX; Ox.135 mg/m², ID, day 1, LV 200 mg /m², | tRR, KPS, AEs |

| First author (year); Trial duration. | Sample size T/C; Gender (M) T/C; Age T/C | TNM (T/C); KPS or ECOG | HM Intervention; dosage; duration | Oxaliplatin (Ox.) regimen; dose, cycles(T/C) | Outcomes; HM AEs. |
|---|---|---|---|---|---|
| 03/2000-11/2005. | 53/54.3 (mean) | ≥60. | decoction per day; for 6 weeks. | ID, day 1-2, 5-FU 2400 mg /m², ID, for 48 hours, day 1, 21 days/cycle; for 2 cycles (all) | (myelosuppression, nausea and vomiting); NS. |

T: treatment group; C: control group; M: male; HM: herbal medicine; N: number; NS: not stated; ID: intravenous drip; tRR: Tumour Response Rate; OS: overall survival; TTP: time to progress; DFS: disease free survival; AEs: adverse events; BW: body weight; IR: immune regulation; TNM: cancer staging system. 'T' for tumour, denotes the extent of invasion of the intestinal wall, 'N' for lymphatic node, the amount of lymphatic node involvement, and 'M' for the metastasis; QoL: quality of live; KPS: Karnofsky Performance Status; ECOG: Eastern Cooperative Oncology Group Performance Status; EORTC QLQ-C30: European Organisation for Research Treatment Of Cancer; HM: herbal medicine; 5-FU: 5-Fluorouracil; LV: Leucovorin; Ox: Oxaliplatin; Xel: Capecitabine; ADM: Adrimycin; HCPT: Hydroxycamptothecine; MeCCNu: Semustine; MMC: Mitomycin; FOLFOX: OXA. + 5-FU + LV; FOLFOX4: 2-hour infusion of LV (200 mg/ m²/d), followed by a 5FU bolus (400 mg/ m²/d) and a 22-hour infusion (600 mg/ m²/d) for 2 consecutive days every 2 weeks, oxaliplatin 85 mg/m² as a 2-hour infusion on day 1,repeated every 14 days; XELOX: Oax. + Capecitabine; TACE: Transcatheter Arterial Chemoembolization; IHPC: intraperitoneal hyperthermic perfusion chemotherapy; ACRC: advanced colorectal cancer; bid: twice per day; tid: three times per day; qd: once per day; med: median.

## Table E3: Risk of bias summary: review authors' judgements about each risk of bias item for each of the 88 included studies

| Study: First author (year) | RSG (selection bias) | AC (selection bias) | BPP (perform. bias) | BOA-subj. (detection bias) | BOA-obj. (detection bias) | IOD (attrition bias) | SR (reporting bias) | Other bias (funding) |
|---|---|---|---|---|---|---|---|---|
| Cao B 2011 | L | L | L | L | L | L | L | U |
| Cao C 2005 | U | U | H | H | L | L | L | U |
| Cazacu M 2003 | U | U | H | L | L | L | L | H |
| Chen X 2005 | U | U | H | H | L | L | L | U |
| Deng D 2010 | U | U | L | H | L | L | L | U |
| Ding X 2010 | L | U | H | H | L | L | L | U |
| Dong J 2011 | L | U | H | H | L | L | L | L |
| Fang M 2008 | L | U | H | H | L | L | L | U |
| Gu C 2009 | U | U | H | L | L | L | L | U |
| Guo Z 1999 | U | U | H | H | L | H | L | U |
| Hou A 2009 | L | U | H | H | L | H | L | U |
| Hu A 2006 | U | U | H | H | L | L | L | U |
| Huang Z 2002 | U | U | H | H | L | L | L | U |
| Huang Z 2005 | L | U | H | H | L | L | L | U |
| Huang Z 2008 | L | U | H | H | L | L | L | U |

| Study: First author (year) | RSG (selection bias) | AC (selection bias) | BPP (perform. bias) | BOA-subj. (detection bias) | BOA-obj. (detection bias) | IOD (attrition bias) | SR (reporting bias) | Other bias (funding) |
|---|---|---|---|---|---|---|---|---|
| Jian X 2005 | U | U | H | H | L | L | L | U |
| Jiang G 2013 | U | U | H | H | L | L | L | U |
| Kono T 2013 | L | L | L | L | L | L | L | U |
| Lao G 2012 | L | U | H | H | L | L | L | U |
| Li H 2000 | U | U | H | L | L | L | L | U |
| Li H 2007 | U | U | H | H | L | L | L | U |
| Li Y 2007 | L | U | H | H | L | L | H | U |
| Liang Q 2009 | L | U | H | H | L | L | L | U |
| Lim M 2012 | U | U | H | H | L | L | L | L |
| Liu H 2009 | L | U | H | H | L | L | L | L |
| Liu H 2013 | L | U | H | H | L | L | L | U |
| Liu J 2000 | U | U | H | H | L | L | L | U |
| Liu J 2005 | L | U | H | H | L | L | L | U |
| Liu J 2005a | L | U | H | H | L | L | L | U |
| Liu W 2011 | U | U | H | H | L | L | L | L |
| Lu Q 2010 | U | U | H | H | L | L | L | U |
| Ma J 2005 | U | U | H | H | L | L | L | U |
| Ma M 2010 | U | U | H | L | L | L | L | U |
| Mao X 2005 | U | U | H | H | L | L | L | U |
| Meng Z 2003 | U | U | H | H | L | L | L | U |
| Pan M 2003 | U | U | H | H | L | H | L | U |
| Qian Y 2009 | U | U | H | H | L | L | L | U |
| Qin Y 2011 | U | U | H | H | L | H | H | U |
| Qiu Z 2011 | U | U | H | H | L | L | L | U |
| Schink M 2007 | U | U | H | L | L | L | L | U |
| Shen H 2003 | U | U | H | H | L | L | L | L |
| Song W 2012 | L | U | H | H | L | L | L | U |
| Tang X 2009 | U | U | H | L | L | L | L | U |
| Tao C 2013 | U | U | H | H | L | L | L | U |
| Torisu M 1990 | U | U | L | L | L | L | L | U |
| Wan H 2013 | U | U | H | H | L | L | L | U |
| Wang C 1999 | U | UI | H | H | L | L | L | U |
| Wang D 2012 | U | U | H | H | L | L | L | U |
| Wang H 2000 | H | U | H | H | L | L | H | U |
| Wang H 2008 | U | U | H | H | L | L | L | U |
| Wang J 2011 | L | U | H | H | L | L | L | U |
| Wang Y 2012 | U | U | H | H | L | L | L | U |
| Wang Y 2013 | U | U | H | H | L | L | L | U |
| Wang Z 2007 | U | U | H | H | L | L | L | U |

| Study: First author (year) | RSG (selection bias) | AC (selection bias) | BPP (perform. bias) | BOA-subj. (detection bias) | BOA-obj. (detection bias) | IOD (attrition bias) | SR (reporting bias) | Other bias (funding) |
|---|---|---|---|---|---|---|---|---|
| Wu G 2010 | L | U | H | H | L | L | L | L |
| Wu Y 2009 | U | U | H | H | L | L | L | U |
| Xiao Y 2008 | U | U | H | H | L | L | L | L |
| Xiao ZQ 1998 | U | U | H | H | L | L | L | U |
| Xion S 2003 | U | U | H | H | L | L | L | U |
| Xu Y 2006 | U | U | H | H | L | H | L | U |
| Xu Y 2010 | U | U | H | H | L | L | L | U |
| Yang Y 2008a | U | U | H | H | L | L | L | L |
| Yang C 2007 | U | U | H | H | L | L | L | L |
| Yang Y 2005 | L | U | H | H | L | L | L | U |
| Yang Y 2008 | L | U | H | H | L | L | L | U |
| Yang Z 2005 | U | U | H | H | L | L | L | U |
| Yin X 2011 | L | U | H | H | L | L | L | L |
| You J 2010 | U | U | H | H | L | L | L | L |
| Zeng B 2008 | U | U | H | L | L | L | L | U |
| Zeng C 2013 | U | U | H | H | L | L | L | U |
| Zeng D 2009 | U | U | H | H | L | L | L | L |
| Zeng J 2008 | U | U | H | H | L | L | L | U |
| Zeng J 2010 | U | U | H | H | L | L | L | L |
| Zhang B 2008 | U | U | H | H | L | L | L | U |
| Zhang H 2008 | U | U | H | H | L | L | L | U |
| Zhang J 2004 | U | U | H | H | L | L | L | U |
| Zhang Q 2006 | L | U | H | H | L | L | L | U |
| Zhang Q 2010 | L | U | H | H | L | L | L | L |
| Zhang S 2007 | U | U | H | H | L | L | L | U |
| Zhang W 2013 | L | U | H | L | L | L | L | U |
| Zhang Yan 2010 | L | U | H | H | L | H | L | L |
| Zhang Yong 2010 | U | U | H | H | L | H | L | U |
| Zheng X 2005 | U | U | H | H | L | L | L | U |
| Zhang Y 2011 | L | U | H | H | L | L | L | U |
| Zhou J 2012 | U | U | H | H | L | L | L | U |
| Zou B 2007 | U | U | H | H | L | L | L | U |

RSG: random sequence generation; AC: allocation concealment; BPP: blinding participants and personnel; BOA-subj.: blinding outcome assessment-subjective outcome; BOA-obj.: blinding outcome assessment-objective outcome; IOD: incomplete outcome data; SR: selective reporting; L: low risk of bias; H: high risk of bias; U: unclear risk of bias

# Appendix F. Characteristics of Studies Included in Chapter 5

## Table F1. Characteristics of the fourteen studies included in the meta-analysis of FOLFOX4 for ACRC

| First author (year); Sample size T/C; Duration. | Gender (M) T/C; Age T/C; KPS all. | Previously untreated T/C; Previously treated T/C; Metastatic sites T/C. | HM: name (manufacturer), ingredients (pinyin, scientific name, part); dosage & administration; FOLFOX4: cycles (T/C). | Outcome measures |
|---|---|---|---|---|
| Cao B (2011); 60/60; 01/2005 to 12/2007. | 32/33; 55.2±13.3/ 58.8±13.7; ECOG 0: 36/37, I: 24/23. | 60/60; 1:26/29, 2: 19/20, 3: 10/8, ≥4: 5/3. | Yiqi Zhuyu Decoction (*Ren shen*, *Panax ginseng* C.A. Mey. Root; *Sophora flavescens* Ait.root; *Huang qi*, *Astragalus membranaceus* (Fisch.) Bge. Root; *Dang shen*, *Codonopsis pilosula* (Franch.) Nannf. root; *Bai zhu*, *Atractylodes macrocephala* Koidz. root; *Ban zhi lian*, *Scutellaria barbata* D. Don.; *Bai hua she she cao*, *Hedyotis diffusa* Willd. herb; *Yu jin*, *Curcuma wenyujin* Y. H. Chen et C. Ling. rhizome; E zhu, *Curcumae phaeocaulis* Val. rhizome; *Bai shao*, *Paeonia lactiflora* Pall. root; *San leng*, *Sparganiumstoloniferum* Buch. Hamil. root; *Chuanxiong*, *Ligusticum chuanxiong* Hort. root; *Dang gui*, *Angelica sinensis* (Oliv.) Diels. root.); 5 mL/kg oral administration, 2 weeks/cycle, for 48 weeks; FOLFOX 4: 24/24. | tRR; PFS; mOS; AEs(neurotoxicity, gastrointestinal events, hematological events). |
| Ding X (2010); 30/30; 09/2007 to 08/2008. | 18/20; 64.5/63; ≥ 70. | NS; NS; NS. | Compound Kushen Injection (Zhendong Jinjing Pharmaceutical Co.): *Ku shen*, *Sophora flavescens* Ait.root; Bai tu ling, *Heterosmilax yunnanensis* Gagnep. root; 20 mL, ID, day 1-7, for 8 cycles; FOLFOX 4: 8/8. | Median OS; Median TTP; AEs (leukopenia, nausea, vomiting, neurotoxicity); T-cell subsets (CD3+, CD4+, CD8+), NK cells. |
| Fang M (2008); 48/45; 05/2002 to 06/2007. | 30/28; 59.5±11.3/ 56.4±10.3; ≥ 70. | NS; NS; NS. | Javanica oil Emulsion Injection (Shenyang Pharmaceutical University Pharmaceutical Co.): *Yan dan zi*, *Brucea javanica* (L.) Merr. seed; 30 mL diluted with saline, 250 mL ID, day 1-14, for 2 cycles; FOLFOX 4: 2/2. | tRR; Chemotherapy AEs (leukopenia, anaemia, thrombocytopenia, nausea, vomiting, diarrhoea, stomatitis, neurotoxicity, alopecia); KPS. |

| **First author (year); Sample size T/C; Duration.** | **Gender (M) T/C; Age T/C; KPS all.** | **Previously untreated T/C; Previously treated T/C; Metastatic sites T/C.** | **HM: name (manufacturer), ingredients (pinyin, scientific name, part); dosage & administration; FOLFOX4: cycles (T/C).** | **Outcome measures** |
|---|---|---|---|---|
| Li HJ (2007); 65/52; 10/2002 to 02/2006. | 43/36; 58/59 (median); 70(median ). | 31/20; 34/32; NS. | Aidi Injection (Guizhou Yibai Pharmaceutical Co): *Ren shen*, *Panax ginseng* C.A. Mey.root; *Huang qi*, *Astragalus membranaceus* (Fisch.) Bge. Root; *Ci wu jia*, *Acanthopanax Senticosus* (Ruper.et Maxim.) Harms. root; *Ban mao*, *Mylabris phalerata* Pallas; 60 mL (0.3g crude drug/mL) diluted with 250 mL 5% glucose injection fluid ID, day 1-10, 14 days/cycle, for 11 weeks;<br>FOLFOX 4:5.5/5.5 (mean) | tRR; Chemotherapy AEs (leukopenia, anaemia, thrombocytopenia, nausea, vomiting, diarrhoea, constipation, stomatitis, liver/kidney impairment, neurotoxicity, alopecia); Median OS; One year OS; KPS. |
| Li YJ (2007); 20/18; 01/2005 to 04/2006. | 22 (all); 72.7 (median, all); ≥ 60. | 28(all); 10(all); NS. | Wenshenjianpi decoction (NS): *Rou cong rong*, *Cistanche deserticola* Y. C. Ma. herb; *Yin yang huo*, *Epimedium grandiflorum* Mot. leaf; Gou ji, *Lycium barbarum* L. fruit; *Dang shen*, *Codonopsis pilosula* (Franch.) Nannf. root; *Huang qi*, *Astragalus membranaceus* (Fisch.) Bge. root; *Bai zhu*, *Atractylodes macrocephala* Koidz. root; *Fu ling*, *Poria cocos* (Schw) Wolf sclerotium; *Yi yi ren*, *Coix lacryma-jobi* L. seed; *Yu jin*, *Curcuma wenyujin* Y. H. Chen et C. Ling. rhizome; *Shan zha*, *Crataegus pinnatifida* Bge. fruit; *Mai ya*, *Hordeum vulgare* L. germinating seed; *Ban zhi lian*, *Scutellaria barbata* D. Don. herb; modified.<br>One decoction per day orally administered concurrently with chemotherapy for median 10-12 weeks;<br>FOLOFOX 4: 6/5.5 cycles (median) | tRR; Chemotherapy AEs (leukopenia, anaemia, thrombocytopenia, liver impairment, nausea, vomiting, diarrhoea neurotoxicity); KPS. |
| Qiu ZC (2011); 22/21*; 01/2005 to 08/2009. | 14/13; 56.9/52.7 (median); ≥ 60. | 22/21; NO; NS. | Kang'ai Injection (Jilin Changbaishan Pharmaceutical Co): *Ku shen*, *Sophora flavescens* Ait.root; *Ren shen*, *Panax ginseng*C.A. Mey.root; *Huang qi*, *Astragalus membranaceus* (Fisch.) Bge. root; 40 mL of Kang'ai injection diluted with 250 mL 5% glucose injection fluid ID, day 1-10, 14 days/cycle, for 4 cycles;<br>FOLFOX 4: 4/4 cycles. | tRR; Chemotherapy AEs (leukopenia, nausea & vomiting); KPS. |
| Wu GL | 23/17; | NS; | Fupiyiwei decoction (NS): *Shi hu*, *Dendrobium loddigesii* Rolfe. stem; *Cang zhu*, *Atractylodes lancea* | tRR; |

| First author (year); Sample size T/C; Duration. | Gender (M) T/C; Age T/C; KPS all. | Previously untreated T/C; Previously treated T/C; Metastatic sites T/C. | HM: name (manufacturer), ingredients (pinyin, scientific name, part); dosage & administration; FOLFOX4: cycles (T/C). | Outcome measures |
|---|---|---|---|---|
| (2010); 33/25; 10/2004 to 05/2008. | 55.4 ±13.6/52.8 ±15.2 (mean); ≥ 60. | NS; NS. | (Thumb.) DC. root; *Yi yi ren*, *Coix lacryma-jobi* L. seed; *Ban xia Pinellia ternata* (Thunb.) Breit. tuber; *Shan yao*, *Dioscorea opposita* Thunb. rhizome; *Fu ling*, *Poria cocos* (Schw) Wolf. sclerotium; *Dou kou*, *Alpinia katsumadai* Hayata. seed; *Jiao gu lan*, *Gynostemma pentaphyllum* (Thunb.) Mak. herb; *Bai shao*, *Paeonia lactiflora* Pall. root; *Huo xiang*, *Pogostemon cablin* (Blanco) Benth. herb; One decoction per day oral-administered concurrently with chemotherapy for 24 weeks;<br>FOLFOX 4:12/12 cycles | Chemotherapy AEs (leukopenia, anaemia, thrombocytopenia, nausea, vomiting, diarrhoea neurotoxicity); KPS; T-cell subsets (CD3+, CD4+, CD8+), NK cells. |
| Xu YX (2010); 61/60; 08/2004 to 11/2008. | 38/37; 53/52 (mean); ≥ 70. | NS; NS; NS. | Jiangniling decoction (NS): *Dang shen*, *Codonopsis pilosula* (Franch.) Nannf. root; *Bai zhu*, *Atractylodes macrocephala* Koidz. root; *Fu ling*, *Poria cocos* (Schw) Wolf. sclerotium; *Bai dou kou*, *Amomum cardamomum*L. seed, *Ban xia*, *Pinellia ternata* (Thunb.) Breit. tuber; *Sha ren*, *Amomum villosum* Lour. Seed; *Xiang fu*, *Cyperus rotundus* L. rhizome; *Chen pi*, *Citrus reticulata* Blanco. peel; *Zhu ru*, *Phyllostachyl nigra* (Lodd.). shaving; *Sheng jiang*, *Zingiber officinale* Rosc. rhizome; *Yi yi ren*, *Coix lacryma-jobi* L. seed; One decoction per day orally administered concurrently with chemotherapy for 22 weeks;<br>FOLFOX 4: 11.1/7.8 cycles (mean). | tRR; Chemotherapy AEs (nausea, vomiting, diarrhoea, anaemia, leukopenia, thrombocytopenia); TTP; KPS. |
| Yang YF (2008); 30/30; 01/2007 to 11/2007. | 16/19; 51.07±10.4 4/51.33 ±10.95 (mean); 40.73±3.49 / 40.90±2.44 | 9/7; 21/23; NS. | Kang'ai Injection (Jilin Changbaishan Pharmaceutical Co): *Ku shen*, *Sophora* flavescens Ait. root; *Ren shen*, *Panax ginseng* C.A. Mey. root; *Huang qi*, *Astragalus membranaceus* (Fisch.) Bge. root; 50 mL of Kang'ai injection diluted with 250 mL 5% glucose injection fluid ID per day, day 1-20, 30 days/course, for 2 courses;<br>FOLFOX 4: 4/4 cycles. | tRR; Chemotherapy AEs (leukopenia, anaemia, thrombocytopenia, liver impairment, nausea, vomiting, diarrhoea, neurotoxicity); QoL, BW. |
| Zeng DX (2009); 35/32; 02/2007 to 12/2008. | 16/19; 50-70 (range); ≥ 70. | 67(all); NO; Liver: 15/19, Lung: 8/7, Others: 9/9. | Ginsenoside Rg3 capsules (Jilin Yatai Pharmaceutical Co): 2 capsules/day, administered concurrently with chemotherapy for 8 weeks.<br>FOLFOX 4: 4X4 cycles | tRR; Chemotherapy AEs (leukopenia, nausea, vomiting, diarrhoea, |

| First author (year); Sample size T/C; Duration. | Gender (M) T/C; Age T/C; KPS all. | Previously untreated T/C; Previously treated T/C; Metastatic sites T/C. | HM: name (manufacturer), ingredients (pinyin, scientific name, part); dosage & administration; FOLFOX4: cycles (T/C). | Outcome measures |
|---|---|---|---|---|
| | | | | neurotoxicity);<br>T cell subsets (CD3+, CD4+, CD8+, CD4+/CD8+). |
| Zeng JQ (2008);<br>30/30;<br>NS. | 19/18;<br>48/60 (median);<br>≥ 60. | NO;<br>30/30;<br>Liver: 14/7,<br>Lung: 6/8,<br>Others: 15/12. | Basic HM formula (NS): *Huang qi*, *Astragalus membranaceus* (Fisch.) Bge. root; *Bai zhu Atractylodes macrocephala* Koidz. root; *Yi yi ren*, *Coix lacryma-jobi* L. seed; *Ban zhi lian*, *Scutellaria barbata* D. Don. herb; *Ku shen*, *Sophora flavescens* Ait. root; *Mu xiang*, *Aucklandia lappa* Decne. root; *Bai hua she she cao*, *Hedyotis diffusa* Willd. herb; modified; One decoction per day orally administered concurrently with chemotherapy for 4 weeks.<br>FOLFOX 4: 2/2 cycles. | tRR;<br>Chemotherapy AEs (leukopenia, gastrointestinal reaction, neurotoxicity);<br>KPS. |
| Zhang HT (2008); 31/29; 03/2003 to 11/2006. | 28/23;<br>52.35/53.4 (mean);<br>≥ 60. | NS;<br>NS;<br>NS. | 3 HM decoctions based on CM 'Zheng' differentiation (NS):<br>1. Damp heat tenesmus decoction: *Ku shen*, *Sophora flavescens* Ait. root; *Zhong jie feng*, *Sarcandra glabra* (Thunb.) Nakai. herb; *Yi yi ren*, *Coix lacryma-jobi* L. seed; *Huai hua*, *Sophora japonica* L. flower-bud; *Di yu*, *Sanguisorba officinalis* L. root; *Bai jiang cao*, *Patrina villosa* Juss. herb; *Jin yin hua*, *Lonicera japonica* Thunb. flower; *Mu mian hua*, *Bombax malabarica* (DC.) Merr. flower; *Bai hua she she cao*, *Hedyotis diffusa* Willd. herb; *Yin chen*, *Artemisia capillaris* Thunb. herb; *Hou po*, *Magnolia officinalis* Rehd. et Wils. bark; *Huang lian*, *Coptis chinensis* Franch. root.<br>2. Toxic stasis in colon decoction: *Ku shen*, *Sophora flavescens* Ait. root; *Zhong jie feng*, *Sarcandra glabra* (Thunb.) herb; *Huai hua*, *Sophora japonica* L. flower-bud; *Di yu*, *Sanguisorba officinalis* L. root; *Bai jiang* cao, *Patrina villosa* Juss. herb; *Jin yin hua*, *Lonicera japonica* Thunb. flower; *Bai hua she she cao*, *Hedyotis diffusa* Willd. herb; *Yan dan zi*, *Brucea javanica* (L.) Merr. seed; *Da ji*, *Cirsium japonicum* DC. herb; *Qi ye yi zhi hua*, *Paris polyphylla* Smith. root; *Chi shao*, *Paeonia* veitchii Lynch. root; *E zhu*, *Curcumae phaeocaulis* Val. rhizome;<br>3. Spleen and kidney deficiencydecoction: *Dang shen*, *Codonopsis pilosula* (Franch.) Nannf. root; *Fu ling*, *Poria cocos* (Schw) Wolf. sclerotium; *Huang qi*, *Astragalus membranaceus* (Fisch.) Bge. root; *Ku shen*, *Sophora flavescens* Ait. root; *Zhong jie feng*, *Sarcandra glabra* (Thunb.) Nakai. herb; *Yi yi ren*, *Coix lacryma-jobi* L. seed; *Sha ren*, *Amomum villosum* Lour. seed, *Lian zi*, *Nelumbo nucifera* Gaertn. Seed; *Bai zhu*, *Atractylodes macrocephala* Koidz. root; *He zi*, *Terminalia chebula* Retz. seed; *He shou wu*, *Polygonum multiflorum* Thunb. root; *Bai shao*, *Paeonia lactiflora* Pall. root; One decoction per day orally administered concurrently with chemotherapy. Started one week before chemotherapy till one week after chemotherapy | tRR;<br>Chemotherapy AEs (gastrointestinal reaction, myelotoxicity);<br>KPS. |

| **First author (year); Sample size T/C; Duration.** | **Gender (M) T/C; Age T/C; KPS all.** | **Previously untreated T/C; Previously treated T/C; Metastatic sites T/C.** | **HM: name (manufacturer), ingredients (pinyin, scientific name, part); dosage & administration; FOLFOX4: cycles (T/C).** | **Outcome measures** |
|---|---|---|---|---|
| | | | completed.<br>FOLFOX 4: 4/4 cycles. | |
| Zhang Q (2010); 60/60; 02/2005 to 10/2007. | 35/33; 56.2 (mean all); ≥ 60. | 32/34; 28/26; Liver: 32/36, Lung: 16/19, Others: 51/39. | Guben Xiaoliu Capsule (Beijing Chinese Medicine Hospital, affiliated with Beijing Medical University): *Dong chong xia cao*, *Cordyceps sinensis* (berk.) Sacc. ascocarp and dead larva; *Ling zhi*, *Ganoderma lucidum* (Leyss.ex Fr.) karst. sporocarp; *Yin yang huo*, *Epimedium grandiflorum* Mot.leaf; *Zhe bei mu*, *Fritillaria thunbergii* Miq. bulb; *Xi yang shen*, *Panax quinquefolium* L. root; *Yi yi ren*, *Coix lacryma-jobi* L. seed; *Shui zhi*, *Hirudo nipponica* Whitman. whole leech; *Quan xie*, *Buthus martensii* Karsch. whole insect; *Long kui*, *Solanum nigrum* L. herb; 4 capsules each time, twice a day, orally administered concurrently with chemotherapy for 8 wks.<br>FOLFOX 4: 4/4 cycles. | tRR; Chemotherapy AEs (leukopenia, anaemia, thrombocytopenia, nausea & vomiting, diarrhoea, neurotoxicity); KPS; T cell subsets CD4+/CD8+, NK cells. |
| Zhang Y (2010); 21/20; 01/2005 to 12/2008. | NS; NS; ≥ 60. | NS; NS; NS. | Jianpi Jiedu decoction (NS): *Huang qi*, *Astragalus membranaceus* (Fisch.) Bge.root; *Bai zhu*, *Atractylodes macrocephala* Koidz. root; *Ba yue zha*, *Akebia quinata* (Thunb.) Decne. fruit; *Shi jian chuan*, *Salvia chinensis* Benth. herb; *Ye pu tao teng*, *Vitis quinquangularis*Rehder. vine and leaf; One decoction per day orally administered concurrently with chemotherapy, for 4 weeks.<br>FOLFOX 4: 2/2 cycles | tRR; Chemotherapy AEs (leukopenia, anaemia, thrombocytopenia, liver impairment, nausea & vomiting, neurotoxicity); TTP; One year OS; Median OS; KPS; BW. |

T: treatment group, C: control group, M: male, N: number, NS: not stated, ID: intravenous drip, H: high risk; L: low risk; U: unclear risk; FOLFOX regimen: 5-FU, Leucovorin, Oxaliplatin. ID: intravenous drip; tRR: Tumour Response Rate; BW: Body Weight; QoL: Quality of Life; TTP: Time to Progression; OS: Overall Survival; mOS: median Overall Survival; mPFS: median Progressive Free Survival; KPS: Karnofsky Performance Status; CM: Chinese medicine; HM: herbal medicine.

* note error in method section which states 20 in control, whereas elsewhere the number is 21.

# Appendix G. Characteristics of Studies Included in Chapter 6

**Table G1.Characteristics of randomised controlled trials of Chinese herbal medicines combined with oxaliplatin-based regimens for colorectal cancer with tumour response rate incidence as an outcome**

| First author (year) | Sample size T/C; Gender (M) T/C;Age T/C | TNM (T/C); KPS/ ECOG | CHM Intervention; dosage & duration | Oxaliplatin (Ox.) regimen; dose, cycles (T/C) |
|---|---|---|---|---|
| Cao B (2013) | 60/60; 32/33; 55.2 ± 13.3/58.8 ± 13.7 | IV (all); ECOG 0-1. | Yiqizhuyu decoction (YZD); one decoction per day orally administered for up to 48 wks. | FOLFOX4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg /m², ID, day 1-2, 5-FU 400 mg /m², bolus, 600 mg /m², ID, 22 hours, day1-2, cycle/14 days, up to 24 cycles (all). |
| Cao C (2005) | 33/29; 20/19; 52/49 (med) | IV (all); NS. | Shengmai injection; 40 mL, ID, day 1–7, NS. | FOLFOX: Ox.130 mg/m², ID, day 1, LV 200 mg /m², ID, day 1-5, 5-FU 750 mg /m², ID, day1-5, cycle/21 days, cycle NS. |
| Chen X (2005) | 47/46; 31/32; 54/53 (med) | II: 11/12, III: 21/20, IV: 15/14; KPS ≥60 | Composite salviae dripping pill; 25mg/pill, 10pills/day, tid, for 3 cycles or more. | FOLFOX: Ox. 130 mg/m² 2hours ID, LV 200 mg/m², 2 hours ID, 5-FU 500 mg, bolus, then 3000 mg/m² ID for 48 hours. 3wks /cycle, for 3cycles (all). |
| Fang M (2008) | 48/45; 30/28; 59.5 ± 11.3/56.4 ± 10.3 | IV (all); KPS ≥70. | Javanica oil emulsion injection; 30 mL, ID, day 1-14 / cycles, for two cycles | FOLFOX4: 2 cycles (all). |
| Hu A (2006) | 28/22; 18/14; 49.3 ± 4.5/48.5 ± 4.3 | IV (all); KPS ≥50. | Treatment with 4 different CHM decoctions according to syndrome differentiation; one decoction per day, for more than 30 days. | FOLFOX: Ox.130 mg/m², ID, day 1, LV 200 mg /m², ID, day 1-2, 5-FU 2400 mg /m², ID, 46 hours, cycle/21 days, 2 cycles (all). |
| Jiang G (2013) | 32/31; 18/18; 53. 2 ± 12. 4/53. 1 ± 12.8 | ACRC; NS. | Unnamed multi-CHM formula; one decoction per day, for 8 weeks. | FOLFOX: Ox.135 mg/m², ID, day 1, LV 200mg /m², ID, day 1-2, 5-FU 400 mg bolus, day 1-2, 2400-3600 mg /m², ID for 48 hours, 14 days/cycle, for 4 cycles (all). |
| Kono T (2013) | 27/23; NS; 67/61 (mean) | NS; ECOG 0–1 | TJ-107 Goshajinkigan; or placebo was administered orally, tid, before each meal (7.5 g/day) for 26 weeks | FOLFOX4, or mFOLFOX6: Ox.85 mg/m², ID, day 1, LV 200 mg /m², ID, 5-FU 400 mg bolus, follow 2400 mg /m², ID for 46 hours, 14 days/cycle, for 8 cycles or more (all). |
| Lao G (2012) | 30/30; 21/23; 35.1 ± 20.2/36.7 ± 20.1. | II:5/7, III: 15/14, IV:10/9; KPS ≥60 | Jianpijiedu decoction; one decoction per day, 21 days /cycle, for two cycles. | FOLFOX: Ox.130 mg/m², ID, day 1, LV 200 mg /m², ID, day 1, 5-FU 500 mg bolus day 1, 2400 mg /m², ID, 48 hours, day 1-2, 21 days /cycle, 2 cycles (all) . |
| Li H (2007) | 65/52; 43/36; 58/59 (med) | III: 27/19, IV: 38/33; KPS ≥60 | Aidi injection; 60 mL, ID, day 1-10, 14days/cycle, for 11weeks. | FOLFOX4: 5.5/5.5 cycles (mean). |
| Li Y (2007) | 20/18; 22 (all); 72.2 (med, all) | III: 15, IV: 23 (all); KPS ≥60 | Wenshenjianpi decoction; one decoction per day, for median 10-12 weeks | FOLOFOX4: 6/5.5 cycles (med). |

| First author (year) | Sample size T/C; Gender (M) T/C;Age T/C | TNM (T/C); KPS/ ECOG | CHM Intervention; dosage & duration | Oxaliplatin (Ox.) regimen; dose, cycles (T/C) |
|---|---|---|---|---|
| Liang Q (2009) | 76/76; 50/51; 53/52 (med) | III: 45/46, IV: 31/30; KPS ≥60 | Shenqifuzheng injection; 250 mL/day, ID, 3wks /cycle, for 2cycles | FOLFOX: Ox. 130 mg/m² 2hours, ID, day 1, LV 200 mg/m², 2 hours, ID, day1, 5-FU 500 mg, bolus, day1, then 3000 mg/m², ID, for 48 hours. 3wks /cycle, for 2cycles (all). |
| Lim M (2012) | 24/23; 17/14; 56.89 ± 14.77/55.37 ± 16.01 | III: 15/16, IV: 9/7; KPS ≥70 | Pianzaihuang capsule; two capsules, bid; 14 days/cycle, 8-10 cycles. | FOLFOX4; 8-10/8-10 cycles. |
| Liu H (2009) | 36/34; 16/18; 50.2 (mean) | ACRC (all); KPS ≥60 | Kang'aifangyi pian; one decoction per day, 21 days/cycle, for 3 cycles. | FOLFOX: Ox.130 mg/m², ID, day 1, LV 200 mg /m², ID, day 1-5, 5-FU 300 mg /m², ID, day 1-5, 21 days / cycle, 3 cycles (all). |
| Liu J(2005b) | 52/26; 24/12; 63.11 ± 11.89/62.78 ± 11.04 | IV (all); KPS ≥50. | Jianpihuoxue formulae; one decoction per day, 30 days/ cycle, 3 cycles. | FOLFOX; Ox.150 mg/m², ID, day 1, LV 200 mg /m², ID, day 1-5, 5-FU 500 mg /m², ID, day 1-5, 30 days/ cycle, 3 cycles (all). |
| Liu J (2005a) | 43/21; 23/10; 61.52 ±10.12 /60.11 ±9.78 | IV (all); KPS ≥50. | Jianpihuoxue formulae; one decoction per day, 30 days/ cycle, 3 cycles. | FOLFOX; Ox.150 mg/m², ID, day 1, LV 200 mg /m², ID, day 1-5, 5-FU 500 mg /m², ID, day 1-5, 30 days/ cycle, 3 cycles (all). |
| Qin Y (2011) | 36/37; NS; NS. | ACRC; NS | Fuzhengguban decoction; one decoction per day, 21 days/ cycle, 2 cycles. | FOLFOX: Ox.135 mg/m², ID, day 1, LV 200 mg /m², ID, day 1-2, 5-FU 2400 mg /m², ID, for 48 hours, 21 days/ cycle, 2 cycles (all). |
| Qiu Z (2011) | 22/21;14/13; 56.9/52.7(med) | IV (all); KPS ≥60. | Kang'ai injection; 40 mL, ID, day 1–10, 14 days/cycle, for four cycles | FOLFOX4, for 4 cycles (all). |
| Song W (2012) | 20/20; 12/13; 56.4 ± 9.1 /48.3 ± 8.2 | ACRC; KPS ≥70. | Xiaoliuhuajichangfang II; one decoction per day, 21 days/ cycle, 2 cycles. | FOLFOX: Ox.135 mg/m², ID, day 1, LV 200 mg /m², ID, day 1-2, 5-FU 2400 mg /m², ID, for 48 hours, 21 days/ cycle, 2 cycles (all). |
| Tao C (2013) | 74/74;51/50;60.1 + 7.9 /60.4 + 8.9. | ACRC; KPS 65.6 + 12.3/66.7 + 14.5 | Co-kushen injection; 15 mL per day, ID, started 14 days before chemotherapy, 5wks/cycle, for 1 cycle. | FOLFOX: Ox.135 mg/m², ID, day 1, LV 200 mg/m², ID, 2 hours, day 1-5, 5-FU 500 mg/m² ,ID, 8-10 hours, day1-5, 3wks/cycle, for 1 cycle (all). |
| Wan H (2013) | 30/30;35(all); 51.7 (mean, all) | III: 40 (all) IV: 20 (all); KPS ≥60. | Kushen injection 40 mL, ID, and Huangqi injection 20 mL, ID, perday, 10 days/cycle, for 6 cycles. | FOLFOX: Ox.100 mg/m², ID, day1, LV 200 mg/m², ID, 5-FU 15-20 mg/kg, ID, day 1-5, 21 days/ cycle, for 6 cycles (all). |
| Wang D (2012) | 49/49; 58(all); 54.5 (mean, all). | ACRC; KPS ≥60. | Unnamed multi-TM formula; one decoction per day, duration NS. | FOLFOX: Ox.120 mg/m², ID, day 1, LV 200 mg /m², ID, day 1-5, 5-FU 300 mg /m², ID, day 1-5, 21 days/ cycle, cycle NS. |
| Wang H (2008) | 34/34; 20/22; 52.58 ± 8.12/51.11 ± 7.72 | IV: 34/34; KPS ≥50. | Yiqiguoxiebuchang decoction; one decoction per day, for 3 mths. | FOLFOX: Ox.85 mg/m², ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 500 mg bolus day 1, 5-FU 2500 mg/m², ID, for 48 hours, 21 days/ cycle, 4 cycles (all). |
| Wang J (2011) | 30/30; 18/21; 52.3 ± 6.2/ 56.7 ± 7.8. | ACRC; KPS ≥60. | Yichangning decoction; one decoction per day, for 2 months. | FOLFOX4, 21 days /cycle, for two cycles (all). |
| Wang Y (2012) | 38/36; 26/25; 52 (mean, all). | ACRC; KPS ≥70. | Aidi injection; 80 mL, ID, per day, 10days/cycle, for 4 cycles | FOLFOX4, for 4 cycles (all). |

| First author (year) | Sample size T/C; Gender (M) T/C;Age T/C | TNM (T/C); KPS/ ECOG | CHM Intervention; dosage & duration | Oxaliplatin (Ox.) regimen; dose, cycles (T/C) |
|---|---|---|---|---|
| Wang Y (2013) | 32/30; 20/19; NS. | ACRC; KPS ≥70. | Xiaoaiping injection; 60 mL, ID, per day, 14 days/ cycle, for two cycles. | XELOX: no details, for two cycles(all) |
| Wang Z (2007) | 34/33; 47 (all); 55 (mean all). | ACRC; KPS ≥60. | Delisheng injection; 40-60 mL, ID per day, 15 days/cycle, for 3 cycles. | FOLFOX: Ox. 130 mg/m², 2hours ID, day 1 ,LV 200 mg/m², 2 hours ID, day1-5,5-FU 500 mg/m², ID day1-5, 3wks/cycle, for 3cycles (all). |
| Wu G(2010) | 33/25; 23/17; 55.4 ± 13.6 /52.8 ± 15.2. | I: 5/3, II: 10/8, III: 15/11, IV: 3/3; KPS ≥60. | Fupiyiwei decoction; one decoction per day, for 24 weeks; | FOLFOX4:12/12 cycles. |
| Xu Y (2006) | 32/20; 21/12; 53/51 (med) | IV: 32/20; KPS ≥60. | Fuzhenghuayujiedusanjie formula; one decoction per day, for 6 mths. | FLOFLX: Ox.100 mg/m², ID, day 1, 8, 5-FU 500 mg bolus, 250 mg /m², ID, day 1-15, or plus HCPT 10 mg/ m², ID, day 1-5, 30 days/ cycle, for 6 cycles (all). |
| Xu Y (2010) | 61/60; 38/37; 53/52 (mean) | ACRC; KPS ≥70. | Jiangniling formula; one decoction per day, 14 days/cycle, for 8-10 cycles | FOLFOX 4: 11.1/7.8 (mean) cycles. |
| Yang C (2007) | 50/50; 29/27; 51.36 ± 10.58 /53.48 ± 9.35. | ACRC; KPS ≥60. | Jianpikangfu pill; 6g, tid, for 4 wks. | FOLFOX: Ox.135 mg/m², ID, day 1, LV 100 mg /m², ID, day 1-5, 5-FU 425 mg /m², ID day 1-5, for 4 weeks (all). |
| Yang Y (2008a) | 30/30;16/19;51.07 ± 10.44 /51.33 ± 10.95. | ACRC; KPS ≥60. | Kang'ai injection; 50 mL, ID, day1-20, 30days/cycle, for 2 cycles. | FOLFOX4, for 4 cycles (all). |
| Yin X(2011) | 32/32; 17/16; 62. 00 ± 5. 23 /61.70 ± 6. 23 | ACRC; NS. | Jianpi formula; one decoction per day, 21 days/cycle, for 2 cycles. | XELOX: Ox.130 mg/m², ID, day 1, Xel250 mg/ m², day 1-14, bid, 21 days/cycle, 2 cycles (all). |
| You J (2010) | 30/30; 14/13; Range: 30-75/31-74 | III: 8/9, IV: 22/21; KPS ≥50. | WD-3 decoction; 50 mL, tid, 4 weeks/cycle, for 4 cycles. | FOLFOX: Ox.125 mg/m², ID, day 1, LV 100 mg /m², ID, day 1-5, 5-FU 500 mg /m², ID day 1-5, 4 weeks/cycle, for 4 cycles (all). |
| Zeng C (2013) | 30/30; 39/19; 54. 3 ±6. 3/53. 2 ± 6. 6 | III: 20/12 IV: 41/18; KPS ≥60. | Fuzhengxiaoji decoction; one decoction per day, 14 days/cycle, for 4-6 cycles. | FOLFOX: Ox.85 mg/m², ID, day 1, LV 200 mg /m², ID, day 1-2, 5-FU 360-500 mg /m² bolus, 600 mg /m², ID, for 22 hours, day 1-2, 14 days/cycle, for 4-6 cycles (all) |
| Zeng D (2009) | 35/32; 25/21; 50<: 4/5, 51-69: 28/25, >70: 3/2. | IV (all); KPS ≥70. | Ginsenoside Rg3 capsules: 2 capsules, bid, for 8 wks. | FOLFOX4, for 4 cycles (all). |
| Zeng J (2008) | 30/30; 19/18; 48/60 (med). | ACRC; KPS ≥60. | Multi-CHM formulae; one decoction per day, for 4wks. | FOLFOX4: 2/2 cycles. |
| Zhang H (2008) | 31/29; 28/23; 52.35/53.4 (mean) | ACRC; KPS ≥60. | 3 CHM decoctions based on symptom differentiation; one decoction per day. Started one week before chemotherapy till one week after chemotherapy completed. | FOLFOX4: 4/4 cycles. |
| Zhang Q (2006) | 38/30; 35 (all); 54.8 (mean, all). | ACRC; KPS:76.5 | Yiqihuoxue formulae; one decoction per day, 21 | FOLFOX: Ox.125 mg/m², ID, day 1, LV 200 mg /m², ID, day 1-2, 5-FU |

| First author (year) | Sample size T/C; Gender (M) T/C;Age T/C | TNM (T/C); KPS/ ECOG | CHM Intervention; dosage & duration | Oxaliplatin (Ox.) regimen; dose, cycles (T/C) |
|---|---|---|---|---|
| | | ± 5.8/73.5 ± 6.0 | days/cycle, for 3 cycles. | 500 mg /m² bolus, day 1-2, 2000 mg /m² ID for 72 hours, 21 days/cycle, for 3 cycles (all). |
| Zhang Q (2010) | 60/60; 35/33; 56.2 (mean, all) | ACRC; KPS: ≥60. | Gubenxiaoliu capsule; 4 capsules, bid, for 8 wks. | FOLFOX4, 4/4 cycles. |
| Zhang Y (2010) | 21/20; NS; NS; | ACRC; KPS: ≥60. | Jianpijiedu decoction; onedecoction per day, for 4weeks. | FOLFOX 4: 2/2 cycles |
| Zhou J (2011) | 34/34; 22/20; 51.2/52.5. | II: 14/13, III: 16/11, IV: 6/5; KPS: ≥60. | Fuzhengjianpi decoction; one decoction per day, for 8 wks. | FOLFOX: Ox.130 mg/m², ID, day 1, LV 100 mg /m², ID, day 1-5, 5-FU 500 mg /m², ID, day 1-5, 28 days/cycle, for 2 cycles (all) |
| Zou B (2007) | 32/27; 29/22; 53/54.3 (mean) | ACRC; KPS: ≥60. | Gubenkang'ai decoction; one decoction per day, for 6 wks. | FOLFOX: Ox.135 mg/m², ID, day 1, LV 200 mg /m², ID, day 1-2, 5-FU 2400 mg /m², ID, for 48 hours, day 1, 21 days/cycle, for 2 cycles (all) |

T: treatment group, C: control group, M: male, N: number, NS: not stated, ID: intravenous drip, tRR: Tumour Response Rate; TNM: cancer staging system. 'T' for tumour, denotes the extent of invasion of the intestinal wall, 'N' for lymphatic node, the amount of lymphatic node involvement, and 'M' for the metastasis. KPS: Karnofsky Performance Status; ECOG: Eastern Cooperative Oncology Group Performance Status; CHM: Chinese herbal medicine. 5-FU: 5-Fluorouracil; LV: Leucovorin; Ox.: Oxaliplatin; Xel: Capecitabine; HCPT: Hydroxycamptothecine; FOLFOX: Ox. + 5-FU + LV; XELOX: Ox. + Capecitabine; ACRC: advanced colorectal cancer; bid: twice per day; tid: three times per day; qd: once per day; wk: week; mth: month; med: median.

## Table G2. Characteristics of randomised controlled trials of Chinese herbal medicine combined with oxaliplatin-based regimens for colorectal cancer with chemotherapy induced nausea and vomiting incidence as an outcome

| First author (year) | Sample size T/C; Gender (M) T/C; Age T/C | CHM Intervention; dosage & duration | Oxaliplatin regimen; dose, cycles (T/C); anti-emetic drug. |
|---|---|---|---|
| Ding X (2010). | 30/30; 18/20; 64.5/63 (med.) | Co-Kushen injection; 20 mL, ID, day 1-7, 14 day/cycle, for 8 cycles. | FOLFOX4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg/m², bolus, 600 mg/m², ID, 22 hours, day1-2, 8/8 cycles. |
| Fang M (2008) | 48/45; 30/28; 59.5 ± 11.3/56.4 ± 10.3 | Javanica oil emulsion injection; 30 mL, ID, day 1-14 / cycles, for two cycles | FOLFOX4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg/m², bolus, 600 mg/m², ID, 22 hours, day1-2, 2 cycles (all); anti-emetic drug used (unknown) |
| Hu A (2006). | 28/22; 18/14; 49.3 ± 4.5/48.5 ± 4.3 | Treatment with 4 different CHM decoctions according to symptom differentiation; one decoction per day, for more than 30 days. | FOLFOX: Ox.130 mg/m², ID, day 1, LV 200 mg /m², ID, day 1-2, 5-FU 2400 mg/m², ID, 46 hours, cycle/21 days, 2/2 cycles; Granisetron, Metoclopramide. |
| Kono T (2013). | 27/23; NS; 67/61 (mean) | TJ-107 Goshajinkigan aqueous extract; or placebo was administered orally, tid, before each meal (7.5 g/day) for 26 wks | FOLFOX4: or mFOLFOX6: Ox.85 mg/m², ID, day 1, LV 200 mg/m², ID, 5-FU 400 mg bolus, follow 2400 mg /m², ID for 46 hours, 14 days/cycle, 8/8 cycles or more. |
| Lao G (2012). | 30/30; 21/23; 35.1 ± | Jianpijiedu decoction; one decoction per day, 21 days /cycle, | FOLFOX: Ox.130 mg/m², ID, day 1, LV 200 mg/m², ID, day 1, 5-FU 500 mg bolus day 1, |

| First author (year) | Sample size T/C; Gender (M) T/C; Age T/C | CHM Intervention; dosage & duration | Oxaliplatin regimen; dose, cycles (T/C); anti-emetic drug. |
|---|---|---|---|
| | 20.2/36.7 ± 20.1. | for two cycles. | 2400 mg/m², ID, 48 hours, day 1-2, 21 days /cycle, 2/2 cycles; 5-HT3 receptor antagonist and dexamethasone. |
| Li H (2007). | 65/52; 43/36; 58/59 (med) | Aidi injection; 60 mL, ID, day 1-10, 14 days/cycle, for 11 weeks. | FOLFOX 4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg/m², bolus, 600 mg/m², ID, 22 hours, day1-2, 5.5/5.5 cycles (mean); Granisetron. |
| Li Y (2007). | 20/18; 22 (all); 72.2 (med, all) | Wenshenjianpi decoction; one decoction per day, for med 10-12 weeks. | FOLFOX 4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg/m², bolus, 600 mg/m², ID, 22 hours, day1-2, 6/5.5 cycles (med). |
| Lim M (2012). | 24/23; 17/14; 56.89 ± 14.77/55.37 ± 16.01 | Pianzaihuang capsule; two capsules, bid; 14 days/cycle, 8-10 cycles. | FOLFOX4:Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg/m², bolus, 600 mg/m², ID, 22 hours, day1-2, 8-10/8-10 cycles. |
| Liu H (2009). | 36/34; 16/18; 50.2 (mean) | Kang'aifangyipian; one decoction per day, 21 days/cycle, for 3 cycles. | FOLFOX: Ox.130 mg/m², ID, day 1, LV 200 mg/m², ID, day 1-5, 5-FU 300 mg/m², ID, day 1-5, 21 days / cycle, 3/3 cycles. |
| Liu J (2005). | 43/21; 23/10; 61.52 ± 10.12 /60.11 ± 9.78 | Jianpihuoxueformulae; one decoction per day, 30 days/ cycle, 3 cycles. | FOLFOX: Ox.150 mg/m², ID, day 1, LV 200 mg/m², ID, day 1-5, 5-FU 500 mg/m², ID, day 1-5, 30 days/ cycle, 3/3 cycles; Ondansetron hydrochloride. |
| Liu W (2011). | 16/16; 11/10; 51/52 (mean) | Yi erkang capsule; 4-6 capsules, bid, for 5-25 months. | FOLFOX: Ox.130 mg/m², ID, day 1, LV 100 mg/m², ID, day 1-5, 5-FU 400 mg/m², ID, day 1-5, 21 days / cycle, 6 /6 cycles; Ondansetron. |
| Ma J (2005). | 28/25; 15/13; 58.1/57.5 (mean) | Jianpixiaoliu decoction; one decoction per day, 90 days/ cycle, 2 cycles. | FOLFOX: Ox.130 mg/m², ID, day 1, LV 200 mg/m², ID, day 1-5, 5-FU 375 mg/m², ID, day 1-5, 21 days / cycle, 6/6 cycles. |
| Qiu Z (2011). | 22/21; 14/13; 56.9/52.7 (med) | Kang'ai injection; 40 mL, ID, day 1–10, 14 days/ cycle, for 4 cycles. | FOLFOX4:Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg/m², bolus, 600 mg/m², ID, 22 hours, day1-2, 4/4 cycles. |
| Song W (2012). | 20/20; 12/13; 56.4 ± 9.1 /48.3 ± 8.2 | Xiaoliuhuajichangfang II; one decoction per day, 21 days/ cycle, 2 cycles. | FOLFOX: Ox.135 mg/m², ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 2400 mg/m², ID, for 48 hours, 21 days/ cycle, 2 /2 cycles; Ramosetron, Metoclopramide. |
| Tao C (2013). | 74/74; 51/50; 60.1 ± 7.9 /60.4 ± 8.9. | Co-kushen injection; 15 mL per day, ID, started 14 days before chemotherapy, 5 weeks/cycle, for 1 cycle. | FOLFOX: Ox.135 mg/m², ID, day 1, LV 200 mg/m², ID, 2 hours, day 1-5, 5-FU 500 mg/m² , ID, 8-10 hours, day1-5, 3wks/cycle, 1/1 cycle. |
| Wang H (2008). | 34/34; 20/22; 52.58 ± 8.12/51.11 ± 7.72 | Yiqiguoxiebuchang decoction; one decoction per day, for 3 months. | FOLFOX: Ox.85 mg/m², ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 500 mg bolus day 1, 5-FU 2500 mg/m², ID, for 48 hours, 21 days/ cycle, 4/4 cycles; Ondansetron hydrochloride. |
| Wang J (2011). | 30/30; 18/21; 52.3 ± 6.2/ 56.7 ± 7.8. | Yichangning decoction; one decoction per day, for 2 months. | FOLFOX4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg/m², bolus, 600 mg/m², ID, 22 hours, day1-2, 21 days /cycle, 2/2 cycles; Ondansetron. |
| Wu G (2010). | 33/25; 23/17; 55.4 ± 13.6 /52.8 ± 15.2. | Fupiyiwei decoction; one decoction per day, for 24 weeks. | FOLFOX4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg/m², bolus, 600 mg/m², ID, 22 hours, day1-2, 12/12 cycles; Ondansetron hydrochloride |
| Xu Y (2010) | 61/60; 38/37; 53/52 (mean) | Jiangniling formula; one decoction per day, 14 days/cycle, for 8-10 cycles | FOLFOX4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg/m², bolus, 600 mg/m², ID, 22 hours, day1-2, 11.1/7.8 (mean) cycles; Granisetron. |

| First author (year) | Sample size T/C; Gender (M) T/C; Age T/C | CHM Intervention; dosage & duration | Oxaliplatin regimen; dose, cycles (T/C); anti-emetic drug. |
|---|---|---|---|
| Yang Y (2008). | 30/30; 16/19; 51.07 ± 10.44 /51.33 ± 10.95. | Kang'ai injection; 50 mL, ID, day1-20, 30days/cycle, for 2 cycles. | FOLFOX4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg/m², bolus, 600 mg/m², ID, 22 hours, day1-2, 4/4 cycles; Granisetron. |
| Yang Z (2005) | 30/30; 18/20; 29-70 /28-69 | Xuesaitong injection, 500 mg, ID; Huangqi injection, 60 mL, ID; Shenmai injection, 50 mL, ID &CHM decoction, one decoction per day, day1-5, 21 days/cycle, for 2 cycles. | FOLFOX: Ox.200 mg/m², ID, day 1, LV 200 mg/m², ID, day 1-5, 5-FU 500 mg/m², ID day 1-5, 2/2 cycles. |
| Zhang H (2008) | 31/29; 28/23; 52.35/53.4 (mean) | 3 CHM decoctions based on syndrome differentiation; one decoction per day. Started one week before chemotherapy till one week after chemotherapy completed. | FOLFOX4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg/m², bolus, 600mg/m², ID, 22 hours, day1-2, 4/4 cycles; Ondansetron hydrochloride. |
| Zhang Q (2006). | 38/30; 35 (all); 54.8 (mean all). | Yiqihuoxue formulae; one decoction per day, 21 days/ cycle, for 3 cycles. | FOLFOX: Ox.125 mg/m², ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 500 mg/m² bolus, day 1-2, 2000 mg/m² ID for 72 hours, 21 days/cycle, 3/3 cycles; Ondansetron hydrochloride |
| Zhang Q (2010). | 60/60; 35/33; 56.2 (mean all); | Gubenxiaoliu capsule; 4 capsules, bid, for 8 wks. | FOLFOX4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400mg/m², bolus, 600 mg/m², ID, 22 hours, day1-2, 4/4 cycles; Ondansetron hydrochloride. |
| Zhang W (2013). | 32/32; 15/16; 56.8 ± 10.1/46.4 ± 9.2. | Xiaoliuhuaji Decoction I; one decoction per day, for 5 mths. | FOLFOX: Ox.135 mg/m², ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 2400 mg/m² , ID for 48 hours, 21 days/cycle, 6/6 cycles; Ramosetron. |
| Zhang Y (2010). | 21/20; NS; NS; | Jianpijiedu decoction; one decoction per day, for 4 wks. | FOLFOX4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg/m², bolus, 600 mg/m², ID, 22 hours, day1-2, 2/2 cycles. |
| Zou B (2007) | 32/27; 29/22; 53/54.3 (mean) | Gubenkang'ai decoction; one decoction per day, for 6 wks. | FOLFOX: Ox.135 mg/m², ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 2400 mg/m², ID, for 48 hours, day 1, 21 days/ cycle, for 2 cycles(all); Granisetron, Metoclopramide. |

RN: reference number (superscript); T: treatment group; C: control group; M: male; N: number; NS: not stated; ID: intravenous drip; CHM: Chinese herbal medicine; CINV: chemotherapy induced nausea and vomiting; 5-FU: 5-Fluorouracil; LV: Leucovorin; Ox.: Oxaliplatin; FOLFOX: Ox. + 5-FU + LV; bid: twice per day; tid: three times per day; qd: once per day; wk: week; mth: month; med: median.

## Table G3. Characteristics of randomised controlled trials of Chinese herbal medicines combined with oxaliplatin-based regimens for colorectal cancer with chemotherapy induced neutropenia incidence as an outcome

| First author (year). | Sample size T/C; Gender (M) T/C; Age T/C | TNM (T/C); KPS/ ECOG | TM Intervention; dosage & duration | Oxaliplatin regimen; dose, cycles (T/C) | Outcome: CIN gr.: T/C |
|---|---|---|---|---|---|
| Deng D (2010) | 18/18; 9/9; 54.17 ± 10.04/53.56 ± 11.10. | IV (all); KPS ≥60. | Yiqixiaoji decoction; one decoction per day, for up to 6 wks. | XELOX: Ox. 130 mg/m², 2hours ID, day 1; Xel. 850 mg/m², bid, for 14 days; 21 days/cycle, for 2 cycles; G-CSF used. | I: 2/4; II: 1/1; III: 0/1; IV: 0/0. |

| First author (year). | Sample size T/C; Gender (M) T/C; Age T/C | TNM (T/C); KPS/ ECOG | TM Intervention; dosage & duration | Oxaliplatin regimen; dose, cycles (T/C) | Outcome: CIN gr.: T/C |
|---|---|---|---|---|---|
| Ding X (2010) | 30/30; 18/20; 64.5/63 (med) | ACRC (all); KPS ≥70 | Co-Kushen injection; 20 mL, ID, day 1-7, 14 day/cycle, for 8 cycles. | FOLFOX4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg/m², bolus, 600 mg/m², ID, 22 hours, day1-2, 8/8 cycles. | I: 9/10; II: 3/6; III: 0/1; IV: 0/0. |
| Fang M (2008) | 48/45; 30/28; 59.5 ± 11.3/56.4 ± 10.3 | IV (all); KPS ≥70. | Javanica oil emulsion injection; 30 mL, ID, day 1-14 / cycles, for two cycles | FOLFOX4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg/m², bolus, 600 mg/m², ID, 22 hours, day1-2. 2/2 cycles; G-CSF used. | I/II: 19/17; III/IV: 2/1. |
| Hu A (2006) | 28/22; 18/14; 49.3 ± 4.5/48.5 ± 4.3 | IV (all); KPS ≥50. | Treatment with 4 different TM decoctions according to syndrome differentiation; one decoction per day, for more than 30 days. | FOLFOX: Ox.130 mg/m², ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 2400 mg/m², ID, 46 hours, cycle/21 days, 2/2 cycles. | I: 4/7; II: 2/6; III: 0/1; IV: 0/0. |
| Kono T (2013) | 44/45; 23/25; 67/61 (med) | NS; ECOG 0–1 | TJ-107 Goshajinkigan aqueous extracts; or placebo was administered orally, tid, before each meal (7.5 g/day) for 26 weeks | FOLFOX4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg/m², bolus, 600 mg/m², ID, 22 hours, day1-2 or mFOLFOX6: Ox.85 mg/m², ID, day 1, LV 200 mg/m², ID, 5-FU 400 mg bolus, follow 2400 mg/m², ID for 46 hours, 14 days/cycle, 8/8 cycles or more. | All gr.: 15/21; ≥III: 10/15. |
| Lao G (2012) | 30/30; 21/23; 35.1 ± 20.2/36.7 ± 20.1. | II:5/7, III: 15/14, IV: 10/9; KPS ≥60 | Jianpijiedu decoction; one decoction per day, 21 days /cycle, for two cycles. | FOLFOX: Ox.130 mg/m², ID, day 1, LV 200 mg/m², ID, day 1, 5-FU 500 mg bolus day 1, 2400 mg/m², ID, 48 hours, day 1-2, 21 days /cycle, 2/2 cycles. | I: 4/9; II: 3/5; III: 1/4; IV: 0/0. |
| Li H (2007) | 65/52; 43/36; 58/59 (med) | III: 27/19, IV: 38/33; KPS ≥60 | Aidi injection; 60 mL, ID, day 1-10, 14 days/cycle, for 11 weeks. | FOLFOX4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg/m², bolus, 600 mg/m², ID, 22 hours, day1-2, 5.5/5.5 cycles (mean). | I: 9/14; II: 5/7; III: 3/5; IV: 0/1. |
| Li Y (2007) | 20/18; 22 (all); 72.2 (med, all) | III: 15, IV: 23 (all); KPS ≥60 | Wenshenjianpi decoction; one decoction per day, for 10-12 weeks. | FOLFOX4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg/m², bolus, 600 mg/m², ID, 22 hours, day1-2, 6/5.5 cycles (med). | I: 5/10; II: 4/4; III: 1/1; IV: 0/0. |
| Lim M (2012) | 24/23; 17/14; 56.89 ± 14.77/55.37 ± 16.01 | III: 15/16, IV: 9/7; KPS 84.78 ± 14.66/83.42 ± 13.09 | Pianzaihuang capsule; two capsules, bid; 14 days/cycle, 8-10 cycles. | FOLFOX4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg/m², bolus, 600 mg/m², ID, 22 hours, day1-2, 8-10/8-10 cycles. | I: 5/7; II: 1/4; III: 0/1; IV: 0/0. |
| Liu H (2009) | 36/34; 16/18; 50.2 (med, all) | ACRC (all); KPS ≥60 | Kang'ai fangyi pian; one decoction per day, 21 days / cycle, for 3 cycles. | FOLFOX: Ox.130 mg/m², ID, day 1, LV 200 mg/m², ID, day 1-5, 5-FU 300 mg/m², ID, day 1-5, 21 days / cycle, 3/3 cycles. | I: 7/9; II: 2/5; III: 1/1; IV: 0/0. |

| First author (year). | Sample size T/C; Gender (M) T/C; Age T/C | TNM (T/C); KPS/ ECOG | TM Intervention; dosage & duration | Oxaliplatin regimen; dose, cycles (T/C) | Outcome: CIN gr.: T/C |
|---|---|---|---|---|---|
| Liu J (2005) | 43/21; 23/10; 61.52 ± 10.12 /60.11 ± 9.78 | IV (all); KPS ≥50. | Jianpihuoxue formulae; one decoction per day, 30 days/cycle, 3 cycles. | FOLFOX: Ox.150 mg/m², ID, day 1, LV 200 mg/m², ID, day 1-5, 5-FU 500 mg/m², ID, day 1-5, 30 days/ cycle, 3/3 cycles. | I: 5/5; II: 2/3; III: 1/1; IV: 0/0. |
| Liu W (2011) | 16/16; 11/10; 51/52 (mean) | IV (all); KPS 40-60 (range). | Yierkang capsule; 4-6 capsules, bid, for 5-25 months. | FOLFOX: Ox.130 mg/m², ID, day 1, LV 100 mg/m², ID, day 1-5, 5-FU 400 mg/m², ID, day 1-5, 21 days / cycle, 6 /6 cycles. | All gr.: 3/12. |
| Ma J (2005) | 28/25; 15/13; 58.1/57.5 (mean) | II:7/4, III: 21/21; KPS ≥60. | Jianpi Xiaoliu decoction; one decoction per day, 90 days / cycle, 2 cycles. | FOLFOX: Ox.130 mg/m², ID, day 1, LV 200 mg/m², ID, day 1-5, 5-FU 375 mg/m², ID, day 1-5, 21 days / cycle, 6/6 cycles. | I: 4/8; II: 2/2; III: 0/0; IV: 0/0. |
| Qiu Z (2011) | 22/21; 14/13; 56.9/52.7 (med) | IV (all); KPS ≥60. | Kang'ai injection; 40 mL, ID, day 1–10, 14 days/cycle, for 4 cycles. | FOLFOX4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg/m², bolus, 600 mg/m², ID, 22 hours, day1-2, 21 days /cycle, 4/4 cycles. | III/IV: 1/8. |
| Song W (2012) | 20/20; 12/13; 56.4 ± 9.1 /48.3 ± 8.2 | ACRC (all); KPS ≥70. | Xiaoliuhuajichangfang II; one decoction per day, 21 days/cycle, 2 cycles. | FOLFOX: Ox.135 mg/m², ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 2400 mg/m², ID, for 48 hours, 21 days/ cycle, 2 /2 cycles. | I: 2/4; II: 3/3; III: 1/2; IV: 0/1. |
| Tao C (2013) | 74/74; 51/50; 60.1 ± 7.9 /60.4 ± 8.9. | ACRC (all); KPS 65.6 ± 12.3/66.7 ± 14.5 | Co-kushen injection; 15 mL per day, ID, started 14 days before chemotherapy, 5 wks/cycle, for 3 cycles. | FOLFOX: Ox.135 mg/m², ID, day 1, LV 200 mg/m², ID, 2 hours, day 1-5, 5-FU 500 mg/m² , ID, 8-10 hours, day1-5, 3 wks/cycle, 3/3 cycle. | I: 10/14; II: 5/16; III: 5/5; IV: 0/0. |
| Wang H (2008) | 34/34; 20/22; 52.58 ± 8.12/51.11 ± 7.72 | IV: 34/34; KPS ≥50. | Yiqiguoxiebuchang decoction; one decoction per day, for 3 months. | FOLFOX: Ox.85 mg/m², ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 500 mg bolus day 1, 5-FU 2500 mg/m², ID, for 48 hours, 21 days/ cycle, 4/4 cycles. | I: 4/8; II: 2/5; III: 1/2; IV: 0/0. |
| Wang J (2011) | 30/30; 18/21; 52.3 ± 6.2/ 56.7 ± 7.8. | ACRC (all); KPS ≥60. | Yichangning decoction; one decoction per day, for 2 months. | FOLFOX4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg/m², bolus, 600 mg/m², ID, 22 hours, day1-2, 21 days /cycle, 2/2 cycles. | I/II: 8/14; III/IV: 1/5. |
| Wang Y (2012) | 38/36; 26/25; 52 (med, all). | ACRC (all); KPS ≥70. | Aidi injection; 80 mL, ID, per day, 10 days/cycle, for 4 cycles. | FOLFOX4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg/m², bolus, 600 mg/m², ID, 22 hours, day1-2, 4/4 cycles. | All gr.: 21/28. |
| Wang Y (2013) | 32/30; 20/19; NS. | ACRC (all); KPS ≥70. | Xiaoaiping injection; 60 mL, ID, per day, 14 days /cycle, for two cycles. | XELOX: no details, 2/2 cycles. | All gr.: 20/26. |
| Wu G (2010) | 33/25; 23/17; 55.4 ±13.6 /52.8 ±15.2. | I: 5/3, II: 10/8, III: 15/11, IV: 3/3; KPS ≥60. | Fupiyiwei decoction; one decoction per day, for 24 wks. | FOLFOX 4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg/m², bolus, 600 mg/m², ID, 22 hours, day1-2, 12/12 | I: 7/8; II: 2/6; III: 1/3; IV: 0/0. |

| First author (year). | Sample size T/C; Gender (M) T/C; Age T/C | TNM (T/C); KPS/ ECOG | TM Intervention; dosage & duration | Oxaliplatin regimen; dose, cycles (T/C) | Outcome: CIN gr.: T/C |
|---|---|---|---|---|---|
| | | | | cycles. | |
| Xu Y (2010) | 61/60; 38/37; 53/52 (mean, all) | ACRC (all); ≥70 | Jiangniling formula; one decoction per day, 14 days/cycle, for 8-10 cycles. | FOLFOX 4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg /m², bolus, 600 mg/m², ID, 22 hours, day1-2, 11.1/7.8 (mean) cycles. | 5.03 ± 2.14/3.03 ± 1.27 (mean) |
| Yang C (2007) | 50/50; 29/27; 51.36 ± 10.58 /53.48 ± 9.35. | ACRC (all); KPS ≥60. | Jianpikangfu pill; 6g, tid, for 4 weeks. | FOLFOX: Ox.135 mg/m², ID, day 1, LV 100 mg/m², ID, day 1-5, 5-FU 425 mg/m², ID day 1-5, 4/4 wks. | I: 18/18; II: 6/14; III: 0/3; IV: 0/0. |
| Yang Y (2008) | 30/30; 16/19; 51.07 ± 10.44 /51.33 ± 10.95. | ACRC (all); KPS ≥60. | Kang'ai injection; 50 mL, ID, day 1-20, 30 days/cycle, for 2 cycles. | FOLFOX4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg/m², bolus, 600 mg/m², ID, 22 hours, day1-2, 4/4 cycles. | I: 9/11;II: 2/8; III: 1/3; IV: 0/0. |
| Zeng C (2013) | 61/30; 39/19; 54. 3 ± 6. 3/53. 2 ± 6. 6 | III: 20/12 IV: 41/18; KPS ≥60. | Fuzhengxiaoji decoction; one decoction per day, 14 days /cycle, for 4 cycles. | FOLFOX: Ox.85 mg/m², ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 360-500 mg/m² bolus, 600 mg/m², ID, for 22 hours, day 1-2, 14 days/cycle, 4/4 cycles. | 4.56 ± 1.33/3.27 ± 1.08 (mean) |
| Zeng D (2009) | 35/32; 25/21; 50<: 4/5, 51-69: 28/25, >70:3/ 2. | IV (all); KPS ≥70. | Ginsenoside Rg3 capsules: 2 capsules, bid, for 8 wks. | FOLFOX4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg/m², bolus, 600 mg/m², ID, 22 hours, day1-2, 21 days /cycle, 4/4 cycles. | All gr.: 22/28. |
| Zeng J (2008) | 30/30; 19/18; 48/60 (med). | ACRC (all); KPS ≥60. | Multi-TM formulae; one decoction per day, for 4wks. | FOLFOX 4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg/m², bolus, 600 mg/m², ID, 22 hours, day1-2, 2/2 cycles. | I: 12/16; II: 7/8; III: 1/2; IV: 0/1. |
| Zhang Q (2006) | 38/30; 35(all); 54.8 (mean, all). | ACRC (all); KPS:76.5 ± 5.8/73.5 ± 6.0 | Yiqihuoxue formula; one decoction per day, 21 days/ cycle, for 3 cycles. | FOLFOX: Ox.125 mg/m², ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 500 mg/m² bolus, day 1-2, 2000 mg/m² ID for 72 hours, 21 days/cycle, 3/3 cycles. | I: 5/9;II: 2/4; III: 1/1; IV: 0/0. |
| Zhang Q (2010) | 60/60; 35/33; 56.2 (mean, all); | ACRC (all); KPS ≥60. | Gubenxiaoliu capsule; 4 capsules, bid, for 8 wks. | FOLFOX4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg/m², bolus, 600 mg/m², ID, 22 hours, day1-2, 4/4 cycles. | I: 10/7; II: 3/8; III: 2/6; IV: 0/0. |
| Zhang W (2013) | 32/32; 15/16; 56.8 ± 10.1/46.4 ± 9.2. | II: 23/22; III: 9/10; KPS ≥70. | Xiaoliuhuaji decoction I; one decoction per day, for 5 mths. | FOLFOX: Ox.135 mg/m², ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 2400 mg/m² , ID for 48 hours, 21 days/cycle, 6/6 cycles. | I: 6/8;II: 4/7; III: 1/4; IV: 0/2. |
| Zhang Y (2010) | 21/20; NS; NS; | ACRC (all); KPS≥60. | Jianpijiedu decoction; one decoction per day, for 4 wks. | FOLFOX 4: Ox.85 mg/m², 2 hours ID, day 1, LV 200 mg/m², ID, day 1-2, 5-FU 400 mg/m², bolus, 600 mg/m², ID, 22 hours, day1-2, 2/2 cycles. | I/II: 4/8; III/IV: 1/3. |
| Zhou J (2011) | 34/34; 22/20; 51.2/52.5. | II: 14/13, III: 16/11, IV: 6/5; KPS ≥60. | Fuzhengjianpi decoction; one decoction per day, for 8 wks. | FOLFOX: Ox.130 mg/m², ID, day 1, LV 100 mg/m², ID, day 1-5, 5-FU 500 mg/m², ID, day 1-5, 28 days/cycle, 2/2 cycles. | I: 1/6;II: 3/7; III: 3/4; IV: 0/2. |

T: treatment group; C: control group; M: male; N: number; CIN: chemotherapy induced neutropenia; gr.: WHO recommendations for grading of acute and subacute toxicity grade; NS: not stated; ID: intravenous drip; TNM: cancer staging system. ‘T’ for tumour, denotes the extent of invasion of the intestinal wall, ‘N’ for lymphatic node, the amount of lymphatic node involvement, and ‘M’ for metastasis; KPS: Karnofsky Performance Status; ECOG: Eastern Cooperative Oncology Group Performance Status; CHM: Chinese herbal medicine; 5-FU: 5-Fluorouracil; LV: Leucovorin; Ox.: Oxaliplatin; Xel: Capecitabine; FOLFOX: Ox. + 5-FU + LV; XELOX: Ox. + Capecitabine; ACRC: advanced colorectal cancer; bid: twice per day; tid: three times per day; qd: once per day; wk: week; mth: month; med.: median.