



# A Review of the Evolution of Systemic Chemotherapy in the Management of Colorectal Cancer<sup>☆</sup>

Bengt Gustavsson,<sup>1</sup> Göran Carlsson,<sup>1</sup> David Machover,<sup>2</sup> Nicholas Petrelli,<sup>3</sup> Arnaud Roth,<sup>4</sup> Hans-Joachim Schmoll,<sup>5</sup> Kjell-Magne Tveit,<sup>6</sup> Fernando Gibson<sup>7</sup>

## Abstract

Herein we present a historical review of the development of systemic chemotherapy for colorectal cancer (CRC) in the metastatic and adjuvant treatment settings. We describe the discovery of 5-fluorouracil (5-FU) by Heidelberger and colleagues in 1957, the potentiation of 5-FU cytotoxicity by the reduced folate leucovorin, and the advent of novel cytotoxic agents, including the topoisomerase I inhibitor irinotecan, the platinum-containing agent oxaliplatin, and the 5-FU prodrug capecitabine. The combination therapies, FOLFOX (5-FU/leucovorin and oxaliplatin) and FOLFIRI (5-FU/leucovorin and irinotecan), have become established as efficacious cytotoxic regimens for the treatment of metastatic CRC, resulting in overall survival times of approximately 2 years. When used as adjuvant therapy, FOLFOX also improves survival and is now the gold standard of care in this setting. Biological agents have been discovered that enhance the effect of cytotoxic therapy, including bevacizumab (a humanized monoclonal antibody that targets vascular endothelial growth factor, a central regulator of angiogenesis) and cetuximab/panitumumab (monoclonal antibodies directed against the epidermal growth factor receptor). Despite the ongoing development of novel anti-tumor agents and therapeutic principles as we enter the era of personalized cancer medicine, systemic chemotherapy involving infusional 5-FU/leucovorin continues to be the cornerstone of treatment for patients with CRC.

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## Introduction

The most recent estimates of the worldwide burden of cancer (GLOBOCAN 2012) indicate that colorectal cancer (CRC) is the third most commonly diagnosed cancer (1.36 million cases; 9.7%)

after lung (1.83 million; 13.0%) and breast cancer (1.68 million; 11.9%), and the fourth highest cause of cancer death (694,000 deaths; 8.5%) after lung (1.59 million; 19.4%), liver (746,000; 9.1%), and stomach cancer (723,000; 8.8%).<sup>1</sup> Despite these statistics, most patients (70%-80%) newly diagnosed with CRC have localized disease that is amenable to curative (R0) surgical resection.<sup>2</sup> After R0 resection, adjuvant chemotherapy with cytotoxic agents is recommended as standard clinical practice for patients with stage III CRC.<sup>3</sup> This recommendation is supported by a pooled analysis of data from the National Surgical Adjuvant Breast and Bowel Project (NSABP) trials,<sup>4</sup> which demonstrated significantly improved survival outcomes after surgery and chemotherapy compared with surgery alone ( $P < .0001$ ).

The remaining 20% to 30% of newly diagnosed patients present with unresectable metastatic disease. In addition, a considerable proportion of patients (40%–50%) experience disease recurrence after surgical resection or develop metastatic disease, typically in the liver or lungs.<sup>5</sup> The management of patients with metastatic CRC (mCRC) requires the systemic administration of cytotoxic drugs.<sup>3</sup> Patients with unresectable mCRC who receive supportive care alone have been shown to have a poor prognosis, with a median

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<sup>1</sup>Department of Surgery, University of Gothenburg, Sahlgrenska University Hospital/Östra Institute of Clinical Sciences, Gothenburg, Sweden

<sup>2</sup>Department of Hematology and Oncology, Institut du Cancer et d'Immunogénétique (ICIG), Hôpital Paul Brousse, Assistance Publique Hôpital de Paris, Villejuif, and University Paris XI, Paris, France

<sup>3</sup>Helen F. Graham Cancer Center at Christiana Care, Newark, NJ

<sup>4</sup>European Society of Surgical Oncology, Oncosurgery Unit, HUG, Geneva, Switzerland

<sup>5</sup>European Society for Medical Oncology, Department of Oncology/Haematology, Martin Luther University, Halle, Germany

<sup>6</sup>Department of Oncology, Oslo University Hospital, Oslo, Norway

<sup>7</sup>PharmaGenesis London, London, UK

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Address for correspondence: Fernando Gibson, PhD, PharmaGenesis London, 9 Whitehall, London SW1A 2DD, UK  
E-mail contact: [fernando.gibson@pharmagenesis.com](mailto:fernando.gibson@pharmagenesis.com)

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overall survival (OS) of 5 months.<sup>6</sup> In contrast, patients with mCRC who receive chemotherapy have been shown to have a median OS of > 2 years.<sup>7</sup>

Herein, we present a historical review of systemic chemotherapy in the adjuvant and metastatic settings, highlighting the key studies that have driven the development of chemotherapy for patients with CRC (Figure 1).

## 5-Fluorouracil and Leucovorin

The German chemist Paul Ehrlich was the first person to coin the term ‘chemotherapy’ during his work on the use of chemical agents to treat infectious diseases in the early 1900s.<sup>8</sup> However, the evolution of chemotherapy for CRC can be said to have begun with the development of 5-fluorouracil (5-FU) in 1957.<sup>9</sup> Charles Heidelberger and colleagues at the University of Wisconsin observed that tumor tissues preferentially used uracil for nucleic acid biosynthesis, and correctly postulated that a fluorouracil analogue would inhibit tumor cell division by blocking the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (thymidylate). Biochemical studies demonstrated that the main route of 5-FU activation proceeds via complex metabolic pathways that result in the formation of 5-fluorodeoxyuridine monophosphate (FdUMP), a potent inhibitor of thymidylate synthase (Figure 2).<sup>10-14</sup> The level of inhibition of thymidylate synthase achieved with FdUMP in patient tumors was shown to correlate with the clinical response to 5-FU treatment.<sup>15,16</sup> Studies of the molecular mechanism of thymidylate formation identified the transient formation of a ternary complex consisting of the substrate dUMP, the folate cofactor 5,10-methylenetetrahydrofolate (MTHF), and thymidylate synthase.<sup>17,18</sup>

The next key advance in the development of 5-FU-based chemotherapy was the finding that inhibition of thymidylate synthase by 5-FU could be potentiated by increased intracellular levels of reduced folates.<sup>12,19-22</sup> At this juncture, it is interesting to note that the antitumor activity of folic acid analogues, including aminopterin and amethopterin (methotrexate), was first demonstrated in 1948 by Sidney Farber and Louis Diamond in children

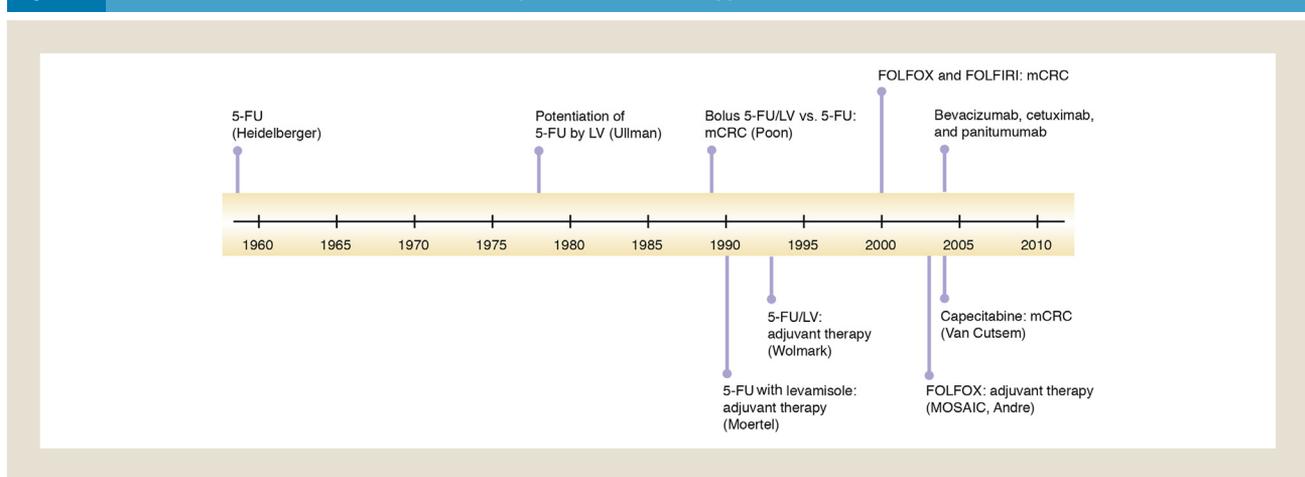
with leukemia.<sup>23</sup> The potentiation of 5-FU activity was shown to be mediated by the formation of a stable ternary complex consisting of FdUMP, MTHF, and thymidylate synthase.<sup>10,13,24</sup> Polyglutamate derivatives of MTHF were shown to substantially increase the efficiency of binding of FdUMP to thymidylate synthase compared with monoglutamate derivatives, in a human colon adenocarcinoma xenograft<sup>25</sup> and human Michigan Cancer Foundation-7 breast cancer cells.<sup>26</sup> In a pivotal in vitro study of the biomodulation of 5-FU activity by the reduced folate leucovorin (5-formyl tetrahydrofolate [THF]), Ullman et al<sup>19</sup> reported that 20 μM leucovorin enhanced 5-FU cytotoxicity approximately fivefold in cultured leukemia cells. Following on from this study, the antitumor activity of 5-FU/leucovorin and 5-FU/methyl THF was established in a number of studies of tumor cell lines, including those of human origin.<sup>20,22,27-31</sup>

The preclinical data on the biomodulation of 5-FU cytotoxicity by leucovorin led to a large number of phase I and II clinical studies in the 1980s.<sup>32</sup> In a pooled analysis of 21 phase II studies of patients with advanced CRC, conducted by Poon et al in 1989, the response rate (RR) of tumors to 5-FU/leucovorin was reported to be 23%.<sup>33</sup> The 2 most commonly used 5-FU/leucovorin treatment regimens in these early studies were those described by Machover et al<sup>34</sup> and Madajewicz et al.<sup>35</sup> Machover et al administered 200 mg/m<sup>2</sup> leucovorin using intravenous (I.V.) bolus and 370 mg/m<sup>2</sup> 5-FU in a 15-minute I.V. infusion daily for 5 days to patients with gastric cancer and mCRC, with courses repeated at 28-day intervals.<sup>34</sup> Madajewicz et al administered 500 mg/m<sup>2</sup> leucovorin as a 2-hour infusion to patients with mCRC, with escalating bolus doses of 5-FU up to a maximum of 750 mg/m<sup>2</sup> given 1 hour after the leucovorin infusion; this schedule was repeated weekly for 6 weeks, followed by a 2-week rest period.<sup>35</sup>

## Treatment of mCRC

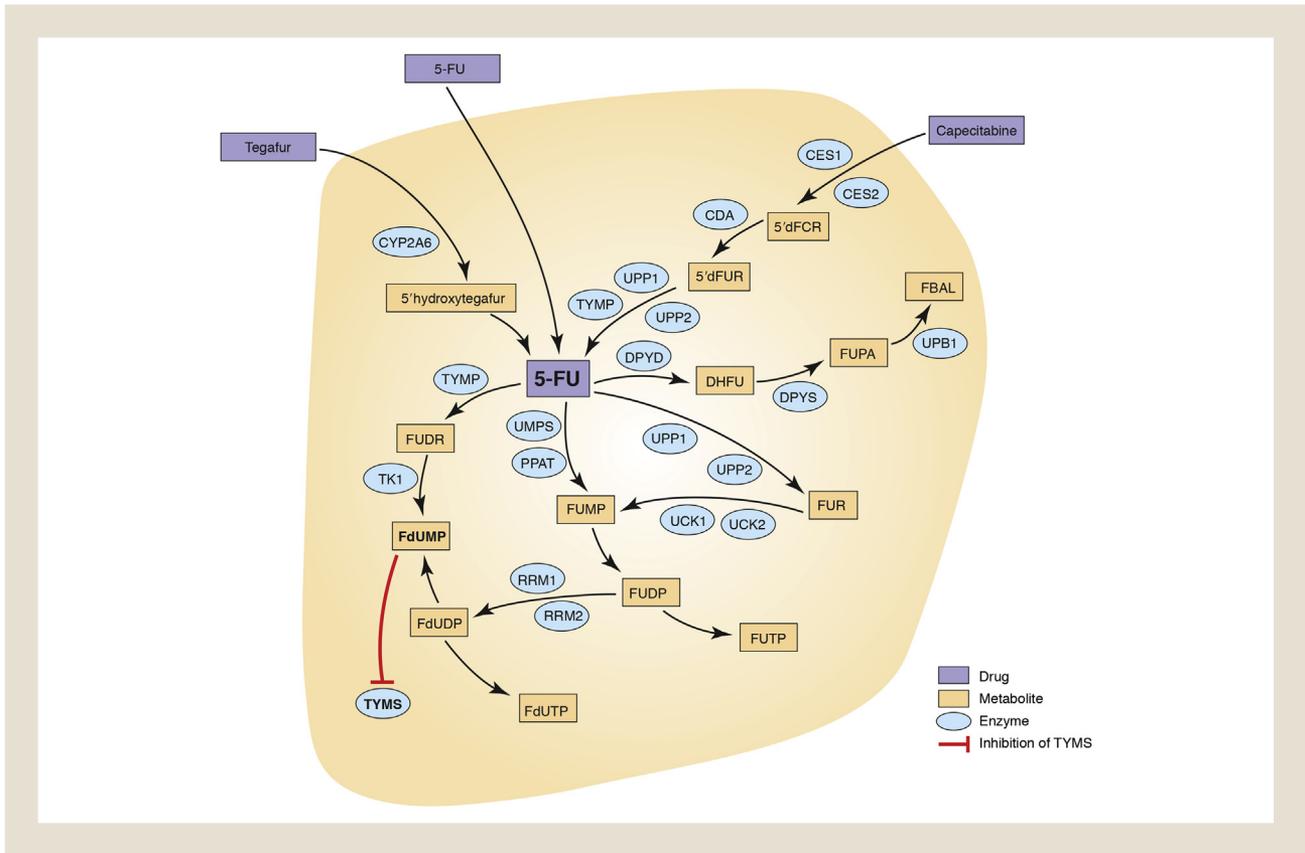
In 1989, the seminal study of Michael Poon and colleagues<sup>33</sup> showed that there was only a trend toward increased OS with I.V. bolus 5-FU/leucovorin, but RR and progression-free survival (PFS) were significantly increased, compared with 5-FU alone in

**Figure 1** Landmark Advances in the Evolution of Systemic Chemotherapy for Patients With CRC



Abbreviations: 5-FU = 5-Fluorouracil; FOLFIRI = Infusional 5-FU/LV With Irinotecan; FOLFOX = 5-FU/LV With Oxaliplatin; LV = Leucovorin; mCRC = Metastatic Colorectal Cancer; MOSAIC = Multicenter International Study of Oxaliplatin/5-FU/Leucovorin in the Adjuvant Treatment of Colon Cancer.

Figure 2 5-Fluorouracil (5-FU) Metabolism



Abbreviations: CDA = Cytidine Deaminase; CES = carboxylesterase; 5'-dFCR = 5'-Deoxy-5-Fluorocytidine; 5'-dFUR = 5'-Deoxy-5-Fluorouridine; DHFU = Dihydrofluorouracil; DPYD = Dihydropyrimidine Dehydrogenase; DPYS = Dihydropyrimidinease; FBAL = Fluoro- $\beta$ -Alanine; FdUDP = 5-Fluorodeoxyuridine Diphosphate; FdUMP = 5-Fluorodeoxyuridine Monophosphate; FdUTP = 5-Fluorodeoxyuridine Triphosphate; 5-FU = 5-Fluorouracil; FUDP = Fluorouridine Diphosphate; FUR = Fluorouridine; FUTP = Fluorouridine Triphosphate; PPAT = Phosphoribosyl Pyrophosphate Amidotransferase; RRM = Ribonucleotide Reductase M; TK = Thymidine Kinase; TYMP = Thymidylate Phosphorylase; TYMS = Thymidylate Synthase; UCK = Uridine-Cytidine Kinase; UUPS = Uridine Monophosphate Synthase; UPB =  $\beta$ -Ureidopropionase; UPP = Uridine Phosphorylase.

Adapted from Whirl-Carrillo, et al. *Pharmacogenomics knowledge for personalized medicine. Clin Pharmacol Ther* 2012; 92:414-7.

patients with mCRC. Median OS was 12.2 months for patients who received 5-FU with high-dose (200 mg/m<sup>2</sup>) leucovorin and 12.0 months for those receiving 5-FU with low-dose (20 mg/m<sup>2</sup>) leucovorin, compared with 7.7 months for 5-FU alone ( $P = .05$ , both leucovorin doses). RRs for 5-FU with high-dose or low-dose leucovorin were 26% ( $P = .04$ ) and 37% ( $P < .001$ ), respectively, compared with 10% for 5-FU alone. The time to progression (TTP) rates for 5-FU with high-dose or low-dose leucovorin were also significantly improved compared with 5-FU alone ( $P = .015$  and  $P = .007$ , respectively).

Another important study, carried out by Petrelli et al,<sup>36</sup> demonstrated that the RR for 5-FU with high-dose leucovorin (48%) was significantly greater than that with 5-FU alone (11%) or 5-FU with methotrexate (5%; overall  $P = .0009$ ). In a subsequent phase III study that compared 5-FU with high-dose or low-dose leucovorin with 5-FU alone, Petrelli et al<sup>37</sup> reported RRs of 12% for 5-FU alone, 30% for 5-FU with high-dose leucovorin ( $P < .01$ ), and 18.8% for 5-FU with low-dose leucovorin ( $P =$  not significant [NS]).

A meta-analysis of 19 randomized trials,<sup>38</sup> involving 3338 patients, reported a twofold increase in RR with 5-FU/leucovorin (21%) compared with 5-FU alone (11%;  $P < .0001$ ) and a small

but statistically significant OS benefit for 5-FU/leucovorin over 5-FU alone (11.7 vs. 10.5 months, respectively;  $P = .004$ ).

Key developments in the early 2000s included the introduction of the topoisomerase I inhibitor irinotecan and the platinum-containing agent oxaliplatin as components of cytotoxic combination therapy for mCRC. Irinotecan was first discovered and synthesized in Japan by Yakult Honsha Ltd in 1983.<sup>39</sup> It is a prodrug analogue (7-ethyl-10-piperidino-piperidino-carboxyloxy derivative) of the alkaloid camptothecin that is converted to the active metabolite SN-38 by liver carboxylesterases.<sup>40</sup> Oxaliplatin was also discovered in Japan at Nagoya City University by Yoshinori Kidani in 1976 by testing the antitumor activity of various platinum (II) complexes of 1,2-diaminocyclohexane isomers.<sup>41</sup>

Saltz et al<sup>42</sup> found that treatment with bolus 5-FU/leucovorin and irinotecan (IFL) resulted in significantly longer PFS (7.0 vs. 4.3 months;  $P = .004$ ), greater RR (39% vs. 21%;  $P < .001$ ), and longer OS (14.8 vs. 12.6 months;  $P = .04$ ) than 5-FU/leucovorin alone as first-line therapy for patients with mCRC. In the Inter-group trial N9741,<sup>43</sup> the efficacy of FOLFOX (5-FU/leucovorin with oxaliplatin) was significantly better than that of IFL with regard to OS (19.5 vs. 15.0 months, respectively;  $P < .0001$ ), TTP

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(8.7 vs. 6.9 months;  $P = .0014$ ), and RR (45% vs. 31%;  $P = .002$ ). The FOLFOX regimen was also associated with significantly lower rates of severe nausea, vomiting, diarrhea, and febrile neutropenia than was the IFL regimen (all  $P < .001$ ). The unfavorable toxicity profile of the IFL regimen led to the development of a regimen comprised of infusional IFL (FOLFIRI). The GOIM (Gruppo Oncologico Dell'Italia Meridionale) study<sup>44</sup> and the GERCOR (Groupe Coopérateur Multidisciplinaire en Oncologie) crossover study<sup>45</sup> each showed similar efficacy for the FOLFIRI and FOLFOX regimens. The GOIM study reported RRs of 31% and 34% ( $P = \text{NS}$ ), OS rates of 14 and 15 months ( $P = \text{NS}$ ), and median TTPs of 7 months (both,  $P = \text{NS}$ ) for FOLFIRI and FOLFOX, respectively. The GERCOR study demonstrated OS rates of 21.5 months in patients allocated to FOLFIRI then FOLFOX, and 20.6 months in those treated with FOLFOX then FOLFIRI ( $P = \text{NS}$ ). As first-line therapy, FOLFIRI achieved an RR of 56% and PFS of 8.5 months, and for FOLFOX the RR was 54% ( $P = \text{NS}$ ) and the PFS was 8.0 months ( $P = \text{NS}$ ).

The combination of infusional 5-FU/leucovorin, oxaliplatin, and irinotecan (FOLFOXIRI) was compared with FOLFIRI in 2 randomized, phase III trials. Souglakos et al<sup>46</sup> reported no significant differences in OS, TTP, or RR between the 2 treatment regimens. Falcone et al<sup>47</sup> showed a significantly greater RR for patients treated with FOLFOXIRI than for those treated with a modified FOLFIRI regimen containing 400 to 600 mg/m<sup>2</sup> 5-FU (60% vs. 34%, respectively;  $P < .0001$ ). PFS (9.8 vs. 6.9 months;  $P = .0006$ ) and OS (22.6 vs. 16.7 months;  $P = .032$ ) were also significantly improved in the FOLFOXIRI arm compared with in the modified FOLFIRI arm, but at the cost of a significant ( $P < .001$ ) increase in toxicity, in terms of increased grades of peripheral neurotoxicity ( $P < .001$ ) and neutropenia ( $P < .001$ ).

The idea of targeting angiogenesis as an anticancer therapy was first proposed by Judah Folkman and colleagues in 1971.<sup>48</sup> However, it was not until 2004 that the pivotal Avastin/Fluorouracil 2107 phase III trial<sup>49</sup> evaluated the humanized monoclonal antibody bevacizumab, which inhibits the action of vascular endothelial growth factor. In this trial, patients were randomized to IFL with bevacizumab or IFL alone. The addition of bevacizumab significantly improved OS (20.3 vs. 15.6 months, respectively;  $P < .001$ ), PFS (10.6 vs. 6.2 months;  $P < .001$ ), and RR (44.8% vs. 34.8%;  $P = .004$ ) compared with IFL alone. In another key trial, the Eastern Cooperative Oncology Group 3200 study<sup>50</sup> enrolled patients previously treated with IFL and found that OS (12.9 vs. 10.8 months, respectively;  $P < .0011$ ), PFS (7.3 vs. 4.7 months;  $P < .0001$ ), and RR (22.7% vs. 8.6%;  $P < .0001$ ) were all significantly improved with bevacizumab and FOLFOX treatment compared with FOLFOX alone.

In 1983 and 1984, John Mendelsohn and Gordon Sato and colleagues proposed epidermal growth factor receptor (EGFR) as a novel target for cancer therapy, based on observations that EGFR was frequently overexpressed in epithelial tumors and that monoclonal antibodies directed against EGFR inhibited the growth of cancer cells.<sup>51-54</sup> The anti-EGFR monoclonal antibodies cetuximab and panitumumab were the first therapeutic agents targeted at a specific molecular pathology: EGFR-positive tumors expressing wild type Kirsten rat sarcoma viral oncogene homolog (KRAS).<sup>55</sup> The efficacy of cetuximab in the treatment of patients

with mCRC was evaluated in the CRYSTAL (Cetuximab Combined with Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer) study,<sup>56,57</sup> in which patients with EGFR-positive tumors were randomized to receive FOLFIRI alone or FOLFIRI with cetuximab. FOLFIRI with cetuximab marginally improved PFS compared with FOLFIRI alone (8.9 vs. 8.0 months, respectively;  $P = .048$ ), but there was no significant difference in OS between the 2 treatments (19.9 vs. 18.6 months;  $P = \text{NS}$ ). In a subset analysis of patients with wild type KRAS (63%), FOLFIRI with cetuximab significantly improved OS (23.5 vs. 20.0 months;  $P = .01$ ), PFS (9.9 vs. 8.4 months;  $P = .001$ ), and RR (57.3% vs. 39.7%;  $P = .001$ ) compared with FOLFIRI alone. No significant difference in efficacy was evident in patients with mutant KRAS.

In the PRIME (Panitumumab Randomized Trial in Combination With Chemotherapy for Metastatic Colorectal Cancer to Determine Efficacy) trial,<sup>58</sup> patients were randomized to treatment with FOLFOX with or without panitumumab, regardless of EGFR or KRAS status. In the subset with wild type KRAS (60% of the study population), panitumumab with FOLFOX significantly improved PFS compared with FOLFOX alone (9.6 vs. 8.0 months, respectively;  $P = .02$ ), but did not lead to a significant improvement in OS (23.9 vs. 19.7 months;  $P = \text{NS}$ ).

The UK Medical Research Council (MRC) Continuous Chemotherapy Plus Cetuximab or Intermittent Chemotherapy trial was a 3-arm randomized controlled trial in which patients were randomized to receive continuous FOLFOX, continuous FOLFOX with cetuximab, or intermittent FOLFOX alone. Maughan et al<sup>59</sup> reported the results for 2 of these regimens: FOLFOX with cetuximab increased RR compared with FOLFOX alone (59% vs. 50%, respectively;  $P = .015$ ), but there was no evidence of improved PFS or OS in patients with wild type KRAS.

Patients in the Nordic-VII study<sup>60</sup> were randomized to receive Nordic FLOX (bolus FOLFOX), Nordic FLOX with cetuximab, or intermittent Nordic FLOX with cetuximab. OS, PFS, and RR were similar in the 3 treatment arms (OS: 20.4, 19.7, and 20.3 months, respectively [ $P = \text{NS}$ ]; PFS: 7.9, 8.3, and 7.3 months [ $P = \text{NS}$ ]; and RR: 41%, 49%, and 47% [ $P = \text{NS}$ ]). In patients with wild type KRAS, cetuximab did not provide any additional benefit compared with Nordic FLOX alone for PFS, OS, or RR.

Findings of several key studies presented at the 2013 Annual Meeting of the American Society of Clinical Oncology (ASCO) provided important updates to the current picture. In the FIRE-3 (FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer) trial,<sup>61</sup> patients with wild type KRAS were randomized to receive first-line FOLFIRI with cetuximab or FOLFIRI with bevacizumab. The primary end points of overall RR (62% vs. 58%, respectively) and PFS (10.0 vs. 10.3 months, respectively) were not significantly different in the 2 treatments arms. However, FOLFIRI with cetuximab provided a statistically significant improvement in OS compared with FOLFIRI with bevacizumab (28.7 vs. 25.0 months, respectively;  $P = .017$ ). A further important contribution to the ongoing first-line therapy debate in mCRC was the TRIBE (Combination Chemotherapy and Bevacizumab as First-line Therapy in Treating Patients with Metastatic Colorectal Cancer) trial.<sup>62</sup> This trial, which evaluated FOLFOXIRI and bevacizumab versus FOLFIRI and bevacizumab,

showed a significant difference in the primary end point of PFS (12.1 vs. 9.7 months, respectively;  $P = .006$ ). The phase II PEAK (Panitumumab Efficacy in Combination with mFOLFOX6

Against Bevacizumab plus mFOLFOX6 in mCRC Subjects with Wild-Type *KRAS* Tumors) trial<sup>63</sup> randomized patients with wild type rat sarcoma (*KRAS* or neuroblastoma rat sarcoma) to first-line

**Table 1** Key Clinical Studies in the Development of Therapy for Patients With mCRC

Study	Publication Date	Study Objective	Patients (n)	Key Efficacy Results
<b>5-FU/LV</b>				
Petrelli et al <sup>36</sup>	1987	To compare the efficacy of 5-FU with high-dose LV (500 mg/m <sup>2</sup> ), 5-FU with methotrexate, and 5-FU alone	74	OS: 12, 10, 11 months, respectively ( $P = \text{NS}$ ) RR: 48%, 5%, 11%, respectively ( $P = .0009$ )
Petrelli et al <sup>37</sup>	1989	To determine whether 5-FU with high-dose (500 mg/m <sup>2</sup> ) or low-dose (25 mg/m <sup>2</sup> ) LV increases efficacy compared with 5-FU alone	343	OS: 13.8, 11.3, 11.5, months, respectively ( $P = \text{NS}$ ) RR: 30%, 19%, 12%, respectively ( $P < .01$ )
Poon et al <sup>33</sup>	1989	To evaluate the efficacy of 5-FU with high-dose (200 mg/m <sup>2</sup> ) LV, 5-FU with low-dose (20 mg/m <sup>2</sup> ) LV, and 5-FU alone	429	OS: 12.2, 12.0, 7.7 months, respectively (adjusted $P = .05$ , both LV doses) RR: 26% ( $P = .04$ ), 37% ( $P < .001$ ), 10%, respectively
<b>5-FU/LV, IFL</b>				
Saltz et al. <sup>42</sup>	2000	To compare the efficacy of IFL versus 5-FU/LV alone	683	OS: 14.8 versus 12.6 months ( $P = .04$ ) PFS: 7.0 versus 4.3 months ( $P = .004$ ) RR: 39% versus 21%; ( $P < .001$ )
<b>FOLFOX, IFL</b>				
Intergroup N9741 <sup>43</sup>	2004	To compare the efficacy and toxicity of FOLFOX versus IFL regimens	795	OS: 19.5 versus 15.0 months ( $P < .0001$ ) TTP: 8.7 versus 6.9 months ( $P = .0014$ ) RR: 45% versus 31% ( $P = .002$ )
<b>FOLFIRI, FOLFOX</b>				
GERCOR <sup>45</sup>	2004	A crossover study to investigate the efficacy of FOLFIRI followed by FOLFOX versus FOLFOX followed by FOLFIRI	222	OS: 21.5 versus 20.6 months ( $P = \text{NS}$ ) PFS: 8.5 versus 8.0 months ( $P = \text{NS}$ ) RR: 56% versus 54% ( $P = \text{NS}$ )
GOIM <sup>44</sup>	2005	To compare the efficacy of FOLFIRI versus FOLFOX regimens	360	OS: 14 versus 15 months ( $P = \text{NS}$ ) RR: 31% versus 34% ( $P = \text{NS}$ ) TTP: 7 versus 7 months ( $P = \text{NS}$ )
<b>FOLFIRI, FOLFOXIRI</b>				
Souglakos et al <sup>46</sup>	2006	To compare the efficacy and toxicity of FOLFIRI versus FOLFOXIRI regimens	283	OS: 19.5 versus 21.5 months ( $P = \text{NS}$ ) TTP: 6.9 versus 8.4 months ( $P = \text{NS}$ ) RR: 34% versus 43% ( $P = \text{NS}$ )
Falcone et al <sup>47</sup>	2007	To compare the efficacy and toxicity of FOLFOXIRI versus FOLFIRI regimens	244	OS: 22.6 versus 16.7 months ( $P = .032$ ) RR: 60% versus 34% ( $P < .0001$ ) PFS: 9.8 versus 6.9 months ( $P = .0006$ )
<b>Bevacizumab</b>				
AVF 2107 <sup>49</sup>	2004	To determine whether bevacizumab with IFL improves survival versus IFL alone	813	OS: 20.3 versus 15.6 months ( $P < .001$ ) PFS: 10.6 versus 6.2 months ( $P < .001$ ) RR: 44.8% versus 34.8% ( $P = .004$ )
ECOG 3200 <sup>50</sup>	2007	To determine the effect of bevacizumab with FOLFOX on survival duration versus FOLFOX alone	829	OS: 12.9 versus 10.8 months; ( $P < .0011$ ) PFS: 7.3 versus 4.7 months ( $P < .0001$ ) RR: 22.7% versus 8.6% ( $P < .0001$ )
<b>Cetuximab, Panitumumab</b>				
CRYSTAL <sup>56,57</sup>	2009, 2011	To investigate the efficacy of cetuximab with FOLFIRI versus FOLFIRI alone; and the association between tumor <i>KRAS</i> mutation status and clinical response to cetuximab	1198	OS: 19.9 versus 18.6 months ( $P = \text{NS}$ ) PFS: 8.9 versus 8.0 months ( $P = .048$ ). In patients with wild type <i>KRAS</i> (63%), OS: 23.5 versus 20.0 months ( $P = .01$ ) PFS: 9.9 versus 8.4 months ( $P = .001$ ) RR: 57.3% versus 39.7% ( $P = .001$ ) No significant difference in efficacy was evident in patients with mutant <i>KRAS</i>
PRIME <sup>58</sup>	2010	To evaluate the efficacy and safety of panitumumab with FOLFOX versus FOLFOX alone	1183	In patients with wild type <i>KRAS</i> (60%) OS: 23.9 versus 19.7 months ( $P = \text{NS}$ ) PFS: 9.6 versus 8.0 months ( $P = .02$ )
COIN <sup>59</sup>	2011	To assess the efficacy of cetuximab with FOLFOX versus FOLFOX alone	1630	OS: 17.0 versus 17.9 months ( $P = \text{NS}$ ) RR: 59% versus 50% ( $P = .015$ ) No evidence of improved PFS or OS in patients with wild type <i>KRAS</i>

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**Table 1** Continued

Study	Publication Date	Study Objective	Patients (n)	Key Efficacy Results
Nordic-VII <sup>60</sup>	2012	To investigate the efficacy of Nordic FLOX, cetuximab with Nordic FLOX, and cetuximab with intermittent Nordic FLOX	571	OS: 20.4, 19.7, 20.3 months, respectively ( $P = NS$ ) PFS: 7.9, 8.3, 7.3 months, respectively ( $P = NS$ ) RR: 41%, 49%, 47%, respectively ( $P = NS$ ) In patients with <i>KRAS</i> mutations, no significant differences were detected

Abbreviations: AVF = Avastin/Fluorouracil; COIN = Continuous Chemotherapy with Cetuximab or Intermittent Chemotherapy; CRYSTAL = Cetuximab Combined with Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer; ECOG = Eastern Cooperative Oncology Group; FLOX = bolus 5-FU/LV with oxaliplatin; FOLFIRI = infusional 5-FU/LV with irinotecan; FOLFOX = 5-FU/LV with oxaliplatin; FOLFOLFOXIRI = 5-FU/LV with oxaliplatin and irinotecan; 5-FU = 5-fluorouracil; GERCOR = Groupe Coopérateur Multidisciplinaire en Oncologie; GOIM = Gruppo Oncologico dell'Italia Meridionale; IFL = bolus 5-FU/LV with irinotecan; *KRAS* = Kirsten rat sarcoma viral oncogene homolog; LV = leucovorin; mCRC = metastatic colorectal cancer; NS = nonsignificant; OS = overall survival; PFS = progression-free survival; PRIME = Panitumumab Randomized Trial in Combination With Chemotherapy for Metastatic Colorectal Cancer to Determine Efficacy; RR = response rate; TTP = time to progression.

panitumumab with FOLFOX or bevacizumab with FOLFOX. PFS for panitumumab with FOLFOX was 13.1 months, compared with 9.5 months for bevacizumab with FOLFOX ( $P = .02$ ). OS for the panitumumab arm was not reached at the time of reporting, but was 29 months for the bevacizumab arm. At ASCO 2012, PEAK data were reported, which suggested that the panitumumab regimen had an adverse effect on PFS in patients with mutated compared with wild type *KRAS*, although the effect was not significant (15.5 vs. 19.3 months, respectively;  $P = NS$ ).<sup>64</sup> Although not validated, the PEAK results suggest that panitumumab should not be used for the treatment of mCRC in patients with *KRAS* mutations or in whom the *KRAS* status is unknown.

Orally administered 5-FU prodrugs were developed to provide a convenient alternative to treatment regimens requiring I.V. infusion of 5-FU. An example of such an oral regimen is the combination of uracil and the 5-FU prodrug tegafur in a 4:1 molar ratio (UFT). Uracil competitively inhibits dihydropyrimidine dehydrogenase, the main catabolic enzyme of 5-FU (Figure 2). In a meta-analysis of 5 randomized controlled trials that compared UFT/leucovorin with bolus 5-FU/leucovorin, Bin et al<sup>65</sup> reported that there were no significant differences in OS and RR between the 2 regimens; however, UFT/leucovorin had a significantly lower toxicity rate than bolus 5-FU/leucovorin ( $P < .001$  for stomatitis/mucositis, Grade 1-4 leukopenia, febrile neutropenia, and infection). These findings are consistent with a pooled efficacy analysis from 2 phase III studies that compared capecitabine (another oral 5-FU prodrug) with bolus 5-FU/leucovorin.<sup>66</sup> A statistically significant difference in RR was reported for capecitabine compared with 5-FU and leucovorin (26% vs. 17%, respectively;  $P < .0002$ ), whereas OS (12.9 vs. 12.8 months;  $P = NS$ ) and TTP (4.6 vs. 4.7 months;  $P = NS$ ) were equivalent in the 2 treatment groups. In Table 1 the findings of the key mCRC studies described in this section are summarized.<sup>33,36,37,42-47,49,50,56-60</sup> Figure 3 shows the temporal trend of OS in these studies. It can be seen that median OS increased sharply from 12.0 months in the early studies of Petrelli et al<sup>36</sup> and Poon et al,<sup>33</sup> to 21.5 months in the GERCOR study,<sup>45</sup> and except for the GOIM study,<sup>44</sup> has remained at 18 to 24 months in recent, large phase III trials.

## Adjuvant Treatment of CRC

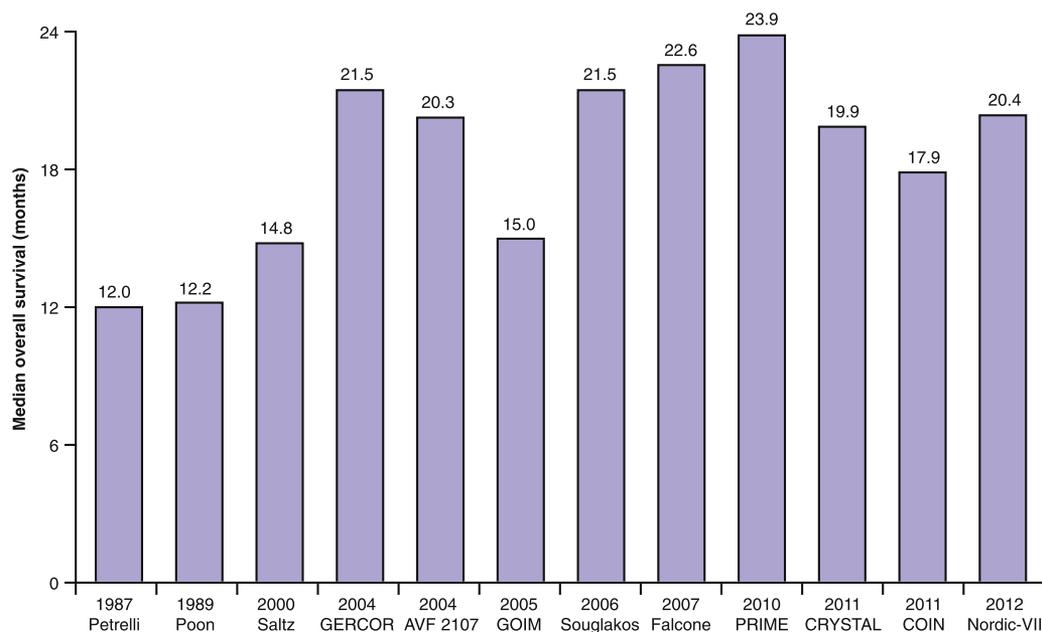
In the 1970s and 1980s, the antihelminthic drug levamisole attracted interest as a possible chemotherapeutic agent because of its

putative immunomodulatory activity.<sup>67,68</sup> In 1989, the North Central Cancer Treatment Group (NCCTG) reported that treatment with levamisole with 5-FU led to a significant reduction in cancer recurrence ( $P = .003$ ) and a significant increase in OS ( $P = .03$ ) when compared with no adjuvant therapy.<sup>69</sup> In 1990, Charles Moertel and colleagues<sup>70</sup> published the results of their seminal study of the efficacy of 5-FU with levamisole versus no adjuvant therapy in patients with stage II or III CRC. 5-FU with levamisole reduced the risk of cancer recurrence by 41% ( $P < .0001$ ) and the overall death rate by 33% ( $P = .006$ ) compared with observation alone. Interestingly, treatment with levamisole alone had no effect. These findings led to the acceptance of 5-FU with levamisole as the standard adjuvant therapy in the 1990s.<sup>71</sup>

The next stage in the evolution of adjuvant therapy involved the evaluation of 5-FU with leucovorin in several key trials. The NSABP C-03 study<sup>72</sup> reported a 3-year disease-free survival (DFS) rate of 73% for patients receiving 5-FU/leucovorin, compared with a rate of 64% for those who received a combination of the alkylating nitrosourea lomustine, the alkaloid vincristine, and 5-FU (MOF;  $P = .0004$ ). The IMPACT (International Multicenter Pooled Analysis of Colorectal Cancer Trials)<sup>73</sup> pooled data from 3 randomized trials that investigated high-dose 5-FU/leucovorin compared with no adjuvant therapy. 5-FU/leucovorin reduced mortality by 22% ( $P = .029$ ) and CRC events by 35% ( $P < .0001$ ) compared with no adjuvant therapy.

A number of randomized trials evaluated the efficacy and safety of the most commonly used 5-FU/leucovorin treatment regimens in the adjuvant setting. The intergroup-0089 study<sup>74</sup> set out to evaluate 4 regimens: (1) the Mayo Clinic regimen, comprised of a daily 20 mg/m<sup>2</sup> (low-dose) I.V. bolus of leucovorin and 425 mg/m<sup>2</sup> I.V. bolus of 5-FU for 5 consecutive days, repeated every 4 to 5 weeks; (2) the Roswell Park regimen, consisting of a weekly 500 mg/m<sup>2</sup> (high-dose) I.V. bolus of leucovorin and 500 mg/m<sup>2</sup> I.V. bolus of 5-FU for 6 weeks, repeated every 8 weeks; (3) low-dose 5-FU/leucovorin with levamisole; and (4) levamisole alone. The main finding was that there were no statistically significant differences among the treatment arms in DFS (9.4, 7.9, 9.2, and 7.1 months, respectively) or OS (11.5, 10.7, 11.4, and 10.3 months, respectively). The MRC study<sup>75</sup> evaluated 3 months of continuous infusion of 5-FU and a 6-month course of the Mayo Clinic regimen. There was no statistically significant difference between the 2 arms in terms of OS (87.9% vs. 83.2%, respectively;  $P = NS$ ). However, patients in the Mayo Clinic regimen arm had

**Figure 3** Temporal Trend of Median Overall (OS) Survival in Key Metastatic Colorectal Cancer Clinical Trials. The OS Values Shown for Each Study Represent the Treatment Arm With the Longest Median Survival



Abbreviations: AVF = Avastin/Fluorouracil; COIN = Continuous Chemotherapy With Cetuximab or Intermittent Chemotherapy; CRYSTAL = Cetuximab Combined With Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer; GERCOR = Groupe Coopérateur Multidisciplinaire en Oncologie; GOIM = Gruppo Oncologico dell'Italia Meridionale; PRIME = Panitumumab Randomized Trial in Combination With Chemotherapy for Metastatic Colorectal Cancer to Determine Efficacy.

significantly lower rates of PFS compared with those who received continuous infusion 5-FU (69% vs. 80%, respectively;  $P = .02$ ). In terms of safety, the frequency of Grade 3 or 4 neutropenia, diarrhea, stomatitis, and severe alopecia were significantly lower ( $P < .0001$ ), and global quality of life scores significantly better ( $P < .001$ ), for patients in the continuous infusion arm compared with the Mayo Clinic regimen arm. The GERCOR C96.1 study<sup>76</sup> compared the Mayo Clinic regimen with LV5FU2 (twice-monthly I.V. infusion of 5-FU/leucovorin; de Gramont regimen<sup>77</sup>). There were no statistically significant differences between the 2 arms in terms of DFS ( $P = \text{NS}$ ) or OS ( $P = \text{NS}$ ), but the de Gramont regimen was significantly less toxic than the Mayo Clinic regimen ( $P = .001$ ).

In the X-ACT (Xeloda in Adjuvant Colon Cancer Therapy) trial,<sup>78</sup> patients were randomized to capecitabine or the Mayo Clinic regimen. There were no statistically significant differences between the 2 arms in terms of DFS (64.2% vs. 60.6%, respectively;  $P = \text{NS}$ ) or OS (81.3% and 77.6%;  $P = .05$ ). However, capecitabine was associated with significantly fewer adverse events than the Mayo Clinic regimen ( $P < .001$ ). The NSABP C-06 study,<sup>79</sup> which compared tegafur with leucovorin versus the Roswell Park regimen, reported that 5-year DFS (68.2% vs. 67.0%, respectively;  $P = \text{NS}$ ) and OS (78.7% vs. 78.5%;  $P = \text{NS}$ ) were similar for the 2 treatments.

In 2004, an interim analysis of data from the pivotal MOSAIC (Multicenter International Study of Oxaliplatin/5-FU/Leucovorin in the Adjuvant Treatment of Colon Cancer) trial<sup>80</sup> showed that

FOLFOX significantly improved 3-year DFS compared with 5-FU/leucovorin (FL regimen: 2-hour I.V. infusion of 200 mg/m<sup>2</sup> of leucovorin followed by an I.V. bolus of 400 mg/m<sup>2</sup> of 5-FU and then a 22-hour I.V. infusion of 600 mg/m<sup>2</sup> of 5-FU given on 2 consecutive days every 14 days); FOLFOX, 78.2% versus FL, 72.9%, respectively ( $P = .002$ ) in patients with stage III CRC, although neutropenia (Grades 3 and 4) was significantly more frequent with FOLFOX than with FL (41.1% vs. 4.7%;  $P < .001$ ). The final analysis of data from MOSAIC in 2009<sup>81</sup> confirmed statistically significant improvements in DFS and OS for FOLFOX compared with FL (5-year DFS: 73.3% vs. 67.4%, respectively [ $P = .003$ ] and 6-year OS: 78.5% vs. 76.0% [ $P = .046$ ]). No survival benefit was detected in patients with stage II disease. The MOSAIC findings established FOLFOX as the standard adjuvant therapy for resected stage III CRC, and, in so doing, suggested that treatments with proven efficacy in the management of mCRC could also be effective in the adjuvant setting. Unfortunately, negative results from a number of large multicenter trials have shown these hopes to be unfounded.

The PETACC (Pan-European Trial in Adjuvant Colorectal Cancer)-3<sup>82</sup> compared FOLFIRI and 5-FU/leucovorin (de Gramont regimen) in patients with stage III disease. FOLFIRI did not produce significant improvements compared with 5-FU/leucovorin in either DFS (56.7% vs. 54.3%, respectively;  $P = \text{NS}$ ) or OS (73.6% vs. 71.3%;  $P = \text{NS}$ ). These findings corroborated those of the Cancer and Leukemia Group B (CALGB) 89803<sup>83</sup> and Action Clinique Coordonnées en Cancérologie Digestive

# Evolution of Systemic Chemotherapy in the Management of CRC

**Table 2** Key Clinical Studies in the Development of Adjuvant Therapy for Patients With CRC

Study	Publication date	Study Objective	Patients (n)	Key Efficacy Results
<b>5-FU With Levamisole</b>				
Moertel et al <sup>70</sup>	1990	To compare the efficacy of 5-FU with levamisole versus observation only in patients with stage II or III CRC	1296	3.5-Year OS: 71% versus 55% Cancer recurrence rate: -41% ( $P < .0001$ ) Overall death rate: -33% ( $P = .006$ )
<b>5-FU/LV</b>				
NSABP C-03 <sup>72</sup>	1993	To evaluate the efficacy of 5-FU/LV versus MOF in patients with stage II or III CRC	1081	3-Year OS: 84% versus 77% ( $P = .007$ )
IMPACT <sup>73</sup>	1995	Pooled analysis of 3 randomized trials to investigate the efficacy of high-dose 5-FU/LV versus no adjuvant therapy in patients with stage II or III CRC	1493	3-Year OS: 83% versus 78% Overall death rate: -22% ( $P = .029$ ) CRC events: -35% ( $P < .0001$ )
INT-0089 <sup>74</sup>	2005	To assess the relative efficacy of 5-FU/LV (Mayo Clinic regimen), 5-FU/LV (Roswell Park regimen), Mayo Clinic regimen with levamisole, and 5-FU with levamisole in patients with stage II or III CRC	3794	5-Year OS: 66%, 66%, 64%, and 54% ( $P = NS$ )
X-ACT <sup>78</sup>	2005	To evaluate the efficacy of capecitabine versus 5-FU/LV (Mayo Clinic regimen) in patients with stage III CRC	1987	3-Year OS: 81% versus 78% ( $P = .05$ ) 3-Year DFS: 64% versus 61% ( $P = NS$ )
NSABP C-06 <sup>79</sup>	2006	To compare the efficacy of tegafur with LV versus 5-FU/LV (Roswell Park regimen) in patients with stage II or III CRC	1608	5-Year OS: 79% versus 79% ( $P = NS$ ) 5-Year DFS: 68% versus 67% ( $P = NS$ )
GERCOR C96.1 <sup>76</sup>	2007	To compare the efficacy of the de Gramont versus Mayo Clinic regimens of 5-FU/LV in patients with stage II or III CRC	905	6-Year OS: 76% versus 78% ( $P = NS$ ) 6-Year DFS: 66% versus 65% ( $P = NS$ )
<b>FOLFOX, FOLFIRI</b>				
MOSAIC <sup>80,81</sup>	2004, 2009	To evaluate the efficacy of FOLFOX versus 5-FU/LV in patients with stage II or III CRC	2246	6-Year OS: 79% versus 76% ( $P = .046$ ) 5-Year DFS: 73% versus 67% ( $P = .003$ )
PETACC-3 <sup>82</sup>	2009	To investigate the efficacy of FOLFIRI versus the de Gramont 5-FU/LV regimen in patients with stage III CRC	2094	5-Year OS: 73.6% versus 71.3% ( $P = NS$ ) 5-Year DFS: 56.7% versus 54.3% ( $P = NS$ )
<b>Bevacizumab</b>				
NSABP C-08 <sup>85</sup>	2011	To investigate the efficacy and safety of bevacizumab with FOLFOX versus FOLFIRI in patients with stage II or III CRC	2672	3-Year DFS: 77% versus 76% ( $P = NS$ )
<b>Cetuximab</b>				
NCCTG/Intergroup N0147 <sup>86</sup>	2012	To assess the benefit of cetuximab with mFOLFOX6 versus mFOLFOX6 in wild type KRAS patients with stage III CRC	2686	3-Year OS: 87% versus 86% ( $P = NS$ ) 3-Year DFS: 75% versus 72% ( $P = NS$ ) (prespecified interim analysis)

Abbreviations: CRC = colorectal cancer; DFS = disease-free survival; FOLFIRI = infusional 5-FU/LV with irinotecan; FOLFOX = 5-FU/LV with oxaliplatin; 5-FU = 5-fluorouracil; GERCOR = Groupe Coopérateur Multidisciplinaire en Oncologie; IMPACT = International Multicentre Pooled Analysis of Colorectal Cancer Trials; INT = intergroup; KRAS = Kirsten rat sarcoma viral oncogene homolog; LV = leucovorin; mFOLFOX6 = modified FOLFOX; MOF = 5-FU with lomustine and vincristine; MOSAIC = Multicenter International Study of Oxaliplatin/5-FU/Leucovorin in the Adjuvant Treatment of Colon Cancer; NCCTG = North Central Cancer Treatment Group; NS = nonsignificant; NSABP = National Surgical Adjuvant Breast and Bowel Project; OS = overall survival; PETACC = Pan-European Trial in Adjuvant Colorectal Cancer; X-ACT = Xeloda in Adjuvant Colon Cancer Therapy.

(ACCORD) 02<sup>84</sup> trials. The CALGB study reported that there was no significant difference in 3-year DFS, the primary end point of the trial, between 5-FU/leucovorin and IFL (60% vs. 63%, respectively;  $P = NS$ ). The main ACCORD02 findings were that 5-year OS rates for 5-FU/leucovorin and FOLFIRI were 67% and 61%, respectively ( $P = NS$ ), and 3-year DFS rates were 60% and 51% ( $P = NS$ ).

Much effort has been expended in investigating the efficacy of bevacizumab and cetuximab in the adjuvant setting. In the NSABP C-08 trial,<sup>85</sup> carried out in patients with stage II or III CRC, treatment with FOLFOX with bevacizumab showed no significant improvement in 3-year DFS compared with FOLFOX alone (77.4% vs. 75.5%, respectively;  $P = NS$ ). In the NCCTG/Intergroup N0147 trial,<sup>86</sup> patients with resected stage III CRC and wild type KRAS were randomly assigned to receive mFOLFOX6 (modified FOLFOX) with cetuximab or mFOLFOX6 alone. The trial was terminated when the prespecified interim analysis

demonstrated that there was no benefit in terms of the primary end point of 3-year DFS from the addition of cetuximab to mFOLFOX6 (74.6% with mFOLFOX6 alone vs. 71.5% with mFOLFOX6 with cetuximab;  $P = NS$ ). In Table 2 the findings of the key adjuvant studies described in this section are summarized.

## Conclusions

The evolution of chemotherapy for patients with CRC has involved a series of landmark advances, including the discovery of 5-FU, the identification of the reduced folate leucovorin as a clinical potentiator of 5-FU cytotoxicity, and the advent of novel cytotoxic and biological agents. As we move into the era of personalized cancer medicine, systemic chemotherapy involving infusional 5-FU/leucovorin remains the cornerstone of treatment for patients with CRC, but there is a need for empirical studies to explore how current treatment regimens can be optimized for individual patients.

## Disclosure

Isfol Medical AB, Gothenburg, Sweden, is currently evaluating a reduced folate (Modufolin) together with 5-FU or methotrexate as a treatment for CRC in clinical trials. Bengt Gustavsson is a director of Isfol Medical AB. Fernando Gibson is an employee of PharmaGenesis London, which received payment from Isfol Medical AB for this work. The remaining authors have stated that they have no conflicts of interest.

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