



LJMU Research Online

Luan, X, Zhang, L-J, Li, X-Q, Rahman, K, Zhang, H, Chen, H-Z and Zhang, W-D

Compound-based Chinese medicine formula: From discovery to compatibility mechanism.

<http://researchonline.ljmu.ac.uk/id/eprint/12359/>

Article

Citation (please note it is advisable to refer to the publisher's version if you intend to cite from this work)

Luan, X, Zhang, L-J, Li, X-Q, Rahman, K, Zhang, H, Chen, H-Z and Zhang, W-D (2020) Compound-based Chinese medicine formula: From discovery to compatibility mechanism. Journal of Ethnopharmacology. ISSN 1872-7573

LJMU has developed **LJMU Research Online** for users to access the research output of the University more effectively. Copyright © and Moral Rights for the papers on this site are retained by the individual authors and/or other copyright owners. Users may download and/or print one copy of any article(s) in LJMU Research Online to facilitate their private study or for non-commercial research. You may not engage in further distribution of the material or use it for any profit-making activities or any commercial gain.

The version presented here may differ from the published version or from the version of the record. Please see the repository URL above for details on accessing the published version and note that access may require a subscription.

For more information please contact researchonline@ljmu.ac.uk

<http://researchonline.ljmu.ac.uk/>

1 **Compound-based Chinese medicine formula: From discovery to**
2 **compatibility mechanism**

3

4 Xin Luan^{1,a}, Li-Jun Zhang^{1,a}, Xiao-Qin Li^{1,3}, Khalid Rahman⁴, Hong Zhang^{1,3*}, Hong-
5 Zhuan Chen^{1*}, Wei-Dong Zhang^{1,2*}

6

7 ¹ *Institute of Interdisciplinary Integrative Medicine Research, Shanghai University of*
8 *Traditional Chinese Medicine, Shanghai 201203, China*

9 ² *School of Pharmacy, Naval Medical University, 325 Guohe Road, Yangpu District,*
10 *Shanghai, 200433, China.*

11 ³ *College of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu,*
12 *611137, China*

13 ⁴ *School of Pharmacy and Biomolecular Sciences, Faculty of Science, Liverpool John*
14 *Moores University, Liverpool L3 3AF, England, UK*

15

16 ^a *These two authors contribute equally to this work.*

17

18 *** Corresponding authors:**

19 No. 1200, Cailun Road, Pudong New Area, Shanghai 201203, China. Telephone: 86-
20 21-51322720. Email Adress: wdzhangy@hotmail.com (W.D. Zhang),
21 hongzhuan_chen@hotmail.com (H.Z. Chen), hqzhang51@126.com (H. Zhang).

22

23

24

25

26

1 **Abstract**

2 *Ethnopharmacological relevance:* Chinese medicine formula (CMF) has a long history
3 of clinical use in the treatment of various diseases under the guidance of traditional
4 Chinese medicine (TCM) theory. The application of CMF can be divided into three
5 levels, crude extracts, homologous compounds mixture, and specific compounds.
6 However, the modern scientific connotation of the CMF theory has not been clarified.

7 *Aim of the review:* To critically evaluate the research strategy for the investigation of
8 compound-based CMF (CCMF).

9 *Materials and methods:* The related information was collected from the scientific
10 databases, including CNKI, Elsevier, ScienceDirect, PubMed, SpringerLink, Web of
11 Science, and Wiley Online.

12 *Results:* The research design including discovery, screening, optimization,
13 pharmacodynamics models, and target research techniques including the targets for
14 compatibility compounds were evaluated. Essentially it has been evaluated that the *in*
15 *vitro* multicellular three-dimensional culture or organoid model has been proposed for
16 the optimization model for compatibility research of CCMF. Based on these, the
17 traditional compatibility theory of CMF, such as Monarch-Minister-Assistant-Guide
18 (Jun-Chen-Zuo-Shi in Chinese), can probably be elucidated by the CCMF research.

19 *Conclusions:* CCMF has the clear advantage of providing the exact composition and
20 controllable quality of modern medicines, in addition to having the characteristics of
21 multi-ingredients and multi-targets synergistic effects of TCM. However, CCMF is still
22 associated with challenges which need to be addressed for its future use.

23 **Keywords:** Chinese medicine formula; compound; target; compatibility; methodology

24

25

26

27

28

29 **Abbreviations**

1 Chinese medicine formula (CMF), traditional Chinese medicine (TCM), homologous
2 compounds mixture-based CMF (HCMF), compound-based Chinese medicine formula
3 (CCMF), traditional Chinese medicine formula (TCMF), Shexiang-Baoxin Pill
4 (SXBXP), Feedback System Control (FSC), Cellular thermal shift assay (CETSA),
5 microscale thermophoresis (MST), Drug affinity responsive target stability (DARTS),
6 high-throughput screening model based on cellular thermal shift assay (HTS-CETSA)

7

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23 **1. Introduction**

1 As a long-standing science and culture, Chinese medicine formula (CMF) has a
2 long history of clinical use in the treatment of various diseases for thousands of years,
3 and has contributed to the prosperity and civilization of the China and the surrounding
4 countries including Japan and Korea.

5 In recent years, acceptance of CMF has gradually increased due to its clinical
6 effect (Liu et al., 2015; Zhu et al., 2018). The proper compatibility of CMF requires
7 strict guidance of traditional Chinese medicine (TCM) theory instead of the use of
8 simply aggregated medicinal plants or compounds. “Monarch-Minister-Assistant-
9 Guide” (Jun-Chen-Zuo-Shi in Chinese) is one of the most typical and important theories,
10 which vividly defines the different roles of the constituents in CMF. In this theory,
11 “Monarch drug” stands for an essential ingredient in a prescription and its leading
12 curative role is aimed at the cause or the main syndrome of a disease. “Minister drug”
13 stands for the ingredient which can strengthen the curative effect of the monarch drug.
14 “Assistant drug” mainly refers to the ingredient which can cooperate with the monarch
15 and minister drugs and inhibit their possible side effects or toxicities. “Guiding drug”
16 mainly refers to the ingredient which can guide other constituents to the pathogenic
17 sites. However, the modern scientific connotation of this theory needs to be further
18 explored and explained.

19 As outlined above, CMF refers to a systemic constitution of Chinese medicines
20 under the guidance of TCM theory for specific types of diseases. The earliest form of
21 CMF is the compatibility of crude extracts, such as Xiao-Chaihu Decoction composed
22 of pieces of Chaihu (*Bupleurum chinense* DC.), Banxia (*Pinellia ternate* (Thunb.)
23 Breit.), Renshen (*Panax ginseng* C. A. Mey.), Gancao (*Glycyrrhiza uralensis* Fisch.),
24 Huangqin (*Scutellaria baicalensis* Georgi), Shengjiang (*Zingiber officinale* Rosc.), and
25 Dazhao (*Ziziphus jujuba* Mill.), which was recorded in the book of Shanghan-Zabing-
26 Lun written by Zhongjing Zhang 1800 year ago. Even now, the crude extracts remain
27 the most common method of CMF application due to its several advantages, including
28 definite and long-term proven effect (China Pharmacopoeia Committee, 2015; Liu, et
29 al., 2015; Zhu et al., 2013; Zhu et al., 2018), relatively easier obtainment, simpler
30 processing and lower cost than chemical agents that cannot be obtained by complete
31 synthesis. However, the unclear chemical composition (active, ineffective and toxic
32 ingredients), unverified functional targets, and indistinct molecular mechanisms hinder
33 the extensive use of CMF globally. To overcome the defects of crude extracts, such as

1 the quality control, safety and inconvenient storage, further research of CMF needs to
2 be undertaken.

3 Zhang and Wang (2005) proposed the concept of homologous compounds
4 mixture-based CMF (HCMF), also known as component-based CMF, such as total
5 saponins from Huangqi (*Astragalus membranaceus* (Fisch.) Bge.), which propelled the
6 CMF research to a new level (). HCMF refers to the partially identified homologous
7 compounds mixture derived from Chinese medicine under the compatibility principles
8 of TCM theory (Zhang et al., 2015). Although HCMF has relatively controllable quality,
9 safety and effectiveness, such as approved Qishen-Yiqi-Diwan, Shouwu-Danshen-
10 Diwan, Zhigan-qin Capsule, and Sanye-Tangzhi Tablet, there still exists unascertained
11 chemical constituents, and unclear targets or mechanisms, which result in the obstacles
12 for successful illustration of the scientific connotation of the compatibility theory at the
13 molecular level.

14 Recently, the emerging technology of protein structure elucidation and target
15 validation methods have helped to further push the pharmacological research of CMF,
16 especially its new form which is composed of specific compounds with clear targets
17 and mechanisms (Wang et al., 2008; Zhang et al., 2010). In view of this, we presented
18 the viewpoint of the promising compound-based Chinese medicine formula (CCMF).
19 This refers to a mixture composed of specific natural compounds derived from Chinese
20 medicines or chemical drugs, under the principles of prescription compatibility of TCM
21 theory. CCMF not only possesses the probable advantages of enhanced efficacy and
22 reduced toxicity in traditional Chinese medicine formula (TCMF), but also has the
23 improved safety, reliable therapeutic effects and clear chemical composition-based
24 stable quality. Furthermore, the therapeutic target and mechanism are most likely to be
25 clarified at the molecular level (Wang et al., 2008; Zhang et al., 2010), and the scientific
26 connotation may be truly revealed for the compatibility theory, hence leading to the
27 modernization of research of TCM. Although TCMF and HCMF also exhibit the
28 probable synergism and attenuation effects similar to CCMF, their effective
29 constituents are yet to be identified and mechanism of action needs to be elucidated
30 (Table 1).

31
32

33 Table 1 Comparison among TCMF and HCMF and CCMF

	TCMF	HCMF	CCMF
Effect		Synergism and attenuation	
Effective constituent	Unclear	Partially clear	Completely clear
Inactive substance	Inclusion	Inclusion	Exclusion
Drug target	Indeterminate	Indeterminate	Determinable
Action Mechanism	Animal level	Animal and cellular levels	Animal, cellular and molecular levels
Elucidation of scientific connotation	No	Difficult	Yes

1

2 **2. Discovery of CCMF**

3 CCMF can be obtained from the following five main sources: classic prescriptions,
4 classic Chinese medicine pair, single Chinese medicine, HCMF, and synergistic active
5 compounds.

6 *2.1. Classic Prescriptions*

7 Classic prescriptions have shown good clinical effect (Liu et al., 2015; Zhu et al.,
8 2013; Zhu et al., 2018). These provide abundance of compounds with potential activity
9 that can be used to comprise CCMF, such as Four-Gentlemen Decoction (Si-Jun-Zi
10 Decoction in Chinese) with the effect of improving digestive system function, Si-Wu
11 Decoction with the effect of improving erythropoiesis, Liuwei-Dihuang Pill
12 traditionally used as a tonic to improve fatigue, lower back pain, menstrual symptoms
13 and night sweats, Jingui-Shenqi Pill used as a warming energy tonic and used to treat
14 various health conditions such as poor circulation, oedema, heart failure and
15 osteoporosis. The information of chemical constituents of TCMF can be obtained from
16 the constituent analysis and related databases. Furthermore, the initial basic
17 composition of CCMF can be obtained from high content screening of these compounds
18 according to the original therapeutic activities of TCMF.

19 Similar to the classic prescriptions, Chinese patent medicines approved by the
20 Chinese Food and Drug Administration (CFDA) for their clinical application with

1 satisfactory effect is another source of CCMF. Chinese patent medicine is the novel
2 Chinese medicine formulation developed through the modern pharmaceutical
3 technology under the TCM principle of syndrome differentiation and treatment, and
4 thus diagnosis and treatment is based on the analysis of the illness and the patient's
5 condition. The advantages of Chinese patent medicine are convenient use, rapid effect,
6 and reduced side effects. For example, Shexiang-Baoxin Pill (SXBXP, China
7 Pharmacopoeia Committee, 2015), consisting of Shexiang (*Moschus berezovskii*
8 Flerov.), Chansu (*Bufo bufo gargarizans* cantor), Renshen (*Panax ginseng* C. A.
9 Mey.), Suhexiang (*Liquidambar orientalis* Mill.), Niu Huang (*Bos taurus domesticus*
10 Gmelin), Rougui (*Cinnamomum cassia* Presl) and Bingpian (borneol), has been
11 successfully used to treat angina pectoris and myocardial infarction. Modern
12 pharmacological studies show that SXBXP can relax blood vessels, reduce myocardial
13 infarction, inhibit vascular calcification and promote angiogenesis (al., 2018). We
14 previously investigated the chemical constituents of SXBXP, 57 non-volatile
15 components were detected by liquid chromatography with diode array detection and
16 electrospray ionisation mass spectrometry (LC-DAD-ESI-MS) and 47 of these were
17 identified. Among them, 20 are triterpene saponins from ginseng, 18 are bufadienolides
18 from toad venom, 5 are cholic acids from bezoar, 1 is bilirubin from bezoar including
19 3 other compounds (Peng et al., 2009). 49 volatile compounds were identified by gas
20 chromatograph-mass spectrometer (GC-MS) analysis and compared with NIST05
21 online database. These findings provide a series of compounds which can be applied in
22 the CCMF research based on SXBXP. Arsenic sulfide, indirubin and tanshinone IIA
23 are the main active compounds of Realgar-Indigo naturalis formula (Zhu et al., 2018),
24 which is a famous Chinese patent medicine and is composed of realgar, indigo naturalis,
25 Danshen (*Salvia miltiorrhiza* Bge.) and Taizishen (*Pseudostellaria heterophylla* (Miq.)
26 Pax ex Pax et Hoffm.). The combination use of these three compounds significantly
27 prolongs the life span of tumor bearing mice compared to their mono- or bi-treatment,
28 displaying probable synergistic effects (Wang et al., 2008).

29 2.2. Classic Chinese Medicine Pair

30 Chinese medicine pair is the fixed drug combination of two commonly used
31 medicines and is the smallest unit of the TCM compatibility. Chinese medicine pair
32 usually exhibits better effect or reduced toxicity compared to their use alone and is the

1 accumulation of clinical experience from ancient times (Yue et al., 2017; Ma et al.,
2 2019). For example, Huangqi (*Astragalus membranaceus* (Fisch.) Bunge) and Danggui
3 (*Angelica sinensis* (Oliv.) Diels) can form a classic Chinese medicine pair, which is
4 commonly used to significantly enhance the effect of reducing oxygen consumption
5 and resisting fatigue (Chang et al., 2018). The main active compounds in this pair
6 includes astragaloside, formononetin, calycosin, calycosin glycoside, and ferulic acid,
7 etc. Zhu et al. (2019) found that the combination of ferulic acid, astragaloside and
8 formononetin can significantly improve proliferation and aging of hematopoietic stem
9 cells, when compared with the single drug. Another example of classic Chinese
10 medicine pair of Fuzi (*Aconitum carmichaelii* Debeaux) and Gancao (*Glycyrrhiza*
11 *uralensis* Fisch.) is from the classic treatise: Shanghan-Zabing-Lun written by
12 Zhongjing Zhang. Hypaconitine and glycyrrhetic acid have been identified as the
13 main effective compounds of Fuzi and Gancao, respectively, and their combination can
14 significantly improve the injured morphology and injury-related indexes of rat
15 myocardial H9c2 cells caused by hypoxia and glucose deficiency at a ratio 1:1 (Wang
16 et al., 2016). These compounds from classic Chinese medicine pair can form the basic
17 composition of CCMF.

18 2.3. Single Chinese Medicine

19 A single Chinese medicine often contains complex chemical compounds, some of
20 which display significant compatible therapeutic effects. Yimucao (*Leonurus artemisia*
21 (Laur.) S. Y. Hu) has heat clearing properties that may help to regulate menstruation,
22 promote diuresis and clear toxins from the body (China Pharmacopoeia Committee,
23 2015) and stachydrine hydrochloride and leonurine hydrochloride are the main active
24 constituents of Yimucao. Li et al. (2019) found that the combination of stachydrine
25 hydrochloride (30, 45 mg/kg) and leonurine hydrochloride (15, 30 mg/kg) can
26 significantly reduce the levels of aspartate transaminase (AST), creatine kinase (CK),
27 CK isoenzyme-MB (CK-MB), lactate dehydrogenase (LDH), hydroxybutyrate
28 dehydrogenase (HBDH), cardiac troponin I (cTnI), and malondialdehyde (MDA)
29 which are serum biomarkers for myocardial injury during ischemia-reperfusion injury
30 in rats. Furthermore the diastolic function of the heart is improved and offered
31 protection against myocardial injury during ischemia-reperfusion, which is similar to
32 the effects of Simvastatin tablets. Danshen (*Salvia miltiorrhiza* Bunge) can improve

1 blood circulation and disperse stasis, and it is also widely used to relieve menalgia.
2 Traditionally, it is used to “clear heart-fire” and may also be used in mental-emotional
3 conditions (China Pharmacopoeia Committee, 2015). The water soluble compounds
4 present include protocatechuic aldehyde, protocatechuic acid, rosmarinic acid, caffeic
5 acid, sodium danshensu, salvianolic acid A, and salvianolic acid B, etc. Tian et al. (2014)
6 found that the combination of these seven compounds can significantly improve the
7 memory impairment caused by cerebral ischemia-reperfusion injury in mice, enhance
8 the hypoxia tolerance, increase the activity of superoxide dismutase (SOD) and catalase
9 (CAT) in brain, and reduce the activity of acetylcholinesterase (AChE) and the level of
10 malonaldehyde. The mechanism of memory protection involved may be related to
11 scavenging of free radicals, apoptosis inhibition of nerve cells, and nerve regeneration
12 in brain tissue. These active compounds can be conducive to form the novel CCMF.

13 2.4. Homologous compounds mixture-based CMF (HCMF)

14 Homologous compounds mixture-based CMF (HCMF), also known as
15 components based-CMF, are composition groups with defined proportion or high
16 homogeneity. The chemical components can be relatively recognized, and the
17 proportion of each component is fixed. However, HCMF still contains a large number
18 of unknown chemical constituents since it is difficult to purify the effective fractions,
19 such as total alkaloids and total saponins. Hence it is necessary to further isolate,
20 identify and screen the compounds contained in these preparations, which may
21 contribute to the novel CCMF optimization.

22 Fan et al. (2017) studied the compatibility law of anti-cancer compounds from
23 total flavonoids of Jingjie (*Schizonepeta tenuifolia* Briq.) by microfluidic chip
24 technology in a well-distributed experiment design. The results shows that the
25 combination of luteolin, luteoloside, quercitrin, hesperidin, apigenin and genistein at a
26 ratio of 3.06: 2.90: 2.04: 4.17: 0.12: 2.75 has better effect of apoptosis induction on
27 lung cancer cell line A549 compared to the same dosage of total flavonoids, and the
28 drug potency order of sequence is luteoloside, quercitrin, luteolin, apigenin, genistein
29 and hesperidin. Cao and Deng (2016) isolated effective glycosides from the original
30 extract of Buyang Huanwu Decocotion by acid-base precipitation and ion exchange
31 chromatography. The mass fractions of astragaloside IV, amygdalin and paeoniflorin
32 in the effective components are 37.98 mg/g, 5.48 mg/g and 103.6 mg/g, respectively.

1 The results suggest that the combination of these three compounds significantly inhibits
2 the proliferation of rat aortic vascular smooth muscle cells (VSMC) induced by platelet
3 derived growth factor (PDGF), and the IC₅₀ is lower than the single compounds or the
4 glycoside component.

5 2.5. Synergistic Active Compounds

6 A TCM formula usually consists of many natural products, such as herbs, animal
7 based products and minerals, each of which contains large number of compounds. One
8 of the biggest challenges in the modernization of TCM is to identify the active
9 compounds that contribute to the therapeutic effects. Therefore, the identification of
10 active compounds is necessary to elucidate the underlying mechanisms and scientific
11 basis of TCM formula. Currently, the pharmacological effects of numerous compounds
12 isolated from Chinese medicines and natural medicines have been studied, such as
13 artemisinin (Bhattacharjee et al., 2018) and arsenic trioxide (Jiang et al., 2019). These
14 compounds can comprise formula according to different key links of etiological and
15 pathological of diseases. Ischemic stroke, also known as cerebral infarction/death,
16 refers to ischemic necrosis or encephalomalacia caused by the ischemia and hypoxia of
17 local brain tissue resulting from cerebral blood supply disorders. Cerebral infarction is
18 characterized by high morbidity, disability, recurrence and mortality. The major
19 treatment measures include thrombolysis, anti-platelet aggregation, anti-inflammatory
20 and neuroprotection. Paeonol, a natural compound, can reduce the levels of
21 inflammatory factors in rat brain tissue and serum after reperfusion injury of ischemic
22 stroke, such as TNF- α , IL-1 β and IL-6, which may offer a protective role by reduction
23 of local excessive inflammation response through inhibition of the production of
24 inflammatory factors (Yang et al., 2010). Paeonol can also significantly decrease the
25 protein and mRNA expressions of ICAM21 and VCAM21 in ischemic brain tissue of
26 rats, which may offer a protective role by reduction of the expression of adhesion
27 molecules in ischemic brain tissue (Zhang et al., 2008). Paeoniflorin can significantly
28 reduce the volume of infarction caused by middle cerebral artery occlusion and improve
29 the MCAO-caused functional deficiencies of the nervous system in rats (Xiao et al.,
30 2005). It also alleviates the free radicals-elicited oxidative damage in rats after
31 reperfusion injury of local ischemic stroke by improving the elimination of free radicals
32 and increasing the expression of Nrf2 (He et al., 2014). In mice hippocampal CA1

1 neurons, paeoniflorin can not only inhibit Na⁺ current in a concentration- and
2 frequency- dependent manner, but also control the gated properties of Na⁺ current, thus
3 antagonizing intracellular Ca²⁺ overload (Zhang et al., 2003). Luteolin has a significant
4 anti-inflammatory effect and can reduce LPS-induced increase of IL-6 by interfering
5 with JNK signaling pathway and activating activator protein-1 (AP-1) in microglial
6 cells (Saebyeol et al., 2008), and inhibiting the NF-κB, MAPK and Akt signaling
7 pathways in activated microglia cells (Zhu et al., 2014). These compounds target
8 different links of stroke and can be prepared by using different chemical mixtures to
9 provide novel CCMF.

10 A compound library is a collection of entity compounds with specific structures
11 or functions and their related information under certain specific standards. PubChem is
12 an open access biological information platform and database supported by the US
13 National Institutes of Health (NIH) with the structure information of more than 30
14 million compounds and 1 million biochemical experimental data. The compound
15 database China Natural Products supported by Ministry of Science and Technology of
16 China has collected more than 10,000 natural compounds. These compound library and
17 database have provided the information for the compatibility formulation of CCMF.

18 **3. Screening approach for compatibility compound**

19 *3.1. Network Pharmacology*

20 TCM network pharmacology is a rising interdisciplinary science and effectively
21 integrates the study of TCM pharmacology with network science, systems biology,
22 computational science and bioinformatics (Li and Zhang, 2013; Zhao et al., 2019).
23 Currently the chemical compounds included in TCM formula can be obtained from
24 several databases, such as TCMSP (Traditional Chinese Medicine Systems
25 Pharmacology Database and Analysis Platform) (Ru et al., 2014), TCM
26 Database@Taiwan (Traditional Chinese Medicine Database@Taiwan) (Chen, 2011),
27 TCMID (Traditional Chinese Medicine Integrated Database) (Xue et al., 2013),
28 NPACT (Naturally occurring Plant based Anticancerous Compound-activity-Target
29 database) (Mangal et al., 2013), CancerHSP (Anticancer Herbs database of Systems
30 Pharmacology) (Tao et al., 2015), and NPASS (Natural Product Activity and Species
31 Source Database) (Zeng et al., 2018). The active compounds can be selected according
32 to the ADME-related parameters (Ru et al., 2014).

1 Kushen is the dry root of *Sophora flavescens* Aiton and Chen et al. (2017) virtually
2 screened the anti-angiogenesis flavonoids from Kushen by molecular docking
3 technology. A ligand database was established by collecting 126 flavonoids compounds
4 which have been separated and identified. A receptor database is composed of 6 targets
5 closely related to angiogenesis such as vascular endothelial growth factor α (VEGF- α),
6 TEK receptor tyrosine kinase (TEK), vascular endothelial growth factor receptor 2
7 (KDR), etc. The small molecule drugs which have inhibitory effect on each target are
8 listed in the DrugBank as a control and the lowest scoring of the listed small molecule
9 drugs to each target is set as the threshold. The Discovery Studio 2.5 (DS 2.5) software
10 is used for molecular docking, and a total of 37 compounds with scores higher than the
11 threshold were identified and top 10% were investigated.

12 3.2. *Biochromatography*

13 Biochromatography is a new chromatographic technique that combines bioactive
14 materials such as receptors, carrier proteins, cell membranes and carriers as stationary
15 phases, and utilizes the specific binding of stationary phase and compounds to screen
16 the drug candidates. Due to the biological activity of the bioactive materials on the
17 stationary phases, the screening compounds can be bound to the stationary phases
18 through the hydrophobic force, van der Waals force, electrostatic action and binding
19 sites of specific ligand, which reflects the pharmacological potential and significance
20 of screening compounds. The biochromatography technology can eliminate the
21 interference of inactive compounds, narrow down the research focus, and identify the
22 structure of leading compounds when combined with HPLC and mass spectrometry.

23 Wu et al. (2019) has established a cell membrane chromatography/ultra-high-
24 performance liquid chromatography-time of flight mass spectrometry (CMC/UPLC-
25 TOF/MS) analysis method for water extracts of Liuwei-Dihuang Decoction and
26 completed rapid identification of 16 potential active compounds including catalpol,
27 paeonol, oleanolic acid, etc. The isolated catalpol displays high affinity and was further
28 proven to induce the growth of mouse osteoblasts and skull mineral area in osteoporotic
29 zebrafish.

30 3.3. *High throughput screening*

1 High throughput screening (HTS) technology is a powerful platform for drug
2 screening with the characteristics of automatic operation, rapid detection,
3 molecular/cellular level, computer analyses and use of micro-sample volumes.
4 Marciano et al. (2019) compared the effect of small molecular compounds on MCF-7
5 breast cancer cell lines with or without sugar in the cell medium on a 384-well
6 microplate. Out of 7,000 compounds, 67 have been successfully identified with the
7 effects of increased sensitivity of MCF7 to the sugar-free culture. Yuliantie et al. (2018)
8 established a high-throughput screening model at the cellular level using secretory
9 embryonic alkaline phosphatase (SEPA) as a reporter gene on 384-well plates, and 25
10 positive compounds were identified from 32,000 compounds. The cytotoxicity,
11 phosphorylation effect on STAT protein and the transcription of IFN regulatory factor
12 (IRF) of these compounds were further investigated.

13 *3.4. Serum pharmacochemistry of Chinese medicine*

14 Serum pharmacochemistry of Chinese medicine uses modern separation and
15 multi-dimensional combination techniques to analyze, identify or characterize the
16 compounds in human/animal serum after administration of Chinese medicines, to
17 reveal the correlation between these compounds and their efficacy (Wang, 2010). In
18 serum pharmacochemistry of Chinese medicine, only the compounds distributed in
19 serum are treated as the research object, which may significantly simplify the number
20 of compounds to be optimized.

21 The common analytical techniques for serum pharmacochemistry include gas
22 chromatography, gas chromatography-mass spectrometry, high performance liquid
23 chromatography, liquid-mass spectrometry, capillary electrochromatography, thin-
24 layer scanning, spectrophotometry, atomic absorption spectrometry, etc. HPLC method
25 can be established for the determination of characteristic components of Chinese
26 medicine and CMF extracts. HPLC-DAD, HPLC-DAD-MS/MS and other analytical
27 techniques are used for classification, analysis of the structure and identification of the
28 migrating components contained in serum. To obtain the accurate results, the
29 fingerprints of standard substance, drug-containing serum and blank serum should be
30 determined, analyzed and compared under the same conditions. Of particular note the
31 serum-containing components may change with time due to the different administration
32 methods, physical and chemical properties, absorption sites and rates of Chinese

1 medicine ingredients (An et al., 2013). By analyzing the serum fingerprints of the blood
2 samples collected at different time points, the time-varying process of the whole blood
3 composition spectrum *in vivo* can be found. It is necessary to establish a dynamic serum
4 pharmacological profile to reflect the changes of chemical composition *in vivo* and
5 the correlation between the prototype drug and metabolic components. The application
6 of ultra-high performance liquid chromatography-mass spectrometry (UPLC-MS) can
7 be used to identify the trace components on line (Wang et al., 2011), and
8 pharmacodynamics can be determined (Yan et al., 2012).

9 In our previous work, the compounds distributed in blood of SXBXP have been
10 successfully investigated, and 17 prototype compounds and 3 metabolites were
11 identified from rat plasma by HPLC-ESI-MS/MS (Jiang et al., 2010). 10 volatile
12 compounds including 6 prototypes and 4 metabolites have been identified by GC/MS
13 and NIST05 database comparison (Guo et al., 2012). Although a total of 96 compounds
14 were identified from SXBXP *in vitro*, only 30 compounds were detected in blood which
15 may simplify the difficulty of SXBXP compatibility research.

16 There are also some limitations in the use of serum pharmacology for
17 Chinese medicine to determine its therapeutic effects. For example, Chinese medicine
18 and formula contain complex chemical constituents, with different physical and
19 chemical properties with different rates of absorption and distribution, therefore, it is
20 difficult to fully determine the dynamic changes of compounds (Chen et al., 2016). The
21 experimental animal species and their physiological and pathological conditions also
22 have great influences on the analysis of results, such as the obvious differences in the
23 absorption under physiological and pathological conditions in rats (Liu et al., 2014; Shi
24 et al., 2014). There are great differences in the pharmacokinetic characteristics of
25 components distributed in blood from Danggui-Buxue Decoction between ischemia
26 rats and normal rats (Shi et al., 2014). In spite of the increased difficulties in animal
27 modeling, the application of pathological animal models has better practical
28 significance compared with the commonly used normal animal models.

29 **4. Optimization method of compatibility compounds**

30 Due to the complex chemical constituents of traditional Chinese medicine, the
31 research on its compatibility lacks relevant experience at the molecular level. The
32 information on how to obtain the optimal combination of these active compounds is a

1 crucial step in the study of CCMF. Therefore, it is necessary to optimize the
 2 compatibility of effective compounds according to the corresponding clinical diseases
 3 and syndromes, so as to screen out the best compatibility formula. Several commonly
 4 used optimization methods are summarized below (Table 2).

5 Table 2 Comparison of common optimization methods

	Advantages	Disadvantages	Range of application
Feedback System Control	Efficient and rapid	The dispersion degree of drug dose has great influence	Focuses on integrative system responses; broad selection of drugs
Orthogonal Design	The data analysis program is simple	Low accuracy	Low level test
Uniform design	The number of trials is greatly reduced	Can not estimate the main and interaction effects in the anova model	The test points are evenly distributed within the test range
Increase-decrease design	Good reliability, large information processing space, and less experiments	Narrow scope of application	The effect is clear and there are only two kinds of TCM compounds
ED-NM-MO trigeminy method	Multi-components optimization ratio	Calculation is complicated	Multi-component compounds

6

7 *4.1. Feedback System Control*

8 Generally, the combination use of drugs can reduce the dosage and side effects or
 9 enhance therapeutic effect compared to the single drug (Ding et al., 2017). As a novel
 10 screening technique for drug combination therapy, Feedback System Control (FSC)
 11 was firstly proposed by Wong et al. (2008). FSC is based on combination of
 12 experimental results under the guidance of differential evolution (DE) algorithm. It uses
 13 parabolic reaction surface (PRS) to define the transfer function of phenotype output and
 14 drug dose input, thereby achieving fast and precise optimization of multiple drug or

1 dose combinations and greatly improving the speed and efficiency. Compared to
2 traditional methods, FSC is more efficient, which significantly reduces the efforts, cost,
3 time and number of experimental subjects (Nowakliwinska et al., 2016; Wong et al.,
4 2008; Silva et al., 2016). Tsutsui and the co-authors used FSC to screen out an optimal
5 composition, consisting of three small molecule inhibitors, which enabled the single
6 cell culture system of maintaining embryonic stem cells through single cell passage on
7 a fibronectin-coated surface (Tsutsui et al., 2011). Yu et al. (2013) identified the
8 potential best combination of four flavonoids and conducted a preliminary evaluation
9 based on the optimization of Huangqi-derived flavonoids by using an engineering
10 approach of the FSC scheme. The optimal combination of flavonoids to maximize
11 hypoxia response element (HRE) -mediated transcriptional activity was quickly
12 acquired. Ding et al. has applied FSC technique to screen out the best combination of
13 four drugs for the treatment of intestinal aphids, so as to effectively alleviate clinical
14 drug resistance (Ding et al., 2017). A very effective “cocktail” program with lower
15 dosage and improved effect has been found only after 4 rounds and 32 combined tests.
16 Therefore, FSC can undertake a significant role in screening of drug combination,
17 which is also suitable to screen for CCMF compatibility.

18 *4.2. Orthogonal Design*

19 Orthogonal design is a method of designing multi-factor and multi-level tests
20 through orthogonal test tables with the characteristics of uniform dispersion, uniformity
21 and comparability. It is suitable for the compatibility optimization of TCM with limited
22 scope and was performed at three factors and four levels for three components
23 (polysaccharides, saponins and phenols) isolated from Banxia-Baizhu-Tianma
24 Decoction (Xu et al., 2019). The fatty and salty diet hypertensive rats with phlegm-
25 dampness congestion symptoms were used as screening models and the parameters of
26 glucolipid metabolism were converted into integrated efficiency according to the equal
27 weight index. The optimal combination dosages of saponins, phenols and
28 polysaccharides are 9.00 mg/kg, 14.50 mg/kg, and 12.95 mg/kg, respectively.

29 *4.3. Uniform design*

30 Uniform design is a method based on the principle of uniformity and can select
31 some representative compounds and uniformly distributed points from the whole range

1 reflecting the major characteristics of the research object. Uniform design can reduce
2 the scope and number of the experiments to a great extent, especially for the multi-
3 factors and multi-levels research, which is more suitable for experiments at tissue level
4 or at whole animal level. However, it lacks the symmetrical comparability compared
5 with the orthogonal design, and the regression analysis method should be used to
6 process the results.

7 Wu et al. (2016) used uniform design method to optimize the compatibility of four
8 main compounds included in *Evodia rutaecarpa* decoction, namely ginsenoside-Rg1
9 (Rg1), ginsenoside-Rb1 (Rb1), evodiamine (EV) and evodiamine (RU) for the mouse
10 disease model of migraine. The results show that Rb1 and EV significantly increase the
11 pharmacodynamics indicators and improve the efficacy, while Rg1 and RU contribute
12 little to the overall formula.

13 *4.4. Other experimental designs*

14 In addition, other experimental designs like increase-decrease design and ED-NM-
15 MO trigeminy method may also be used in CCMF study. Shang et al. (2003) established
16 this method of experiment with a baseline geometric proportion property for
17 optimization and screening of the proportion of small formula. The advantages are the
18 comprehensive and reliable information analysis, the large information processing
19 space and the relatively small number of experiments. However, the increase-decrease
20 design is used only in small CMF with clear therapeutic effects, such as formula with
21 only two ingredients.

22 The ED-NM-MO trigeminy method is proposed on the basis of experimental
23 design (ED)-nonlinear modeling (NM)-multi-objective optimization (MO). It uses
24 three related links to achieve nonlinear multi-objective optimization of prescription
25 dose ratio. The ED-NM-MO method is suitable for the investigation of medicine
26 properties and can be used to optimize the dosage and ratio of CMF. The compatibility
27 composition of Danshen and Sanqi was optimized by this method, and the optimal ratio
28 is obtained for 7 pharmacodynamic indicators and 6 pharmacodynamic indicators,
29 respectively (Wang et al., 2006).

30 **5. Therapeutic evaluation model**

1 Drug screening can be conducted at the levels of molecules, cells, tissues and
2 animals. However, the use of tissues and animals are not suitable for the composition
3 screening for CCMF owing to their low throughput. The screening at molecular level
4 is also inappropriate for CCMF due to its characteristics of single-target. Hence, some
5 common models for cancer research, including patient-derived cancer cell lines (PDX),
6 patient-derived xenografts (PDX), multicellular three-dimensional (3D) cultures and
7 organoids, are introduced. Especially, the cell 3D-culture and organoid models have
8 tremendous potential for CCMF research.

9 *5.1. Multicellular three-Dimensional culture model*

10 In recent years, cell 3D-culture has attracted considerable attention as it can
11 simulate physiology and pathology state better than traditional two-dimensional (2D)
12 culture. It has a higher degree of interaction between cells and maintains more orderly
13 tissue processing of tiny structures of organs in the body (Nath and Devi, 2016). The
14 multicellular tumor spheroids (MCTS) is a type of models most commonly used in
15 tumor research since its 3D structure can simulate the angiogenic tumor region
16 composed of proliferating cells and necrotic cells (Froehlich et al., 2016). There are two
17 construction methods for MCTS, including material assisted and mechanical device
18 assisted construction. The low melting point agarose and Matrigel with appropriate
19 growth factors or extracellular matrix are the commonly used materials for 3D-culture
20 (Matte et al., 2016). The mechanical means are used to construct a physical
21 environment conducive to cell aggregation into pellets, including suspension drop
22 method, rotating culture system method, and so on.

23 The formation of cell spheroids is a cell-dependent manner, although not all kinds
24 of cells can be used in 3D-culture model. Froehlich et al. (2016) has studied the ellipsoid
25 formations of three breast cancer cell lines MCF-7, MDA-MB-231 and SK-BR-3 under
26 different culture conditions, including hanging drop, liquid overlay and suspension
27 culture and 25% methocel was recommended as the most reliable and effective
28 condition. MCF-7 cells can form spheroids under nearly all of analytical conditions.
29 MDA-MB-231 cells form spheroids under only one scheme (liquid overlay technique,
30 3.5% Matrigel), but SK-BR-3 cells are not spherical under any condition. Galateanu et
31 al. (2016) used human colorectal cancer multi-cells to establish a 3D spheroid model
32 which was applied to evaluate the effect of the clinical drug combination of folinic acid,

1 oxaliplatin and 5-fluorouracil. These results demonstrate the effect of carrying
2 liposomes loaded with drugs on multicellular tumor spheres. However, the dose range
3 needs to be confirmed through *in vivo* studies for combinational use of three drugs and
4 their subsequent encapsulation into liposomes.

5 The MCTS establishes a stereoscopic information exchange system similar to the
6 real tumor, which plays a great role in the connection between cells and clinical trials,
7 and enhances the early prediction of the potential of candidate drugs (Luo and Gao,
8 2018). However, there are still some limitations for this out-of-body model. For
9 example, not all immortal cell lines spontaneously form MCTS, which requires
10 extensive optimization and validation, limiting the use of this model (Juergen et al.,
11 2009). To address this problem, more complex *in vitro* systems are currently being
12 evaluated, including co-culture with other cell types, such as fibroblasts, to promote the
13 formation of MCTS and the replication of complex signals that occur *in vivo* (Cui et al.,
14 2017; Park J et al., 2016). On the other hand, single cell type model cannot reflect the
15 multi-target effect characteristics of CCMF, establishment of the 3D model of multi-
16 type cells co-culture is the development direction in future.

17 As a powerful *in vitro* drug evaluation model, 3D cell culture has made many
18 gratifying achievements since its emergence. Zhang et al. (2014) found that the
19 pathological changes in the redistribution of phosphorylated p21 activated kinase
20 (pPAK) and other related proteins in nerve cells could only be observed under the
21 condition of 3D culture, which could not be found in the conventional 2D cell culture
22 model. APP and PSEN1 mutations are expressed successfully in ReNcell VM cell lines,
23 generating FAD ReNcell lines, and then obtaining cell model that could accelerate
24 neuronal cell differentiation to form neural network through 3D cell culture method
25 (Choi et al., 2014; 2016). This research successfully reproduces the pathological
26 process of A β deposit and drive tau protein extracellular accumulation, which provides
27 more effective methods and techniques for the treatment of alzheimer's disease.

28 Cell culture is an indispensable experimental technique during drug development
29 process. 3D cell culture has become a valuable tool, which is closer to the real situations
30 *in vivo*, helping to bridge the gap between the *in vitro* and *in vivo* models with reduced
31 animal number used in the early stage of experiments and the possibilities of error (Yu
32 and Zhou, 2019). However, most 3D cell cultures rely on gel substrates, and the
33 applications are limited by their solids and opacity, as well as the inconsistencies of the

1 cells' exposure to environmental stimulus. Therefore, before 3D cell cultures can be
2 widely accepted and regarded as standard in the drug development and precision
3 medicine related research, there are still many difficulties and obstacles which need to
4 be tackled and solved.

5 5.2. Organoid Model

6 Organoid is a 3D “micro-organ model” prepared by self-organization of different
7 types of stem cells and can mimic the structure and function of original organs
8 (Fatehullah et al., 2016). Such *in vitro* culture systems include a self-renewing stem cell
9 population that can differentiate into multiple organ-specific cell types with similar
10 spatial tissue to the corresponding organ and be able to reproduce some of its functions
11 thus providing highly physiologically relevant systems (Lancaster and Knoblich, 2014).
12 In recent years, investigations on organoid have mainly focused on *in vitro* models of
13 diseases and *in vitro* organ reconstruction, which will open up new approaches for
14 biological research. The development and application of organoid models will be one
15 of the hot topics in tumor and stem cell research in future (Xu et al., 2018).

16 Organoid can be prepared using somatic cells, adult stem cells (including
17 progenitor cells) or pluripotent stem cells. In 2009, the intestinal organ simulation
18 technology made the technological breakthroughs. Toshiro found that adult intestinal
19 stem cells could proliferate and spontaneously organize *in vitro* (Toshiro et al., 2009),
20 hence the researchers developed a 3D culture system capable of reconstructing the
21 appropriate environment for intestinal stem cells *in vitro* and differentiating from
22 intestinal epithelial cells or single LGR5+ stem cells into organoids with self-renewal
23 ability and maintaining the villous structure of epithelial tissues. Huch et al. optimized
24 the condition of the mouse liver organ culture system and successfully obtained liver
25 organs using liver duct cells derived from humans (Huch et al., 2015). This type of
26 organ maintains the stability of the genome during long-term culture and can be
27 converted into functional hepatocytes under conditions of *in vitro* culture and
28 transplantation. After the differentiation of ESCs into the endoderm induced by activin
29 A, Noggin and transforming growth factor- β (TGF- β) inhibitors, the expression levels
30 of SOX2 and FOXA2 both increased, which activated the HH pathway and inhibited
31 the FGF pathway respectively, and then the quasi-organs of the lung were obtained
32 (Dye et al., 2015). Fong et al. (2016) co-cultured tumor cells derived from PDX model

1 of prostate cancer with osteoblasts, and the 3D model formed in this culture system
2 could well maintain the proliferation activity of cells and the state of osteogenesis,
3 which is consistent with the phenotype of bone metastasis of prostate cancer. Therefore,
4 this organ model can be used to study the interaction between tumor cells and
5 microenvironment including stromal cells.

6 Compared with traditional 2D culture models, the organoid represents an
7 innovative technique that summarizes the physiological processes of the entire
8 organism, with such advantages as closer proximity to physiological cell composition
9 and behavior, more stable genome, and more suitable for biological transfection and
10 high-throughput screening. Compared with animal model, the organoid model is
11 simpler to operate and can be used to study the mechanisms of disease occurrence and
12 development. At present, although organoids show the ability of self-renewal and
13 differentiation *in vitro* experiments, but its passage times are limited. However, the
14 major challenge is the improvement of passage ability and the maintenance of
15 proliferation and undifferentiation in patients. On the other hand, the effects of matrix
16 and the vascular system have not been considered in the present organoid culture.
17 Although organoid technology is still in its infancy, it has great potential to study a
18 wide range of subjects, including developmental biology, disease pathology, cell
19 biology, reproductive mechanisms, precision medicine, drug toxicity and efficacy trials.
20 It is believed that with the continuous innovation of technology, quasi-organs as an
21 ideal model will play an increasingly important role in human disease research.

22 5.3. PDC and PDX Models

23 Since the successful establishment of the HeLa cell line in 1951, a new field of
24 cancer research has been opened, making PDC a major model for cancer research.
25 Tumor cells need only simple culture conditions, can proliferate indefinitely *in vitro*,
26 and are suitable for large-scale drug screening. However, the tumor cell lines also have
27 serious defects in that the heterogeneity and the characteristics of tumor cells *in vivo*
28 are lost during *in vitro* culture. The PDX model refers that the tumor tissue derived
29 from patients is cut into small pieces and then transplanted into immune-deficient mice
30 (Lai et al., 2017). Some tumor cell lines originated from mice can also be transplanted
31 into mice to form tumor. Although the PDX model can maintain the heterogeneity of
32 tumors to a large extent and has been applied to drug screening, it still faces many

1 problems, such as low success rate of transplantation, low screening throughput, large
2 sample size of tumors and long experimental period (Gao and Chen, 2015; Xu et al.,
3 2018).

4 **6. Research methods of molecular targets**

5 Many publications have systematically introduced the techniques for active targets
6 of natural products (Zeng and Peng, 2018; Zhou and Xiao, 2018), including affinity-
7 based protein profiling (A/BPP), drug affinity responsive target stability (DARTS),
8 cellular thermal shift assay (CETSA), stability of proteins from rates of oxidation
9 (SPROX), target identification by chromatographic co-elution (TICC), microscale
10 thermophoresis (MST), chemical genomics screening, chemical genomics
11 bioinformatics prediction, differential genomics screening, differential proteomics
12 screening, and so on. Among these, CETSA and DARTS as the validation methods can
13 be applied to screen drug targets when combined with other assistant techniques such
14 as quantitative mass spectrometry. We briefly introduce the frontier techniques in
15 CCMF research below, such as CETSA, DARTS and MST.

16 *6.1. Cellular Thermal Shift Assay*

17 Cellular thermal shift assay (CETSA) was first proposed in 2013 (Daniel et al.,
18 2013). The basic principle is that when a small molecule compound binds to a protein,
19 the thermal stability of the protein molecule is significantly improved. This kind of
20 binding protein needs higher temperature to be aggregated and degenerated. When
21 heated to a certain temperature, the binding protein and the unbound protein can be
22 separated by high-speed centrifugation. The known proteins can be validated by
23 western-blot analysis. This unique technique can directly measure drug-protein binding,
24 and has been widely used to verify conjugation (Dai et al., 2018; Jafari et al., 2014).
25 Currently, more than 98.3% of 558 known small molecular drug targets belong to
26 proteins (Santos et al., 2017), and more than 3000 potential target proteins can be
27 combined with small molecule compounds to exert pharmacological effects (Yue et al.,
28 2012). However, CETSA can only be applied to validate the known proteins of target
29 due to the limitation of western-blot technique, and it is not suitable for high-throughput
30 screening.

1 Currently, there are two kinds of high-throughput methods for drug targets
2 screening based on improved CETSA technique. The first is to screen drugs according
3 to the target and isolate specific compounds that bind to target proteins from candidate
4 compounds. The novel techniques can be used to increase the throughput of screening,
5 such as ALPHAScreen based on the interaction between donor and receptor microbeads
6 (Jafari et al., 2014) and ligand-stabilized soluble target protein detection based on
7 enzyme fragment chemiluminescence quantification (Martinez et al., 2018). Another is
8 to screen targets by known drugs. Some proteins combined with a small molecule
9 compound are obtained through high-throughput screening, which is very suitable for
10 the target screening of CMF compounds. However, it is difficult to construct high-
11 throughput screening models based on cellular thermal shift assay (HTS-CETSA) for
12 screening target proteins owing to the large number of candidate target proteins, their
13 physical and chemical properties and the difficulty of biotin labeling of small molecule
14 compounds.

15 6.2. *Drug affinity responsive target stability (DARTS)*

16 Drug affinity responsive target stability (DARTS) analysis technique was
17 proposed by Brett et al. (2009). The basic principle is that the binding of natural small
18 molecule compounds to target proteins can prevent proteases from digesting the target
19 proteins. The antienzymatic stability changes of the target proteins before or after the
20 binding is detected by electrophoresis, and then the target proteins directly bound to
21 small molecule compounds can be analyzed by bio-mass spectrometry. The advantages
22 of this technique are easy operation without drug modification, independence of the
23 drugs' action mechanism, and the technique is especially suitable for validation of
24 protein targets with poor affinity but high abundance. For example, this technique was
25 used to discover the binding target proteins eIF4A for resveratrol (Brett et al., 2009).
26 However, these results may be disturbed by non-direct target proteins, especially in
27 living cells. DARTS can also be used to identify the binding target proteins of small
28 molecule compounds when combined with western-blot analysis (Park Y et al., 2016).
29 Nitazoxanide, an anti-parasite drug, was proved to effectively inhibit the Wnt signaling
30 pathway independent of adenomatous polyposis coli by targeting peptidyl arginine
31 deiminase 2 through DARTS and western-blot analyses, suggesting its potential in the
32 treatment of Wnt-pathway mutations in cancer patients (Qu et al., 2018). On the other

1 hand, the binding targets of plant extracts could also be identified by DARTS. With the
2 combined DARTS and bio-mass spectrum analysis, the grape seed extract was found
3 to down-regulate the whole proteins involved in translation process through the
4 endoplasmic reticulum (ER) stress response protein of human colon cancer cells,
5 resulting in the modification of oxidized protein, especially the amino acid residues of
6 the protein target (Derry et al., 2014).

7 6.3. *Microscale thermophoresis*

8 Microscale Thermophoresis (MST) refers to the directional motion of molecules
9 in the microscopic temperature gradient field. The change of biomolecular structure or
10 conformation will cause the differences of its hydration layer, molecular weight and
11 surface charges, which will influence the molecular distribution in the temperature
12 gradient field. This kind of changes can be used to analyze molecular interactions and
13 various stoichiometric parameters. MST can directly measure molecular interactions in
14 similarly natural environments (serum and cell lysate) and in many biological solutions.
15 MST can not only analyze the interaction between proteins, but also measure the
16 combination of small molecular compounds and proteins. The affinity of small
17 molecule inhibitor quercetin with kinase PKA was measured in buffer solution and
18 human serum respectively. The affinity in serum was reduced by 400 times, indicating
19 that the biological matrix can affect the combination of drugs with proteins, thereby
20 interfering with the drug function (Wienken et al., 2011). Hexokinase 2 (HK2) is the
21 rate-limiting enzyme in the first step of the glycolytic pathway, and is highly expressed
22 in cancer cells and plays a key role in oncogenesis and metastasis. Bao et al. has verified
23 that the target of a compound isolated from *Ganoderma lucidum* is HK2 protein by
24 MST analysis (Bao et al., 2018). This compound significantly inhibits the activity of
25 HK2, suggesting that it may be a potential drug for cancer therapy. However, MST
26 analysis can only provide the indirect evidence of binding between small molecules and
27 proteins through the affinity value, hence it still needs to be confirmed by other methods.

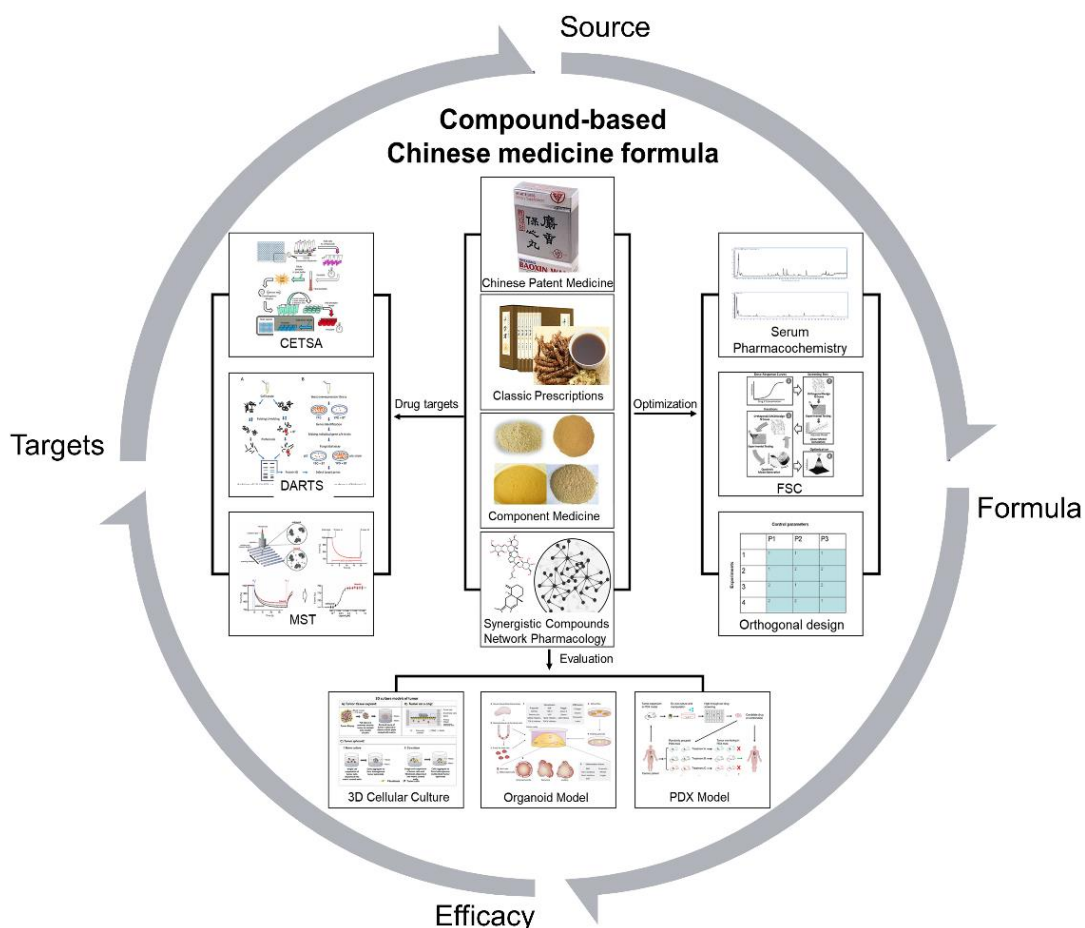
28 7. Discussion

29 Unclear modern scientific connotation of TCM is a major scientific issue limiting
30 the breakthrough of TCM modernization and internationalization. The compatibility
31 application of CMF is an important feature for TCM (Fan et al., 2014), but it is difficult

1 to clearly elucidate the compatibility mechanism at the levels of decoction pieces and
2 components.

3 Hence, we proposed that compound-based Chinese medicine formula (CCMF)
4 (Figure 1), which can be obtained from optimized screening of compounds from
5 decoction pieces, components-based Chinese medicine or activity compounds with
6 synergistic effects, can become the future direction of TCM. The complex and unclear
7 composition of TCM is difficult to control its safety, effectiveness and quality, which
8 limits its further clinical application. For example, TCM injections probably have
9 shown good clinical therapeutic effects, such as the antiviral effect and improvement
10 of myocardial ischemia. However, due to the complex constitution of unknown and
11 ineffective materials which has been injected directly into circulation system, TCM
12 injections have caused series of adverse drug reactions and concerns. According to the
13 2017 Annual Report for National Adverse Drug Reaction Monitoring by CFDA, TCM
14 injections alone accounts for 84.1% of reported TCM averse dug reactions which leads
15 to the restriction and banning of some effective TCM injections. The CCMF research
16 based on TCM injections can help to solve this safety issues.

17



1
2
3
4
5
6
7
8
9
10
11

Figure 1. Key study contents involved in the research of compound-based Chinese medicine formula. In this review, Chinese patent medicine, classic prescriptions, component medicine and synergistic compounds are introduced as the common sources of compound-based Chinese medicine formula (CCMF). Serum pharmacochemistry, feedback system control (FSC) (Liu et al., 2014) and orthogonal design are the recommended optimization method for CCMF. The cellular thermal shift assay (CETSA) (Matte et al., 2016), drug affinity responsive target stability (DARTS) (Luo and Gao, 2018) and microscale thermophoresis (MST) (Cui et al., 2017) has been introduced as the screen method for drug targets. The three-dimensional (3D) cellular culture (Xu et al., 2018), organoid model (Daniel et al., 2013) and PDX model (Park J et al., 2016) has been summarized as the promising method for the efficacy evaluation.

CCMF not only has the advantages of clear chemical composition and controllable quality of modern medicines, but also has the characteristics of synergistic effect of TCM. Furthermore, the compatibility mechanism of its Monarch-Minister-Assistant-Guide (Jun-Chen-Zuo-Shi in Chinese) can be investigated on the basis of the therapeutic targets to elucidate the scientific connotation. For example, the Realgar-Indigo naturalis formula (RIF) has been proven clinically to show good therapeutic effects on acute promyelocytic leukemia in combination with all-trans retinoic acid

1 (Zhu et al., 2013; 2018). To further investigate molecular mechanism, a systematic
2 study of RIF at compounds-level was carried out by Wang et al. (2008). Arsenic sulfide,
3 indirubin and tanshinone IIA have been identified as the main active ingredients of the
4 formula. Arsenic sulfide alone can prolong the survival time of the acute promyelocytic
5 leukemia mice, but the combination with indirubin and tanshinone IIA shows more
6 significant therapeutic effect through the enhanced degradation of PML-RAR α
7 oncoprotein caused by arsenic sulfide which serves as “monarch drug”, increased
8 expression of genes related to the leukocyte differentiation and maturation which
9 caused by tanshinone IIA as “minister drug”, and decreased expression of protein
10 promoted cell cycle by indirubin as “assistant drug”. As the “guiding drugs”, tanshinone
11 IIA and indirubin can also increase the expression aquaglyceroporin 9 which is
12 responsible for arsenic sulfide transportation and cell uptake. Wang et al. (2008) has
13 clearly elucidated the synergistic molecular mechanisms of RIF, and provided an
14 excellent example for CCMF research which should be the future direction for TCM
15 modernization.

16 There are three core contents in the compatibility study of CCMF, including the
17 screening model, compatibility optimization, and targets validation. Although the
18 complex disease environments can be better simulated in animals, they are not suitable
19 for compatibility optimization of formula because of the limited throughput. The *in*
20 *vitro* model of single type of cells can usually neither conform to the multifactor-caused
21 disease progression, nor can it reflect the characteristics of multi-component and multi-
22 target synergism of CMF. The multicellular co-culture and organoid *in vitro* could be
23 the ideal models for formula screening, which can not only simulate multi-factor
24 disease environments, but also have characteristics of high screening throughput. The
25 FSC introduced in this review also can be combined with multicellular co-culture for
26 screening of compatibility formula of CCMF.

27 **8. Conclusions**

28 Compatibility is the advantage of Chinese medicine formula in the treatment of
29 diseases, but its scientific connotation has not been elucidated owing to unclear active
30 components and binding target. Although TCMF has the advantages of easier
31 obtainment, simpler processing and lower cost, it is difficult to control its quality, safety
32 and effectiveness due to the presence of numerous ineffective and unknown

1 components. HCMF still contains partially inactive and unknown substance, which is
2 responsible for the indeterminate binding target. The quality, safety and effectiveness
3 of CCMF can be controlled by the use of well defined compounds. Moreover, its target
4 and mechanism of action can also be clarified further. Although there are many
5 approaches to investigate the action targets, including CETSA, DARTS, and MST, it
6 still lacks the high throughput screening methods for binding targets of CCMF. The
7 combination of mass spectrometry with CETSA or DARTS has improved the
8 throughput in a certain extent, but there still exist some defects such as isotope labeling,
9 complicated operation, high technical requirement and cost. Multicellular culture and
10 organoid are better *vitro* models for investigation of CCMF, but are currently limited
11 in their use. There is an urgent need to establish screening models of compatibility and
12 screening targets for CCMF.

13 **Acknowledgments**

14 This work was supported by funds from the National Natural Science Foundation
15 of China (No. 81773941, 81903654 and 81703755), National Key Subject of
16 Drug Innovation (2019ZX09201005-007), National key R & D program for key
17 research project of modernization of traditional Chinese medicine (2019YFC1711602),
18 Program for Professor of Special Appointment (Young Eastern Scholar) at Shanghai
19 Institutions of Higher Learning, Shanghai “Chenguang Program” of Education
20 Commission of Shanghai Municipality (No.18CG46), and “Yangfan Program”
21 (No.19YF1449400) of Science and Technology Commission of Shanghai Municipality.

22 **Conflicts of interest**

23 The authors have declared that there is no conflict of interests.

24 **Author contributions**

25 Xin Luan wrote and revised the manuscript. Li-Jun Zhang collected the literature
26 and wrote part of the manuscript. Xiao-Qin Li collected the literature. Khalid Rahman
27 revised the manuscript. Hong Zhang, Hong-Zhuan Chen and Wei-Dong Zhang
28 presented the research ideas and revised the manuscript.

1 **References**

- 2 An, L.P., Dou, Z.H., Hou, J.Y., 2013. Research progress in serum pharmacology and serum
3 pharmacochemistry of traditional Chinese medicine. *Cen. South Pharm.* 7, 521-524.
- 4 Bao, F., Yang, K., Wu, C., Gao, S., Wang, P., Chen, L., Li, H., 2018. New natural inhibitors of
5 hexokinase 2 (HK2): Steroids from *Ganoderma sinense*. *Fitoterapia* 125, 123-129.
- 6 Bhattacharjee, S., Coppens, I., Mbengue, A., Suresh, N., Ghorbal, M., Slouka, Z., Safeukui, I., Tang,
7 H.Y., Speicher, D.W., Stahelin, R.V., 2018. Remodeling of the malaria parasite and host human red
8 cell by vesicle amplification that induces artemisinin resistance. *Blood* 131, 1234-1247.
- 9 Brett, L., Rui, H., Nao, J., Chin, R.M., Mariam, A., Sarah, W., Jianing, W., Wu, R.P., Fernando, G., Loo,
10 J.A., 2009. Target identification using drug affinity responsive target stability (DARTS). *P. Natl.*
11 *Acad. Sci. USA* 106, 21984-21989.
- 12 Cao, L., Deng, C.Q., 2016. Effects of glycosides components and combinations of Buyang Huanwu
13 decoction on vascular smooth muscle cells proliferation and related signaling pathway. *J. Chin.*
14 *Mater. Med.* 41, 1889-1897.
- 15 Chang, C.W., Chen, C.Y., Yen, C.C., Wu, Y.T., Hsu, M.C., 2018. Repressed exercise-induced hepcidin
16 levels after danggui buxue tang supplementation in male recreational runners. *Nutrients* 10. pii:
17 E1318. doi: 10.3390/nu10091318.
- 18 Chen, C.Y., 2011. TCM Database@Taiwan: the world's largest traditional Chinese medicine database
19 for drug screening in silico. *PloS one* 6, e15939.
- 20 Chen XX, Liu Y, Huang Y, Zhao LL, Chen L, SM, W., 2017. Virtual screening of anti-angiogenesis
21 flavonoids from *Sophora flavescens*. *Chin. J. Chin. Mat. Med.* 42, 1140-1144.
- 22 Chen, Z., Pan, M.J., Xing, X.F., Zhang, Y.J., 2016. Research progress on serum pharmacochemistry
23 studies in pharmacodynamics material basis of Chinese materia medica and Chinese herbal formula.
24 *Drug Evaluation Res.* 39, 143-147.
- 25 China Pharmacopoeia Committee, 2015. *Pharmacopoeia of the people's Republic of China, Volume I.*
26 Chinese Medical Science and Technology Press, Beijing, 76, 290, 575, 1738.
- 27 Choi, S.H., Kim, Y.H., Hebisch, M., Sliwinski, C., Lee, S., D'Avanzo, C., Chen, H., Hooli, B., Asselin,
28 C., Muffat, J., 2014. A three-dimensional human neural cell culture model of Alzheimer's disease.
29 *Nature* 515, 274-278.
- 30 Choi, S.H., Kim, Y.H., Quinti, L., Tanzi, R.E., Kim, D.Y., 2016. 3D culture models of Alzheimer's
31 disease: a road map to a "cure-in-a-dish". *Mol. Neurodegener.* 11, 75. doi: 10.1186/s13024-016-
32 0139-7
- 33 Cui, X., Hartanto, Y., Zhang, H., 2017. Advances in multicellular spheroids formation. *J. R. Soc.*
34 *Interface* 14, 20160877.
- 35 Dai, L., Zhao, T., Bisteau, X., Sun, W., Prabhu, N., Lim, Y.T., Sobota, R.M., Kaldis, P., Nordlund, P.,
36 2018. Modulation of Protein-Interaction States through the Cell Cycle. *Cell* 173, 1481-1494 e1413.
- 37 Daniel, M.M., Rozbeh, J., Marina, I., Takahiro, S., E Andreas, L., Chen, D., Lekshmy, S., Yihai, C., P?R,
38 N., 2013. Monitoring drug target engagement in cells and tissues using the cellular thermal shift
39 assay. *Science* 341, 84-87.

1 Derry, M.M., Somasagara, R.R., Raina, K., Kumar, S., Gomez, J., Patel, M., Agarwal, R., Agarwal, C.,
2 2014. Target Identification of Grape Seed Extract in Colorectal Cancer Using Drug Affinity
3 Responsive Target Stability (DARTS) Technique: Role of Endoplasmic Reticulum Stress Response
4 Proteins. *Curr. Cancer Drug Targets* 14, 323-336.

5 Ding, X., Njus, Z., Kong, T., Su, W., Ho, C.M., Pandey, S., 2017. Effective drug combination for
6 *Caenorhabditis elegans* nematodes discovered by output-driven feedback system control technique.
7 *Sci. Adv.* 3, eaao1254.

8 Dye, B.R., Hill, D.R., Ferguson, M.A., Tsai, Y.H., Nagy, M.S., Dyal, R., Wells, J.M., Mayhew, C.N.,
9 Nattiv, R., Klein, O.D., 2015. In vitro generation of human pluripotent stem cell derived lung
10 organoids. *eLife* 4(4). doi: 10.7554/eLife.05098

11 Fan, J.X., Wang, S., Meng, X.S., Bao, Y.R., Li, T.J., 2017. Study of cancer cell apoptosis induced by
12 *Schizonepeta tenuifolia* with microfluidic chip technology. *Yao Xue Xue Bao* 52, 126-131.

13 Fan, T.P., Briggs, J., Liu, L., Lu, A.P., Greef, J.v.d., Xu, A.L., 2014. The art and science of traditional
14 medicine Part 1: TCM today - A case for integration. Integrating traditional medicine into modern
15 health care. *Science* 346, S4.

16 Fatehullah, A., Tan, S.H., Barker, N., 2016. Organoids as an in vitro model of human development and
17 disease. *Nat. Cell Biol.* 18, 246-254.

18 Fong, E.L., Wan, X., Yang, J., Morgado, M., Mikos, A.G., Harrington, D.A., Navone, N.M., Farach-
19 Carson, M.C., 2016. A 3D in vitro model of patient-derived prostate cancer xenograft for controlled
20 interrogation of in vivo tumor-stromal interactions. *Biomaterials* 77, 164-172.

21 Froehlich, K., Haeger, J.D., Heger, J., Pastuschek, J., Photini, S.M., Yan, Y., Lupp, A., Pfarrer, C.,
22 Mrowka, R., Schleußner, E., 2016. Generation of Multicellular Breast Cancer Tumor Spheroids:
23 Comparison of Different Protocols. *J. Mammary Gland. Biol. Neoplasia.* 21, 89-98.

24 Galateanu, B., Hudita, A., Negrei, C., Ion, R.M., Costache, M., Stan, M., Nikitovic, D., Hayes, A.W.,
25 Spandidos, D.A., Tsatsakis, A.M., 2016. Impact of multicellular tumor spheroids as an in vivo-like
26 tumor model on anticancer drug response. *Int. J. Oncol.* 48, 2295-2302.

27 Gao, D., Chen, Y., 2015. Organoid development in cancer genome discovery. *Curr. Opin. Genet. Dev.*
28 30, 42-48.

29 Guo, L.A., Wang, S.P., Xiang, L., Jiang, P., Zhang, W.D., Liu, R.H., 2012. Identification of volatility
30 components of Shexiang Baixin pill in rat plasma by GC-MS. *J. Pharma. Practice* 30, 207-210.

31 He, J.Y., Rao, M., Lin, Tang, M., Dong, Z., 2014. Effect of paeoniflorin on superoxide dismutase and
32 nuclear factor E2-related factor 2 expression in brain tissues of rats with middle cerebral artery
33 occlusion and its neuroprotection. *J. Chongqing Med. Univ.* 38, 178-182.

34 Huch, M., Gehart, H., Vanboxtel, R., Hamer, K., Blokzijl, F., Verstegen, M.A., Ellis, E., Vanwenum,
35 M., Fuchs, S., Deligt, J., 2015. Long-Term Culture of Genome-Stable Bipotent Stem Cells from
36 Adult Human Liver. *Cell* 160, 299-312.

37 Jafari, R., Almqvist, H., Axelsson, H., Ignatushchenko, M., Lundback, T., Nordlund, P., Martinez
38 Molina, D., 2014. The cellular thermal shift assay for evaluating drug target interactions in cells.
39 *Nat. Protoc.* 9, 2100-2122.

- 1 Jiang, F., Li, Y., Si, L., Zhang, Z., Li, Z., 2019. Interaction of EZH2 and P65 is involved in the arsenic
2 trioxide-induced anti-angiogenesis in human triple-negative breast cancer cells. *Cell Biol. Toxicol.*
3 1-11.
- 4 Jiang, P., Liu, R.H., Dou, S., Liu, L., Zhang, W.D., Chen, Z.L., Xu, R.L., Ding, J.M., 2010. Analysis of
5 the constituents in rat plasma after oral administration of Shexiang Baoxin pill by HPLC-ESI-
6 MS/MS. *Biomed. Chromatog.* 23, 1333-1343.
- 7 Juergen, F., Claudia, S., Reinhard, E., Kunz-Schughart, L.A., 2009. Spheroid-based drug screen:
8 considerations and practical approach. *Nat. Protoc.* 4, 309-324.
- 9 Lai, Y., Wei, X., Lin, S., Qin, L., Cheng, L., Li, P., 2017. Current status and perspectives of patient-
10 derived xenograft models in cancer research. *J. Hematol. Oncol.* 10, 106.
- 11 Lancaster, M.A., Knoblich, J.A., 2014. Organogenesis in a dish: modeling development and disease
12 using organoid technologies. *Science* 345, 1247125.
- 13 Li, C.P., Xuan, C.P., Yuan, J.L., Shi, J.J., Deng, L., Wang, W.Y., Li, N.N., YC, L., 2019. Protective
14 effects of the active components of *Leonurus japonicas* Houtt. in myocardial ischemia-reperfusion
15 injury. *Chin. J. Hosp. Pharm.* 39, 708-712.
- 16 Li, S., Zhang, B., 2013. Traditional Chinese medicine network pharmacology: theory, methodology and
17 application. *Chin. J. Nat. Med.* 11, 110-120.
- 18 Liu, P., Li, W., Li, Z.H., Qian, D.W., Guo, J.M., Shang, E.X., Su, S.L., Tang, Y.P., Duan, J.A., 2014.
19 Comparisons of pharmacokinetic and tissue distribution profile of four major bioactive components
20 after oral administration of Xiang-Fu-Si-Wu Decoction effective fraction in normal and
21 dysmenorrheal symptom rats. *J. Ethnopharmacol.* 154, 696-703.
- 22 Liu, R., Runyon, R.S., Wang, Y., Oliver, S.G., Fan, T., Zhang, W., 2015. Deciphering ancient
23 combinatorial formulas: the shexiang baoxin pill. *Science* 347(6219 suppl): s40-s42.
- 24 Lu, L., Sun, X., Chen, C., Qin, Y., Guo, X., 2018. Shexiang Baoxin Pill, Derived From the Traditional
25 Chinese Medicine, Provides Protective Roles Against Cardiovascular Diseases. *Front. Pharmacol.*
26 9, 1161. doi: 10.3389/fphar.2018.01161.
- 27 Luo, J., Gao, J.Q., 2018. Construction of 3D Multicellular tumor spheroid model and its Application in
28 drug screening and preparation evaluation. *Chin. J. Mod. Appl. Pharm.* 35, 609-614.
- 29 Ma, T., Gu, Z., Shen, D., Xu, A., Ge, B., 2019. Research progress in herb-pair compatibility mechanism
30 in recent decade. *Chin. J. Inf. Tradit. Chin. Med.* 26, 132-136.
- 31 Mangal, M., Sagar, P., Singh, H., Raghava, G.P., Agarwal, S.M., 2013. NPACT: Naturally Occurring
32 Plant-based Anti-cancer Compound-Activity-Target database. *Nucleic Acids Res.* 41(Database
33 issue), D1124-1129.
- 34 Marciano, R., Prasad, M., Ievy, T., Tzadok, S., Leprivier, G., Elkabets, M., Rotblat, B., 2019. High-
35 throughput screening identified compounds sensitizing tumor cells to glucose starvation in culture
36 and vegf inhibitors in vivo. *Cancers* 11. pii: E156. doi: 10.3390/cancers11020156.
- 37 Martinez, N.J., Asawa, R.R., Cyr, M.G., Zakharov, A., Urban, D.J., Roth, J.S., Wallgren, E., Klumpp-
38 Thomas, C., Coussens, N.P., Rai, G., 2018. A widely-applicable high-throughput cellular thermal
39 shift assay (CETSA) using split Nano Luciferase. *Sci. Rep.* 8, 9472.

1 Matte, I., Legault, C.M., Garde-Granger, P., Laplante, C., Bessette, P., Rancourt, C., Piche, A., 2016.
2 Mesothelial cells interact with tumor cells for the formation of ovarian cancer multicellular
3 spheroids in peritoneal effusions. *Clin. Exp. Metastasis* 33, 839-852.

4 Nath, S., Devi, G.R., 2016. Three-dimensional culture systems in cancer research: Focus on tumor
5 spheroid model. *Pharmacol. Ther.* 163, 94-108.

6 Nowaksliwinska, P., Weiss, A., Ding, X., Dyson, P.J., Bergh, H.V.D., Griffioen, A.W., Ho, C.M., 2016.
7 Optimization of drug combinations using Feedback System Control. *Nat. Protoc.* 11, 302-315.

8 Park, J.I., Lee, J., Kwon, J.L., Park, H.B., Lee, S.Y., Kim, J.Y., Sung, J., Jin, M.K., Song, K.S., Kim,
9 K.H., 2016. Scaffold-free coculture spheroids of human colonic adenocarcinoma cells and normal
10 colonic fibroblasts promote tumorigenicity in nude mice. *Transl. Oncol.* 9, 79-88.

11 Park, Y.D., Sun, W., Salas, A., Antia, A., Carvajal, C., Wang, A., Xu, X., Meng, Z., Zhou, M., Tawa,
12 G.J., Dehdashti, J., Zheng, W., Henderson, C.M., Zelazny, A.M., Williamson, P.R., 2016.
13 Identification of multiple cryptococcal fungicidal drug targets by combined gene dosing and drug
14 affinity responsive target stability screening. *MBio* 7, pii: e01073-16. doi: 10.1128/mBio.01073-16.

15 Peng, J., Dou, S., Lei, L., Zhang, W., Chen, Z., Xu, R., Ding, J., Liu, R., 2009. Identification of multiple
16 constituents in the TCM-formula Shexiang Baoxin Pill by LC coupled with DAD-ESI-MS-MS.
17 *Chromatographia* 70, 133-142.

18 Qu, Y., Olsen, J.R., Yuan, X., Cheng, P.F., Levesque, M.P., Brokstad, K.A., Hoffman, P.S., Oyan, A.M.,
19 Zhang, W., Kalland, K.H., 2018. Small molecule promotes β -catenin citrullination and inhibits Wnt
20 signaling in cancer. *Nat. Chem. Biol.* 14, 94-101.

21 Ru, J., Li, P., Wang, J., Zhou, W., Li, B., Huang, C., Li, P., Guo, Z., Tao, W., Yang, Y., Xu, X., Li, Y.,
22 Wang, Y., Yang, L., 2014. TCMSP: a database of systems pharmacology for drug discovery from
23 herbal medicines. *J. Cheminform.* 6,13. doi: 10.1186/1758-2946-6-13.

24 Saebyeol, J., Kelley, K.W., Johnson, R.W., 2008. Luteolin reduces IL-6 production in microglia by
25 inhibiting JNK phosphorylation and activation of AP-1. *P. Natl. Acad. Sci. USA* 105, 7534-7539.

26 Santos, R., Ursu, O., Gaulton, A., Bento, A.P., Donadi, R.S., Bologa, C.G., Karlsson, A., Allazikani, B.,
27 Hersey, A., Oprea, T.I., 2017. A comprehensive map of molecular drug targets. *Nat. Rev. Drug*
28 *Disco.* 16, 19-34.

29 Shang, H.C., Zhang, B., Wang, Y.Y., Gao, X.M., Zhao, Y.J., 2003. A method for proportion screening
30 of TCM small prescriptions. *Chin. J. Exper. Trad. Med. Form.* 9, 1-3

31 Shi, X., Tang, Y., Zhu, H., Li, W., Li, Z., Li, W., Duan, J.A., 2014. Comparative tissue distribution
32 profiles of five major bio-active components in normal and blood deficiency rats after oral
33 administration of Danggui Buxue Decoction by UPLC-TQ/MS. *J. Ethnopharmacol.* 153, 169-177.

34 Silva, A., Lee, B.Y., Clemens, D.L., Kee, T., Ding, X., Ho, C.M., Horwitz, M.A., 2016. Output-driven
35 feedback system control platform optimizes combinatorial therapy of tuberculosis using a
36 macrophage cell culture model. *P. Natl. Acad. Sci. USA* 113, E2172-2179.

37 Tao, W., Li, B., Gao, S., Bai, Y., Shar, P.A., Zhang, W., Guo, Z., Sun, K., Fu, Y., Huang, C., Zheng, C.,
38 Mu, J., Pei, T., Wang, Y., Li, Y., Wang, Y., 2015. CancerHSP: anticancer herbs database of systems
39 pharmacology. *Sci. Rep.* 5, 11481.

- 1 Tian H, Wang Q, Mei YF, Zhang DS, GP, X., 2014. Influence of radix salviae multiorrhizae hydrosoluble
2 combinations on the memory dysfunction induced by ischemia-reperfusion in mice. *Acta*
3 *Neuropharmacol.* 4, 5-16.
- 4 Toshiro, S., Vries, R.G., Snippert, H.J., Marc, V.D.W., Nick, B., Stange, D.E., Es, J.H., Van, Arie, A.,
5 Pekka, K., Peters, P.J., 2009. Single Lgr5 stem cells build crypt-villus structures in vitro without a
6 mesenchymal niche. *Nature* 459, 262-265.
- 7 Tsutsui, H., Valamehr, B., Hindoyan, A., Qiao, R., Ding, X., Guo, S., Witte, O.N., Liu, X., Ho, C.M.,
8 Wu, H., 2011. An optimized small molecule inhibitor cocktail supports long-term maintenance of
9 human embryonic stem cells. *Nat. Commun.* 2(1), 167.
- 10 Wang, L., Zhou, G.B., Liu, P., Song, J.H., Liang, Y., Yan, X.J., Xu, F., Wang, B.S., Mao, J.H., Shen,
11 Z.X., Chen, S.J., Chen, Z., 2008. Dissection of mechanisms of Chinese medicinal formula Realgar-
12 Indigo naturalis as an effective treatment for promyelocytic leukemia. *P. Natl. Acad. Sci. USA* 105,
13 4826-4831.
- 14 Wang, L.Q., Zhang, Y.Y., He, Y., Wan, H.T., Zhou, H.F., Yang, J.H., 2016. Effect of different
15 compatibility proportion of active constituent of fuzi (*radix aconiti carmichaeli*) and gancao
16 (*glycyrrhiza uralensis*) on h9c2 myocardial cell with oxygen-glucose deprivation. *J. Trad. Chin.*
17 *Med.* 57, 1327-1331.
- 18 Wang, R., Shang, H.C., Wang, Y.Y., Zhang, B.L., Gao, X.M., Zhao, Y.J., 2006. Multi-objective
19 optimization research of the ratio of Danshen and Sanchi with ED-NM-MO trigeminy method.
20 *Tianjin J. Trad. Chin. Med.* 23, 242-247.
- 21 Wang, X.J., 2010. The formation and development of traditional chinese medicine serum medicinal
22 chemistry. *Mod. Trad. Chin. Med. Mater. Medica-World Sci. Technol.* 12, 632-633.
- 23 Wang, X., Sun, H., Zhang, A., Wang, P., Han, Y., 2011. Ultra-performance liquid chromatography
24 coupled to mass spectrometry as a sensitive and powerful technology for metabolomic studies. *J.*
25 *Sep. Sci.* 34, 3451-3459.
- 26 Wienken, C.J., Baaske, P., Rothbauer, U., Braun, D., Duhr, S., 2011. Protein-binding assays in biological
27 liquids using microscale thermophoresis. *Nat. Commun.* 1, 100.
- 28 Wong, P.K., Yu, F., Shahangian, A., Cheng, G., Sun, R., Ho, C.M., 2008. Closed-loop control of cellular
29 functions using combinatory drugs guided by a stochastic search algorithm. *P. Natl. Acad. Sci. USA*
30 105, 5105-5110.
- 31 Wu C, Xu PC, Yao WX, Shou D, Zhu Y, NN, W., 2019. Rapid screening of anti-osteoporosis active
32 ingredients from Liuwei Dihuang Decoction by osteoblast membrane chromatography/ultra-high
33 performance liquid chromatography-time of flight mass spectrometry. *Chin. J. Chromatogr.* 37,
34 305-312.
- 35 Wu, Y., Pan, X., Xu, Y., Lu, X., He, S., He, R., Gong, M., 2016. Optimization of combinations of
36 ginsenoside-Rg1, ginsenoside-Rb1, evodiamine and rutaecarpine for effective therapy of mouse
37 migraine. *J. Nat. Med.* 70, 207-216.
- 38 Xiao, L., Wang, Y.Z., Liu, J., Luo, X.T., Ye, Y., Zhu, X.Z., 2005. Effects of paeoniflorin on the cerebral
39 infarction, behavioral and cognitive impairments at the chronic stage of transient middle cerebral
40 artery occlusion in rats. *Life Sci.* 78, 413-420.

- 1 Xu, H., Lyu, X., Yi, M., Zhao, W., Song, Y., Wu, K., 2018. Organoid technology and applications in
2 cancer research. *J. Hematol. Oncol.* 11, 116. doi: 10.1186/s13045-018-0662-9.
- 3 Xu N, Shi HY, Wang SL, Wang L, Zhang CB, Y, S., 2019. Comprehensive Evaluation of Compatibility
4 of Effective Components in Banxia Baizhu Tianma Tang for phlegm dampness hypertension based
5 on orthogonal test combined with multiple pharmacodynamic indexes. *Chin. J. Exper. Trad. Med.*
6 *Formul.* 24, 7-13.
- 7 Xue, R., Fang, Z., Zhang, M., Yi, Z., Wen, C., Shi, T., 2013. TCMID: Traditional Chinese Medicine
8 integrative database for herb molecular mechanism analysis. *Nucleic Acids Res.* 41(Database issue),
9 D1089-1095.
- 10 Yan, G., Hang, Y., Wang, X., 2012. Identification technique for in vivo ingredients of traditional Chinese
11 medicines based on LC-MS analysis. *Chin. J. Chin. Mater. Med.* 37, 1765-1770.
- 12 Yang, Q., Wu, J.B., Zhang, Y.M., 2010. Effect of paeonol on inflammatory factors after focal cerebral
13 ischemia-reperfusion injury in rats. *Chin. J. Biochem. Pharma.* 31, 111-113.
- 14 Yu, H., Zhang, W.L., Ding, X.T., Zheng, K.Y.Z., Ho, C.M., Tsim, K.W.K., Lee, Y.K., 2013. Optimizing
15 combinations of flavonoids deriving from astragali radix in activating the regulatory element of
16 erythropoietin by a feedback system control scheme. *Evid. Compl. Alter. Med.* (2013), 1-10.
- 17 Yu, K., Zhou, Y., 2019. Research progress of 3D cell culture in drug research and development. *Chin.*
18 *Med. Pharm.* 9, 36-39.
- 19 Yue, R., Shan, L., Yang, X., Zhang, W., 2012. Approaches to target profiling of natural products. *Curr.*
20 *Med. Chem.* 19, 3841-3855.
- 21 Yue, S.J., Xin, L.T., Fan, Y.C., Li, S.J., Tang, Y.P., Duan, J.A., Guan, H.S., Wang, C.Y., 2017. Herb
22 pair Danggui-Honghua: mechanisms underlying blood stasis syndrome by system pharmacology
23 approach. *Sci. Rep.* 7, 40318. doi: 10.1038/srep40318.
- 24 Yuliantie, E., Dai, X., Yang, D., Crack, P.J., Wang, M.W., 2018. High-throughput screening for small
25 molecule inhibitors of the type-I interferon signaling pathway. *Acta Pharm. Sinica B* 8, 889-899.
- 26 Zeng, K.W., Peng, F.T., 2018. Recent progress on the methodology for target study of traditional Chinese
27 medicine. *Scientia Sinica Chimica* 48, 1420-1428.
- 28 Zeng, X., Zhang, P., He, W., Qin, C., Chen, S., Tao, L., Wang, Y., Tan, Y., Gao, D., Wang, B., Chen,
29 Z., Chen, W., Jiang, Y.Y., Chen, Y.Z., 2018. NPASS: natural product activity and species source
30 database for natural product research, discovery and tool development. *Nucleic Acids Res.* 46(D1),
31 D1217-D1222.
- 32 Zhang, B.L., Wang, Y.Y., 2005. Basic research on the key scientific problems of prescriptions-the
33 development of modern Chinese medicine by compositional compatibility. *Chin. J. Chin. Mater.*
34 *Med.* 3, 258-261.
- 35 Zhang, D., Pekkanen-Mattila, M., Shahsavani, M., Falk, A., Teixeira, A.I., Herland, A., 2014. A 3D
36 Alzheimer's disease culture model and the induction of P21-activated kinase mediated sensing in
37 iPSC derived neurons. *Biomaterials* 35, 1420-1428.
- 38 Zhang, G.Q., Hao, X.M., Chen, S.Z., Zhou, P.A., Cheng, H.P., Wu, C.H., 2003. Blockade of paeoniflorin
39 on sodium current in mouse hippocampal CA1 neurons. *Acta Pharma. Sinica* 24, 1248-1252.

- 1 Zhang, J.H., Zhu, Y., Fan, X.H., Zhang, B.L., 2015. Efficacy-oriented compatibility for component-
2 based Chinese medicine. *Acta Pharma. Sinica* 36, 654-658.
- 3 Zhang, S., Gao, H.Q., Zhang, X.M., 2008. Peaonol reduces the expression of ICAM-1 and VCAM-1
4 after focal brain ischemia-reperfusion injury in rats. *Chin. J. Biochem. Pharma.* 01, 5-8.
- 5 Zhang, X.W., Yan, X.J., Zhou, Z.R., Yang, F.F., Wu, Z.Y., Sun, H.B., Liang, W.X., Song, A.X.,
6 Lallemand-Breitenbach, V., Jeanne, M., Zhang, Q.Y., Yang, H.Y., Huang, Q.H., Zhou, G.B., Tong,
7 J.H., Zhang, Y., Wu, J.H., Hu, H.Y., de Thé, H., Chen, S.J., Chen, Z., 2010. Arsenic Trioxide
8 Controls the Fate of the PML-RAR Oncoprotein by Directly Binding PML. *Science* 328, 240-243.
- 9 Zhao, J., Yang, J., Tian, s., 2019. A survey of web resources and tools for the study of TCM network
10 pharmacology. *Quantita. Biol.* 7, 17-29.
- 11 Zhou, Y.Q., Xiao, Y.L., 2018. Target Identification of Bioactive Natural Products. *Acta Chimica Sinica*
12 76, 177-189.
- 13 Zhu, H.H., Wu, D.P., Jin, J., Li, J.Y., Ma, J., Wang, J.X., Jiang, H., Chen, S.J., Huang, X.J., 2013. Oral
14 Tetra-Arsenic Tetra-Sulfide Formula Versus Intravenous Arsenic Trioxide As First-Line Treatment
15 of Acute Promyelocytic Leukemia: A multicenter randomized controlled trial. *J. Clin. Oncol.* 31,
16 4215-4221.
- 17 Zhu, H.H., Wu, D.P., Du, X., Zhang, X., Liu, L., Ma, J., Shao, Z.H., Ren, H.Y., Hu, J.D., Xu, K.L.,
18 Wang, J.W., Song, Y.P., Fang, M.Y., Li, J., Yan, X.Y., Huang, X.J., 2018. Oral arsenic plus retinoic
19 acid versus intravenous arsenic plus retinoic acid for non-high-risk acute promyelocytic leukaemia:
20 a non-inferiority, randomised phase 3 trial. *Lancet Oncol.* 19, 871-879.
- 21 Zhu JH, Huang XP, CQ, D., 2019. Main active components combination of *Astragalus membranaceus*
22 and *Angelica sinensis* promotes proliferation of aging hematopoietic stem cells. *Chin. Trad. Herb.*
23 *Drugs* 50, 111-119.
- 24 Zhu, L.H., Wei, B., Lu, D., Zhang, C.J., Shu, X.M., Lu, D.X., 2014. Luteolin inhibits SH-SY5Y cell
25 apoptosis through suppression of the nuclear transcription factor- κ B, mitogen-activated protein
26 kinase and protein kinase B pathways in lipopolysaccharide-stimulated cocultured BV2 cells. *Exp.*
27 *Ther. Med.* 7, 1065-1070.