

The Art and Science of Traditional Medicine

Part 2: Multidisciplinary Approaches for Studying Traditional Medicine



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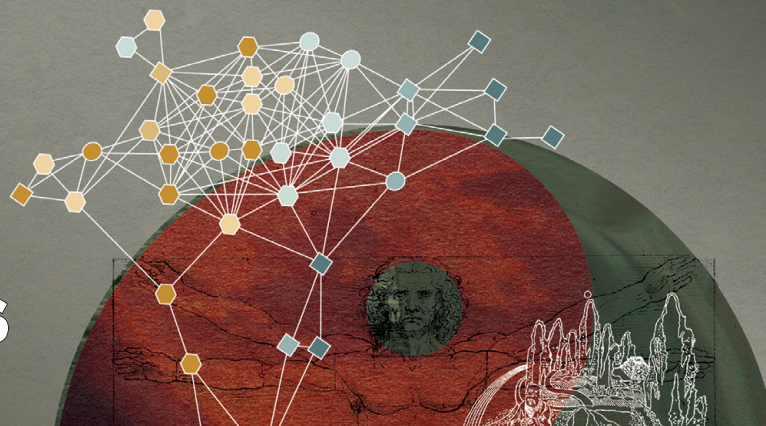


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Contents



In this second of three special supplements, herbal genomics as a novel approach for revolutionizing research on, and ultimately use of, traditional herbal medicines and other materia medica, as well as advances in their quality control and standardization, is highlighted. A prominent focus is the U.S. Food and Drug Administration's practical framework for developing botanicals (including traditional medicines) into new drugs based on the same standards as small molecule drugs. The application of mechanistic studies to drug discovery and development from traditional therapies is discussed, with an emphasis on preclinical toxicology assessments, pharmacovigilance, comparative effectiveness research, and the practice of "P4" medicine, particularly in the context of influenza, ischemic heart disease, stroke, and cancer.

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Herbal genomics: Examining the biology of traditional medicines

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Traditional herbal medicines, such as plant- and fungi-based remedies, have been used for more than 5,000 years. However, the genetic background, the agricultural traits, and the medicinal quality of most traditional herbs are poorly understood. With rapid advances in high throughput sequencing technologies and greatly reduced costs, a new discipline called “herbal genomics” has emerged. Researchers are now systematically categorizing medicinal herbs by sequencing, assembling, and annotating their genomes, and by analyzing their genes’ functions. The genomes of some commonly used herbs have already been sequenced, such as Lingzhi (*Ganoderma lucidum* or “mushroom of immortality”). This species has provided an effective model system that has facilitated the study of the biosynthetic pathways of secondary metabolites in medicinal fungal species (1). Genomic information, together with transcriptomic, proteomic, and metabolomic data, can therefore be used to predict secondary metabolite biosynthetic pathways and their regulation, triggering a revolution in discovery-based research aiming to understand the genetics and biology of herbs.

Herbal genomics provides an effective platform to support the chemical and biological analyses of complex herbal products that may contain more than one active component. Therefore, it is now being applied to many areas of herb-related biological research to help understand the quality of traditional medicines and for molecular herb identification through the establishment of an herbal gene bank. Moreover, functional herbal genomics can contribute to model herb research platforms, geoherb research, genomics-assisted herb breeding, and herbal synthetic biology, all of which are important for securing the sourcing of the medicinal plants and their active compounds in the future.

Creating model herbs

With the recent developments in biotechnology and genomics, several species including *Ganoderma lucidum*, *Salvia miltiorrhiza*, and *Catharanthus roseus* have emerged as valuable models for studying the genetics and metabolic activities of herbs. These species have been shown to synthesize active pharmaceutical components, including triterpenes, diterpene quinone, and indole alkaloids. Although the core biosynthetic pathways of secondary metabolites in herbs are conserved, downstream pathways have evolved and became highly diverse (2). Therefore genes from different cultivars of medicinal herbs or evolutionarily

related species can be evaluated using these herbal models to understand the mechanisms underlying natural variation. These model systems can also be used to identify novel biosynthetic pathways for convergent secondary metabolites in closely related herb species. Recent advances in genome editing have provided feasible approaches by introducing or altering specific alleles; hence, genetic control over metabolites can be investigated (3). Although the elucidation of biosynthetic pathways is one of their most appealing features, model herbs can also provide information on perennial habits, development patterns, cultivation requirements, and resistance to environmental or biological insult (Figure 1).

Biological basis of geoherbism

The Chinese concept of geoherbism encompasses the use of “authentic” or “superior” herbs, which are produced in a specific region or environment, to generate remedial products that have a high efficacy. Through the application of new ‘omics technologies to geoherbism, information can be obtained concerning the optimal growth conditions of medicinal products and specific herb genotypes, allowing both genetic and environmental factors to be taken into account when considering herbal growth. ‘Omics provide new and powerful tools to elucidate the molecular basis underlying geoherbism and to select elite varieties. Creating herb pangenomes—the entire genetic code for all of the strains within a given species—can provide insights into identifying the “core genomes” and “dispensable genomes” of the species as well as the individual genetic variations that exist in different regions or ecological circumstances. Environmental stressors can lead to epigenetic modifications; techniques such as DNA methylome analysis, chromatin immunoprecipitation (ChIP)-sequencing, and small RNA sequencing are useful for investigating the influence of epigenetic factors. In addition, soil microbes can affect an herb’s environment, and metagenomic analysis of soil microbial populations can point to important interactions between microbes and herbs that may alter growth conditions (4).

Targeted herb breeding

Molecular breeding requires the availability of polymorphic markers and/or information about trait-associated genes. Since they are considered minor crops, herbs have been limited in genomics-assisted improvements due to the high cost; however, next generation sequencing and its increasing affordability have dramatically accelerated marker selection breeding programs through the sequencing of wild varieties and different cultivars of herbs that represent a valuable reservoir of genetic diversity. Herbal ‘omics research has

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accelerated the identification of many functional genes in model species and has also allowed the development of functional markers specific to the production of desired compounds, information that can be used for targeted molecular breeding (5).

Researching herbal synthetic biology

Although herbs are sources of novel and known therapeutic compounds, problems in sourcing are common. Biotechnology and genetic engineering offer approaches to alternative production methods. Metabolic engineering of medicinal plants has been studied extensively, resulting in, for example, *Atropa belladonna* plants producing scopolamine instead of atropine. However, it is clear that in order to improve the overall production of a plant compound, the overexpression of the primary coding genes or even regulatory genes in the pathway are not sufficient, since compartmentalization, transport, storage, and co-factor availability may be important rate limiting factors. A better understanding of pathways involved would build a foundation for a more comprehensive approach to metabolic engineering. This is the goal of herbal synthetic biology, which involves the alteration or de novo synthesis of genomes, with the potential to address resource and purity issues. Furthermore, natural products for drug discovery can be structurally diversified by combining and introducing plant metabolic pathways into other organisms, such as bacteria or yeast (6). The conventional practice in herbal synthetic biology is to introduce the heterologous biosynthetic pathway into an expression system able to produce the products. However, a different approach for the large-scale production of a pure compound could be the engineering of an entirely novel synthetic genome, as described for *Mycoplasma* (7).

Defining a molecular identity

DNA barcoding is revolutionizing the practice of herbal identification, utilizing the concept of "one sequence, one species." Standardized DNA barcoding identification systems are available, but the process can be tedious. Analysis of a plastid genome as a superbarcode is a promising alternative for closely related species or cultivars that cannot be unambiguously distinguished by traditional DNA barcoding (8, 9). With the increasing availability of DNA barcodes, current market issues with herbal medicines that result from the use of inferior substitutes, adulterants, and counterfeits could be resolved. Overall, a standardized identification system based on DNA barcoding can play an important role in controlling the quality of traditional

medicines through the accurate identification of herbal materials.

Constructing an herbal gene bank

Herbal genetic information is being accumulated with increasing speed, making the need for a common platform for integrated and consolidated access to all 'omics data paramount. Several herb-related databases have already been developed to categorize current resources, including genomic information (<http://herbalgenomics.org>), transcriptomic information (<http://medicinalplantgenomics.msu.edu>), a DNA barcode database (<http://tcmbarcode.cn/en>), and a metabolic pathway database (<http://cathacyc.org>). However, these distributed resources are not subjected to long-term maintenance and require bioinformatics skills to use. A comprehensive and easily accessible database is required that stores molecular and biological data for herbal medicines. The DNA/protein sequences and metabolomes of herbs can be integrated into such a database (10, 11). With improved bioinformatics approaches, genomic and chemical information can be used to identify the biosynthetic pathways of secondary metabolites leading to the design of more efficient and targeted searches for plant- and fungi-based remedies (12).

Despite its success thus far, herbal genomics still faces significant technological and ethical challenges. For instance, there have been only a few well-assembled herbal genomes released to date, partly because of their complexity. High

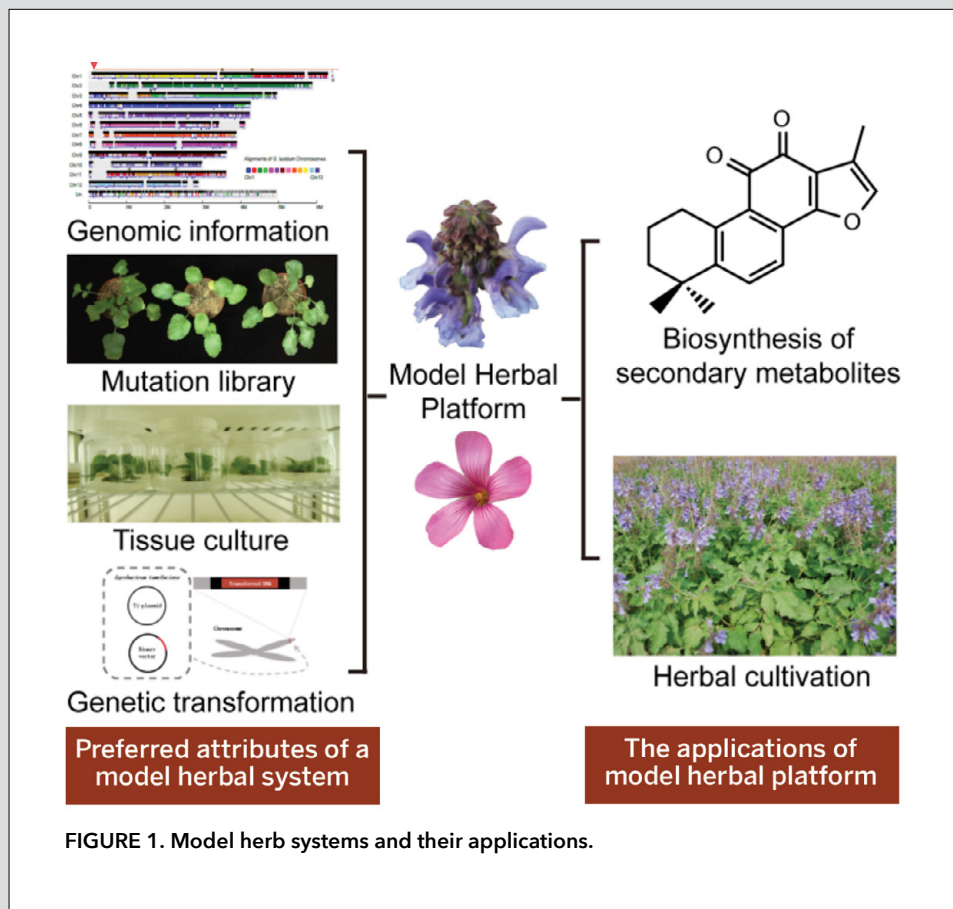


FIGURE 1. Model herb systems and their applications.

heterozygosity, repetition-rich DNA sequences, and polyploidy are factors that impede data assembly from short-read, whole-genome shotgun sequencing. Furthermore, the lack of high throughput methods reduces the efficiency of identifying enzymes and pathways involved in the biosynthesis of secondary metabolites. There have also been ethical and biosecurity concerns regarding synthetic biology expressed by the scientific community and the public. Nevertheless, herbal genomics provides an unprecedented opportunity to revolutionize the use and acceptance of traditional herbal medicines, while contributing to the knowledge base essential for further proteomic and metabolomic studies.

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A holistic approach to the quality control of traditional Chinese medicines

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Plants and traditional medicines (TMs) are used around the world for the prevention and treatment of diseases as well as the sources of numerous prescription and over-the-counter therapeutics (1). In many cases, these TMs have been used for thousands of years and

are still largely harvested from the wild. However, the quality control for the growth and isolation of most TMs is poor or nonexistent. Patients and practitioners alike need to be confident of the quality, safety, efficacy, and consistency (QSEC) of TMs, which requires standardization of all aspects of the plant preparation. This begins with the identification of the correct plant, and includes isolation and characterization of all bioactive constituents. The evidence-based criteria to determine the QSEC of TMs differs considerably across the globe. As a result, the evidentiary standards required for the marketing of TM products can vary greatly from country to country.

As acknowledged by the famous Chicago architect, Louis Sullivan, “form forever follows function.” For patients and practitioners, the expectation for the TMs is that they must be effective (function). Strategies (form) to assure this outcome require a strong evidence base and necessitate overcoming certain barriers to success. In many societies, the acceptance of TMs precedes integration into the health care system, causing them to face philosophical and regulatory barriers due, at least in part, to the absence of an evidence base.

The World Health Organization has recognized some of these issues (1, 2) and, through the Beijing Declaration, has encouraged the integration of evidence-based TMs into national health care systems and promoted regulations and standards that “ensure appropriate, safe, and effective use of traditional medicine” (3).

In order to fully integrate TMs into national health care systems, and provide an evidence-based justification for their use, certain entrenched customs and beliefs need to be addressed. These include the ideas that a traditional medicine used for thousands of years must be safe and effective; that simply using the “correct” plant is adequate; that the biological

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effects will be consistent, irrespective of the geographic origin of the plant, the plant part used, or the method of plant preparation; that older or cultivated plant material is less effective than fresh, wild plants; that complex plant mixtures are necessary for effectiveness, but cannot be standardized; and that the traditional knowledge and the particular medicinal plant will always be available. The application of contemporary information, together with botanical, chemical, biological, and clinical research, provides an evidence-based research agenda for traditional medicine that serves both the practitioner and the patient (4). The next step, standardization (5), includes proper plant authentication through DNA barcoding (6, 7), and the chemical profiling and quantification of all bioactive constituents in the material (8, 9).

Traditional Chinese medicine (TCM) is an ancient, holistic treatment system established through empirical evaluation, and exists in many related forms in Greater China, Japan, Korea, Vietnam, Malaysia, and Singapore. It seeks to restore energy (*qi*) and balance (yin and yang) through the use of medicinal plants, fungi, animal products, and minerals, and, superficially, appears quite different from the reductionist approach of Western medicine.

However, modern biomedical science is now embracing the concept of systems biology, which views human diseases as the result of a multifactorial instability in homeostasis (10). Treatment of cancer or HIV-AIDS now involves a cocktail of drugs targeting different mechanisms of action. At the same time, TCM is embracing network pharmacology, which investigates how the major constituents in a plant (or plants) act on various biological pathways to produce multiple, synergetic actions (11, 12).

The quality control (QC) of TCMs should begin in the field and continue throughout the production process. Developing a QC system for a TCM preparation is a critical, foundational step for the manufacture of a standardized product suitable for biological and clinical studies. A typical TCM preparation, often consisting of an admixture of multiple plants, represents a vast array of chemical constituents that work synergistically to bring about the observed therapeutic effects. Establishing a chemical and biological quality standard for such a complex TCM preparation represents a daunting analytical challenge. A comprehensive analytical approach, integrating chemical, metabolic, and biological methods, was therefore developed to serve as a paradigm for establishing quality standards for TCMs.

Development of systemic analytical methods

A comprehensive analytical assay that can provide the chemical fingerprint of each individual component of a complex preparation is necessary to monitor quality and biological consistency of TCMs (Figure 1) (13, 14).

In our laboratory, liquid chromatography/multiple-stage mass spectrometry (LC-MSⁿ) techniques have been employed in order to explore the chemical profile of various TCM plant materials. Ginseng, notoginseng, and American ginseng (*Panax* species, Araliaceae) are commonly used in TCM formulae and contain ginsenosides like triterpene and steroid saponins as their active ingredients. These three similar plants have differing clinical efficacies and are easily confused, particularly in their post-processing forms.

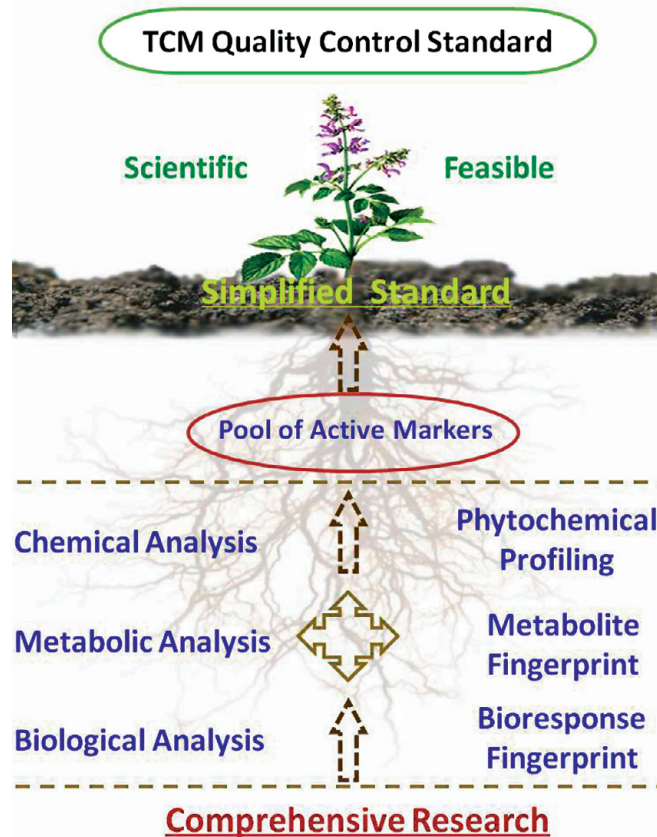
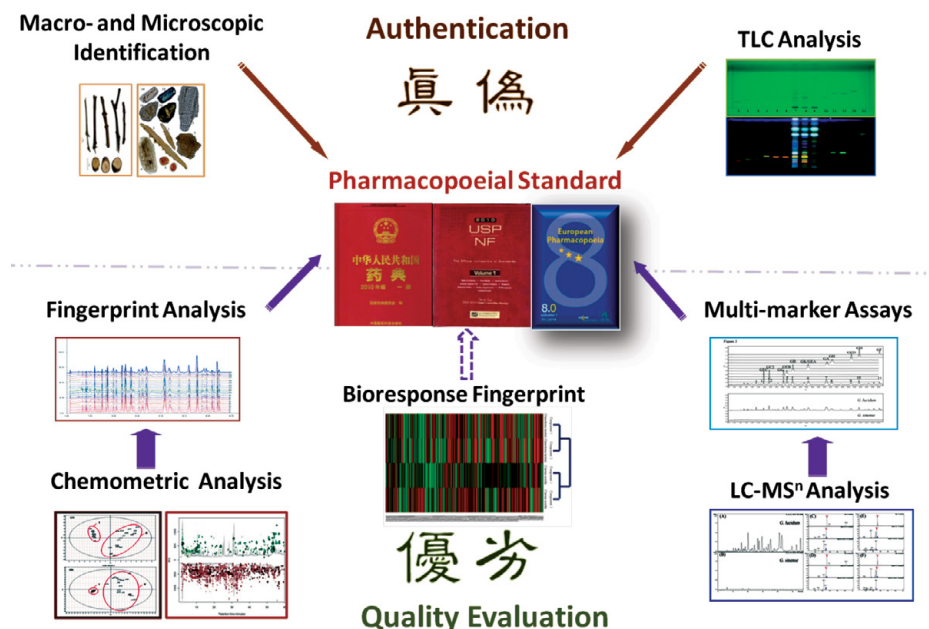


FIGURE 1. A systemic traditional Chinese medicine (TCM) quality research approach: from comprehensive research to simplified standard.

A total of 623 ginsenosides, including 437 potential new ginsenosides, were characterized from the three plants, allowing specific biomarkers to be developed that can unequivocally differentiate between them (15). This technique was similarly applied to a number of TCM herbs, including *Salvia miltiorrhiza*, *Ganoderma lucidum*, *Glycyrrhiza uralensis*, and *Rheum palmatum*.

In one case, a metabolic fingerprinting technique identified seventy metabolites of the major tanshinones and salvianolic acids of *S. miltiorrhiza* in rats after oral administration of the plant, enabling the determination of metabolic pathways and excretion routes (16, 17). Combining both chemical and biological analyses provides an effective strategy for revealing active components. Given the complex metabolic matrix of *S. miltiorrhiza*, a three-tier strategy involving analysis of single compounds, extracted fractions, and the whole herb was adopted. A multi-level biological approach was used that integrated pharmacology, molecular biology, and systems biology. The target proteins and mechanisms of action were found to be impacted at the molecular, cellular, tissue, and whole animal levels, supporting the contention that TCMs work on multiple targets through multiple pathways. In the case of *S. miltiorrhiza*, salvianolic acid B was determined to modulate

FIGURE 2. Holistic quality control model for traditional Chinese medicines. TLC, thin layer chromatography; LC-MSⁿ, liquid chromatography/multiple-stage mass spectrometry.



several molecular targets, including matrix metalloproteinase 9, epidermal growth factor receptor, and integrin (18), and the major tanshinone derivatives were found to be cardioprotective and antioxidant agents (19).

Elaboration of an overall quality standard

A new quality control model that combines analytical fingerprinting to monitor batch-to-batch consistency, and a multi-component assay to assure authenticity and quality, has been developed and effectively applied to several Chinese herbal materials and their preparations (Figure 2). Based on the salvianolic acids and the tanshinones, an overall quality standard for *S. miltiorrhiza* was established using the single standard to determine multicomponents (SSDMC) method, which uses a single reference standard to quantify the content of many related compounds in a mixture, and has been adopted by both the Chinese and United States pharmacopoeias. It is recommended that only pharmacopoeial quality material be used in clinically evaluations in order to ensure product consistency and safety.

Conclusions

A multitude of intrinsic and extrinsic factors affect the quality, consistency, and stability of medicinal plants and their metabolites (12, 13). The regulated application of Good Agricultural and Collection Practices, Good Laboratory Practices, Good Manufacturing Practices, and Good Clinical Practices, are necessary to assure high quality, safe, effective, and consistent TCM products for practitioners and patients (5). In order for TCM products to be accepted into a global, evidence-based health care system, it is imperative for robust international standards and procedures to be developed that govern their growth, collection, processing, and administration. Furthermore, the extreme complexity of TCMs necessitates the integration of new technologies

and strategies for proper analysis of bioactive constituents, allowing the targets and pathways impacted to be fully understood (20).

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Evolution of traditional medicines to botanical drugs

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Botanicals constitute an important source for new drugs (1, 2). To facilitate botanical drug development, the Center for Drug Evaluation and Research (CDER) of the U.S. Food and Drug Administration (FDA) established the Botanical Review Team in 2003 and published its first *Guidance for Industry: Botanical Drug Products* in 2004 (3). This guidance represents FDA's thinking and provides recommenda-

tions on quality, nonclinical, clinical, and other unique aspects associated with botanical new drug development through the investigational new drug (IND) and new drug application (NDA) processes. From 2004 to 2013, CDER received over 400 botanical IND applications and pre-IND meeting requests (Table 1). Most of the INDs were allowed to enter phase 2 clinical trials for evaluation of preliminary safety and efficacy of the investigational botanical products in patients. FDA approved the first botanical NDA for Veregen (sinecatechins) in 2006 (4, 5) and the second botanical NDA for Fulyzaq (crofelemer) in 2012 (6, 7). These two NDA approvals show that new therapies derived from natural complex mixtures can be developed to meet modern FDA standards of quality, safety, and efficacy. A small number of INDs are currently in phase 3 clinical trials, which may lead to more NDAs in the future.

"Totality-of-evidence" approach

For new botanical products intended to be marketed as drugs in the United States, applicants need to provide evidence of safety and efficacy at the same level as is expected for small-molecule products. Botanical products also need to meet standards for product quality, so the marketed product batches deliver a therapeutic effect consistent with that observed for product batches tested in the clinical studies (i.e., therapeutic consistency). However, quality control of botanical products is challenging because these products contain complex mixtures in which the active components may not be known and may also have considerable batch-to-batch variations (e.g., in chemical composition). The conventional chemistry, manufacturing, and controls (CMC) data (primarily from chemical testing) used to ensure the quality of small-molecule products may be insufficient for botanical products. To address this challenge, FDA has developed a "totality-of-evidence" approach based on knowledge and experience acquired from the review of botanical IND and NDA submissions. In addition to conventional CMC data, this integrated approach considers other evidence including raw material control, clinically relevant bioassay(s), and other non-CMC data (including clinical data on the dose-response

generated based on multiple batches of the drug product). The degree of reliance on these other data for ensuring consistency of quality depends on the extent to which the botanical mixture can be characterized and quantified. Such an integrated approach is best explained and illustrated through the FDA's experience with the first two botanical NDAs.

Veregen

Veregen (sinecatechins) ointment was approved for topical treatment of external genital and perianal warts (4, 5). It contains 15% (w/w) sinecatechins, a botanical substance that is a partially purified fraction of the water extract of green tea leaves from *Camellia sinensis* (L.) Kuntze (Theaceae). Sinecatechins is a mixture of catechins (85% to 95% by weight of the total drug substance) and other green tea components. These catechins include more than 55% of epigallocatechin gallate and other catechin derivatives (Figure 1A) (4).

For FDA approval, the safety and efficacy of Veregen were established in two randomized, double-blind, vehicle-controlled clinical studies. The study results showed that the treatment group receiving Veregen demonstrated a significantly higher response rate compared with the vehicle-control group (53.6% vs. 35.3%) (4). The response rate was defined as the proportion of patients with complete clinical (visual) clearance of all external genital and perianal warts (baseline and new) by week 16. Although extensive human consumption of green tea and literature research data on catechins provided considerable reassurance of the safety of topical use of catechins, this information was not considered sufficient to support the NDA approval for Veregen. Standard nonclinical studies were conducted to support the safety of Veregen.

As a naturally occurring mixture in which the active components are not well defined, the identified individual major and minor chemical components in Veregen need to be monitored and controlled for each marketed product batch. In the absence of data correlating chemical properties and clinical response, the acceptance ranges for these components were primarily established based on their levels observed in the multiple batches tested in the clinical studies. As significant variations of catechins and other chemical components have been identified from the tea leaves of different cultivars (8), this botanical product can have considerable batch-to-batch variability (e.g., in total catechins and ratios of different catechins). In addition, although the majority of components can be adequately characterized and quantified, there may still be some residual uncertainties about the chemical nature of minor components in sinecatechins. Therefore, to ensure consistent quality for Veregen, FDA considered two other important pieces of information from the application, summarized below:

- The botanical raw materials for future marketed product

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TABLE 1. Number of botanical pre-INDs and INDs submitted to CDER (2004–2013).

Submissions	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	Total
Pre-IND	12	6	11	7	14	10	10	8	5	8	91
IND	21	38	22	39	27	27	27	33	33	51	318
Pre-IND and IND	33	44	33	46	41	37	37	41	38	59	409

batches were limited to the cultivars used in clinical studies of Veregen, which were identified by botanists and tea experts based on the characteristics and history of the specimens collected at the tea farms. It was emphasized that cultivation sites or farms should follow the principles of good agricultural and collection practices (GACP). These control measures reduce the variability in chemical composition at the plant and raw material levels, so the marketed product batches will exhibit a natural variability similar to the range observed in batches used in clinical studies.

- The efficacy results of clinical studies showed no significant difference in clinical response between two doses (i.e., 10% and 15%). This observation suggests that subtle variations in any uncharacterized fractions may not have an impact on the therapeutic effect.

Fulyzaq

Fulyzaq (crofelemer) is the first FDA-approved drug for symptomatic relief of noninfectious diarrhea in patients with HIV/AIDS on antiretroviral therapy (6, 7). It is available as a delayed-release tablet containing 125 mg crofelemer, a botanical drug substance derived from the red latex of *Croton lechleri* Müll.Arg. (Euphorbiaceae). Crofelemer is an oligomeric proanthocyanidin mixture primarily composed of (+)-catechin, (-)-epicatechin, (+)-gallocatechin, and (-)-epigallocatechin monomer units linked in random sequence (Figure 1B) (6). The red latex, commonly known as Dragon's Blood, has been commonly used in South America as an herbal medicine for treating diarrhea (9, 10). Results from the randomized, double-blind, placebo-controlled and placebo-free clinical study showed that a proportion of HIV-positive patients (who were on stable antiretroviral therapy with a history of diarrhea) in the crofelemer 125 mg bid group was significantly higher than that in the placebo group in experiencing clinical response (17.6% vs. 8.0%) (6). The clinical response was defined as less than or equal to two watery bowel movements per week during at least two of the four weeks of the placebo-controlled phase.

Compared with sin catechins, the chemical characterization of crofelemer presented an even greater challenge. This drug consists of a mixture of oligomers that vary in composition, sequence, and length, which precluded adequate separation and quantitation of proanthocyanidin oligomers based on multiple conventional chromatographic methods. Additional chromatographic, spectroscopic, spectrometric, and acid hydrolysis methods, which likely represented the state of the art of analytical technologies at the time, were needed to provide a comprehensive characterization of crofelemer. These analytical methods collectively revealed extensive information on the structural signatures of crofelemer (e.g., the composition of proanthocyanidin oligomers), but they were ultimately considered insufficient to support the character-

ization and quality control of this complex botanical mixture (e.g., difficulty of quantification of proanthocyanidins at the molecular level and limitations of the hydrolysis method that provided less than optimal yields) (11).

Because the degree of uncertainty regarding chemical characterization of crofelemer was much greater compared with sin catechins, FDA concluded that in addition to raw material control and clinical data from multiple doses and product batches, a clinically relevant bioassay to assess the drug product activity was warranted for the approval of Fulyzaq. The non-CMC data that FDA relied on to ensure consistency of quality for Fulyzaq are summarized below:

- Similar to Veregen, botanical raw material controls included implementation of GACP and restricted harvesting of botanical raw material to specific eco-geographic regions (EGRs). These control measures help to reduce the variability at the plant and raw material levels.

- Crofelemer's mechanism of action (MOA) was reported in the literature as a potent inhibitor of both the cyclic adenosine monophosphate-stimulated cystic fibrosis transmembrane conductance regulator chloride ion channel and the calcium-activated chloride ion channel at the luminal membrane of enterocytes (12). Knowledge of crofelemer's MOA led to an important development of a clinically relevant bioassay, which enabled the establishment of clinically relevant specifications. The existence of a clinically relevant bioassay could potentially provide more flexibility for the manufacturer to make postapproval changes (e.g., expansion of EGRs to increase and diversify the botanical raw material supply).

- The clinical data from multiple doses (125–500 mg bid) showed that the drug's effects were not sensitive to the tested doses. This is consistent with in vitro observations and clinical pharmacology data, suggesting that the estimated drug concentrations in the gastrointestinal tract after oral dosing (125 mg bid) are several-fold higher than the concentrations used for maximum chloride ion channel inhibition, which leads to drug saturation at the action sites (7, 12, 13). The clinical data from multiple batches of crofelemer also did not reveal any noticeable differences among drug product batches manufactured by using different drug substance batches. The above information suggested that the natural variations observed in crofelemer were unlikely to have significant impact on the efficacy of Fulyzaq.

Conclusion

The approvals of Veregen and Fulyzaq demonstrated that FDA's science-based "totality-of-evidence" regulatory approach can ensure the consistency of product quality and thereby therapeutic effect of botanical drugs. For quality control purposes, relying on other evidence including raw material control, clinically relevant bioassay(s), and/or clinical

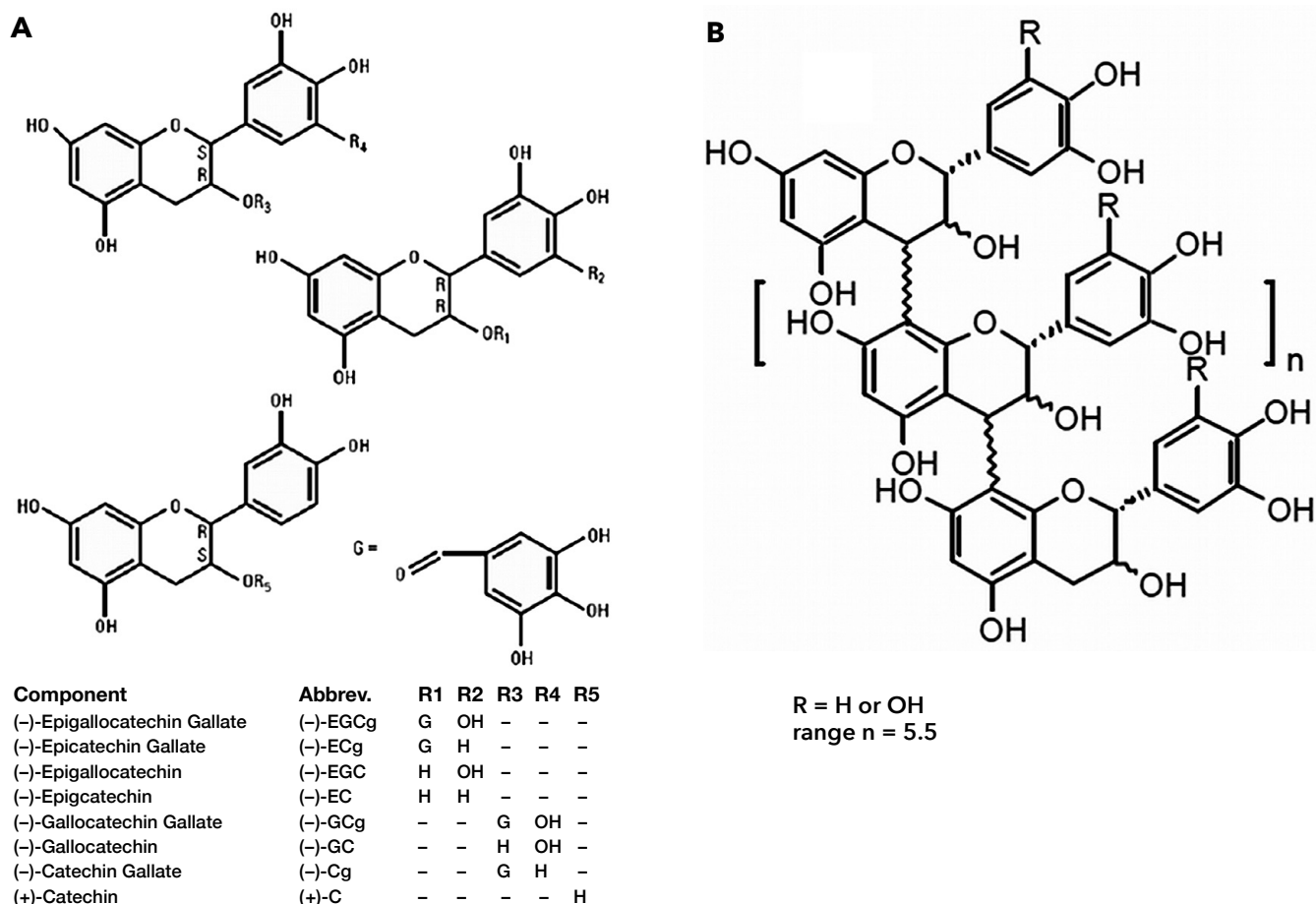


FIGURE 1. (A) Known catechins in sinecatechins (4). **(B)** Proanthocyanidins in Crofelemer (6).

data can overcome the limited ability to characterize the entire botanical mixture or its active components, based on the analytical technology available. The approval of these two botanical NDAs demonstrates the success of an integrated approach and provides the industry with a practical framework for developing botanicals (including traditional medicines) to new drugs that are held to the same FDA standards as small-molecule drugs.

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Developing influenza treatments using traditional Chinese medicine

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Humans have been faced the threat of epidemics such as influenza throughout their existence. Traditional Chinese medicine (TCM) practitioners began documenting their diagnostic and treatment principles related to epidemic diseases in the classic Chinese medical book,

“Emperor Internal Medical Classic” (1). The unique treatments and herbal formulas used to combat influenza may serve as a source of information and inspiration for the development of new drugs (2).

Chinese herbal medicines and influenza

A major difference between Western and Chinese influenza treatments is the mode and targets of their actions. The first antiviral chemical drugs appeared in the West in the mid-1960s. Since then, many single-target therapeutics have been designed, but drug resistance is common. To circumvent this, Western medicine has incorporated multiple molecular targets into a single treatment using combination therapies, a practice now well accepted in the West.

Chinese herbal formulas (CHF), on the other hand, often act via multiple modes that target not only the virus, but also various components of the host's immune response (Table 1), creating a synergistic effect. For example, *jinchai* capsules blunt viral replication by blocking adsorption of virions and preventing virus hyperalgesia-induced membrane fusion (3), while evodiamine blocks viral action by interfering with the AMPK/TSC2/mTOR signaling pathway, which is associated with virus-induced autophagy (4). Figure 1 summarizes the points of action of CHF when treating influenza.

Isatis indigotica roots and influenza

Isatis indigotica roots (IIR) (*Banlangen*) have long been used to treat seasonal influenza in China. Currently, more than 100 chemical constituents of IIR have been identified. Among them, the compounds of epigotrin; 2,4(1H,3H)-quinazolinone; 4(3H)-quinazolinone; and clemastanin B have been demonstrated to kill or significantly inhibit the influenza virus. Studies from our laboratory have shown that polysaccharides extracted from IIR can prevent the influenza virus from attaching to host cell surfaces through a process involving hemagglutinins (5). Moreover, an indole alkaloid has been found to play a major role in preventing viral infection of host cells (6), while compounds derived from IIR can block translocation of the nucleocapsid protein at the early stage of replication, primarily through modulation of NF- κ B signaling, thus inhibiting viral replication (7). In addition, IIR has been

shown to exert immune modulatory effects in vitro and in vivo. In lipopolysaccharide (LPS)-stimulated RAW264.7 murine macrophages, the methanolic extracts of IIR inhibited degradation of I κ B α and production of nitric oxide, prostaglandin E₂, and interleukin (IL) 6 (8). The polysaccharides from IIR could promote proliferation of lymphocytes and macrophages, as well as production of IL-2 and interferon (IFN) γ in mouse models (9). Indirubin and its derivatives can suppress a number of pro-inflammatory cytokines/chemokines in infected human bronchial epithelial cells, human peripheral blood-derived macrophages, and alveolar epithelial cells (Table 1) (10, 11). Taken together, these data imply that IIRs play a variety of roles protecting against viral infection by targeting both the virus and the host—a markedly different effect than that of marketed chemically synthesized drugs.

Drug development strategies using TCM

High-quality consistency, treatment effectiveness, safety assurance, and patient affordability are the key factors for drug development. TCM can inform research into these areas in the following ways.

Firstly, the strategies and principles underpinning the translational research used in TCM-based influenza treatments could be applied more broadly. Two possible approaches can be taken: the standard, bottom up bench-to-bedside strategy, or a more innovative approach that transitions empirical medical knowledge from TCM into an evidence-based research strategy. We proffer that the latter better reflects the real-world interaction between basic science and the TCM clinical experience.

Secondly, basic research and clinical studies on CHF could be conducted in parallel. For example, the effects of extracts and/or combinations of the active compounds from commonly prescribed CHF could be investigated concurrently with standardized clinical trials based on documented clinical experience.

Thirdly, well-defined methodologies for standardized assessment of the quality, efficacy, and safety of CHF are still lacking. It is important to standardize the composition and level of active components of herbs in CHF before including them in a basic research project or clinical trial so as to maintain the data integrity.

Finally, TCM research is complex. It therefore behooves all researchers to develop interdisciplinary, innovative, and collaborative research projects, through which the scientific foundation of TCMs can be elucidated and a new framework that incorporates modern medical science can be built.

We have been pioneers in an attempt to implement the abovementioned strategies using IIR, launching the first randomized control trial in China in 2010. Various 'omics

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TABLE 1. Examples of TCM and Western anti-influenza drugs.

Antivirals				Target	Target subject	Mechanism of action or therapeutic effect	Year Documented	Reference
Western anti-influenza compounds	Cyanovirin-N			Surface glycoproteins of enveloped viruses	Virus	Inhibits entry of virus	1997	12
	Nitazoxanide			Influenza hemagglutinin	Virus	Impairs hemagglutinin intracellular trafficking	2009	13
	DAS181			Influenza viral receptor	Host	Removes viral receptor	2006	14
	Amantadine/Rimantadine			Influenza M2 protein	Virus	Inhibits proton conductance of M2	1965	15, 16
	Favipiravir(T-705)			Influenza RNA polymerase	Virus	Inhibits viral RNA polymerase activity	2002	17, 18
	Oseltamivir/Peramivir/Zanamivir/Laninamivir			Influenza neuraminidase	Virus	Blocks release of virus	1996/2000/1993/2005	19-22
Chinese anti-influenza herbal formulas, compounds, and constituents	Single herb	Ban Lan Gen (<i>Isatis indigotica</i> root)	Methanol extract	NF- κ B signaling	Host	Inhibits nitric oxide and prostaglandin E ₂ production, and NF- κ B signaling in macrophages	1260s	8
			Polysaccharides	—	Host	Promotes transformation of lymphocytes and production of IL-2 and IFN- γ		9
			Clemastanin B	—	—	Blocks influenza ribonucleoprotein nuclear export, prolongs mean lifespan of infected mice		7, 23
			Indirubin	NF- κ B signaling	Host	Interrupts virus-induced p38 MAP kinase activation and NF- κ B translocation, and reduces expression of CCL5 in human bronchial epithelial cells		10
			Indirubin derivatives	—	Host	Suppresses pro-inflammatory cytokines and chemokines		11
	Complex herbal formula	Da Qing Ye (<i>Folium isatidis</i>)	Monomer	—	—	Reduces mortality rate of influenza virus-infected mice	202-220 B.C.E.	24
		Jin Yin Hua (<i>Lonicera japonica</i>)	Ethanol extracts	Antiviral, immune-modulatory, and anti-inflammatory protein in mouse serum	Host	Reduces lung index and alleviates lung lesions in influenza virus-infected mice	1400s	25
		LianQiao (<i>Forsythia suspense</i>)	Ethanol and water extracts	—	Host	Regulates CCL5 and MCP-1 secretion in H1N1 virus-infected A549 cells		26
		Ma Xing Shi Gan Tang + Yin Qiao San		—	—	Reduces time to fever resolution in patients with H1N1 influenza virus infection	2011	27
		Lian Hua Qing Wen capsule		—	—	Reduces time to fever resolution in patients with H1N1 influenza virus infection	2004	28

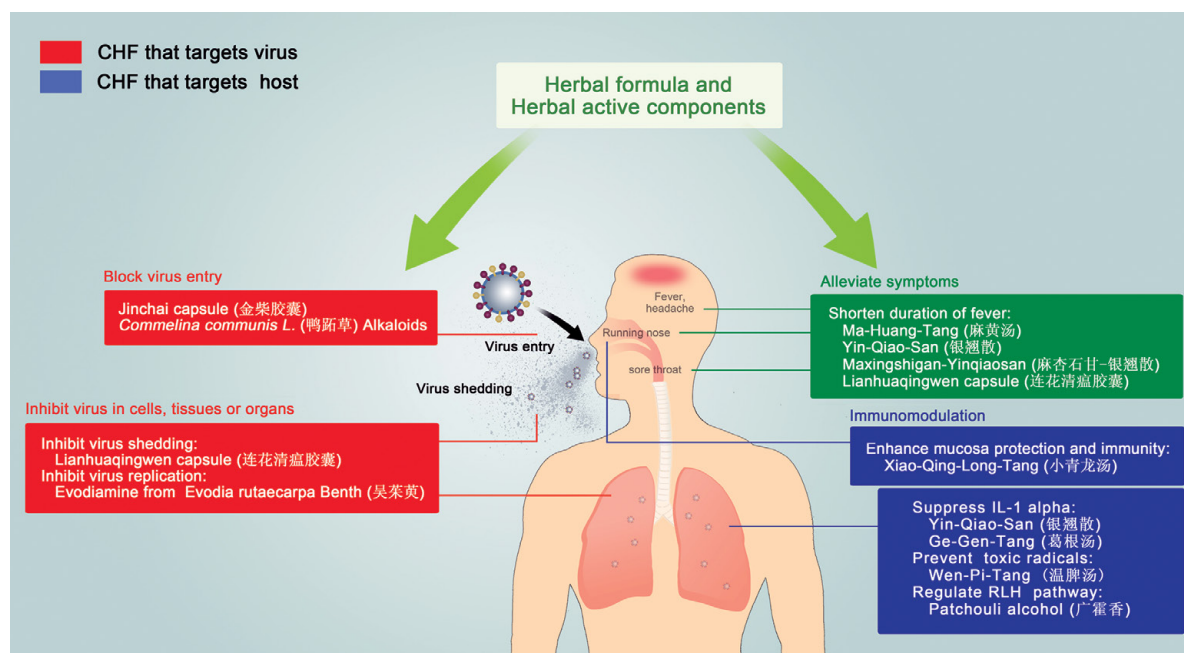


FIGURE 1.
Holistic
intervention
to treat
influenza.

technologies have been concurrently used to search for bioactive compounds, and we expect that additional active constituents with unique pharmaceutical activities will be found in the future. We have also combined the application of modern technologies with TCM clinical experience. For example, practitioners have noted that IIR appears to display beneficial clinical effects if administered during early onset of the disease (9). These studies suggest that further investigation of the mechanisms of IIR action is warranted. Importantly, using treatments with multiple sites of action may prevent or delay the generation of resistant viral strains.

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A novel drug discovery strategy inspired by traditional medicine philosophies

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For thousands of years, traditional medicines in China (traditional Chinese medicine, TCM), Japan (Kampō medicine), Korea (traditional Korean medicine), Indonesia (Jamu), India (Ayurvedic medicine), North America (phytotherapy), and Europe (herbalism) have been the primary means for maintaining health as well as preventing and treating human diseases (1). Over time, experiential knowledge derived from the medical application of natural products led to their incorporation into complex medical knowledge bodies—*materia medica*—characterized by the understanding of nature unique to each culture. For almost 200 years, the traditional use of natural products has also represented a source of effective drugs (2–5). This strategy represents a successful approach to novel drug identification and development through isolation and purification of active ingredients from natural products, high-throughput and high-content screening, and subsequent analysis and testing according to the guidelines of the U.S. Food and Drug Administration and other regulatory agencies (6–8). However, the pharmacologically active ingredients of a phytocomplex are not always the original natural molecules, but may be their host-specific metabolites or molecular complexes formed following co-administration with other herbs. This complexity has generated significant scientific challenges in the study of natural products (9, 10). The multicomponent nature of traditional medicines leading to multiple potential molecular interactions, multiple targets, and numerous metabolic byproducts, suggests that a conventional reductionist approach will have limitations in identifying active ingredients, making a more network-oriented, holistic approach preferable (12).

The *Jun-Shi* medicinal compatibility model

To address the challenge innate in the complex composition of natural products, inspiration was taken from the theoretical principles underlying TCM (13). This includes meticulous documentation of clinical observations, which can then inform the practice of traditional healing and help to develop guidelines and principles. The principles of these practices will be validated when successfully applied to practical clinical problems (13). We applied this approach to analyzing the *Jun-Chen-Zuo-Shi* principle of combining different *materia medica* in a specific manner when creating TCM compound formulations (*Fufang*). Additionally, as a strategy for drug discovery, we propose a simplified *Jun-Shi*

model to identify the ingredients from TCM formulations that reach the bloodstream and the pharmacological effects they may have in the body.

Jun-Chen-Zuo-Shi and *Qiqing* (seven ways of pairing compatible herbs) are the basic theories behind the formulation of TCM treatments. *Jun-Chen-Zuo-Shi* theory guides the combination of different herbal medicines in *Fufang*, based on the healing/pharmacological properties and constituents of each herb. The *Jun* (emperor) component is the principal phytocomplex targeting the major symptom of the disease. There are only a few varieties of *Jun* medicinals that are administered as a single formula, usually in large doses. The *Chen* (minister) herbs synergize with *Jun* to strengthen its therapeutic effects, and may also treat secondary symptoms. The *Zuo* (assistant) medicinal reduces or eliminates possible adverse or toxic effects of the *Jun* and/or *Chen* components, while also enhancing their effects and sometimes treating secondary symptoms. Finally, the *Shi* (courier) herbs facilitate delivery of the principal components to the lesion sites, or facilitate the overall action of the other components (14–15). The principles of *Qiqing* describe how herbs can be used independently, to reinforce (when both herbs have similar properties) or enhance (when the efficacy of the primary medicinal is improved) the effects of other herbs, or to antagonize certain unwanted, negative side-effects. In practice, *Qiqing* helps to determine the optimal pairing and proportions of two medicinals in a formulation.

We propose combining the principles of *Jun-Chen-Zuo-Shi* and *Qiqing* to create the notion of a *Jun-Shi* medicinal pair in order to provide better therapeutic efficacy when compared with a single medicinal (16). A *Jun-Shi* medicinal pair also has the synergistic characteristics of a *Fufang*, targeting the active phytochemicals to their designated sites of action. At the same time, the simpler composition of the *Jun-Shi* medicinal pair provides a less complex formulation for scientific analysis, which should reduce the complexity and difficulty when seeking new drugs from TCM-related sources. Based on these theories, we propose an innovative strategy for new drug discovery through the screening of *in vivo* effector compounds from *Jun-Shi* medicinal pairs. The *Jun* herb performs the primary action while the *Shi* herb potentiates this activity either by modifying the physicochemical properties of the *Jun* herb or facilitating its interaction with the pharmacological target. The identification of pharmacologically meaningful differences could therefore be made by comparing action and efficacy of the *Jun* herb with or without the *Shi* herb.

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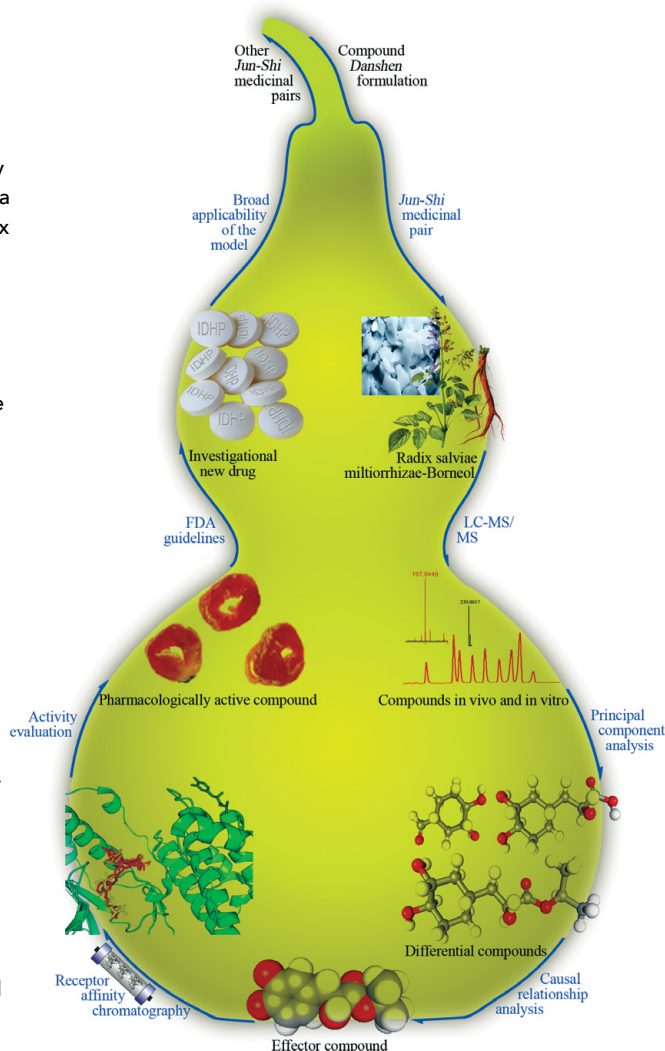
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FIGURE 1. Schematic of the strategy for drug discovery based on the identification of an in vivo effector compound using the *Jun-Shi* medicinal compatibility model. We firstly selected *Jun* and *Shi* herbs as a research target from a complex *Fufang* using the principles of the *Jun-Shi* medicinal compatibility model. In vivo and in vitro compounds of the *Jun-Shi* medicinal pair were analyzed by high-performance liquid chromatography-mass spectrometry (LC-MS) and nuclear magnetic resonance. The data obtained by LC-MS was extracted and further analyzed by principal component analysis (a variance-focused procedure seeking to reproduce the total parameter variance, in which components reflect both common and unique variance of each parameter) to uncover the differential compounds. The resulting compounds were then examined using causal relationship analysis such as the Granger causality test. The bioactivity of those effector compounds identified was screened by receptor affinity chromatography and confirmed by classical functional pharmacological assays to identify leads of interest. One lead, isopropyl 3-(3,4-dihydroxyphenyl)-2-hydroxypropanoate (IDHP), was evaluated further according to guidelines established by the U.S. Food and Drug Administration and other regulatory agencies for the development of an investigational new drug. We hope that the mechanism of action of IDHP will further confirm the broad applicability of the *Jun-Shi* medicinal compatibility model to drug discovery.



(*Danshensu*) were found to be increased in rabbit heart by co-administration of *Bingpian*. Additionally, isopropyl 3-(3,4-dihydroxyphenyl)-2-hydroxypropanoate (IDHP), a novel metabolite of *Danshensu* in *Fufang Danshen Diwan* was identified (18). Pharmacokinetic studies showed that this new compound was preferentially found in heart and brain tissues, in agreement with the lesion sites expected to be targeted by this formulation according to TCM principles. Furthermore, synthetic IDHP was generated and shown to have a protective function against myocardial and cerebral ischemia injury (19, 20), strongly suggesting that IDHP is the effector compound in the *Danshen-Bingpian* medicinal formulation in *Fufang Danshen Diwan*.

Conclusions

The studies described here propose a novel strategy for drug discovery based on the identification of active substances in TCM herbal formulations. The strategy consists of the selection of *Jun-Shi* medicinal pairs, the establishment of a quantitative model for analysis of causal relationships, and a system for the

Testing the *Jun-Shi* model

The *Fufang Danshen Diwan* (Dantonic pill) has successfully completed phase 2 clinical trials in the United States and is currently undergoing phase 3 trials (17), which made this a perfect candidate on which to test our strategy of drug discovery. We choose to focus on the *Danshen* (*Radix Salviae miltiorrhizae*) plus *Bingpian* (Borneol) pair of medicinals present in this pill to investigate our *Jun-Shi* compatibility model. The process is described in Figure 1. The levels of *Danshen*-derived phenolic acids such as 3-(3,4-dihydroxyphenyl)-2-hydroxypropionic acid

identification of active substances by receptor affinity chromatography (Figure 1) (21, 22). Compared with the conventional procedure of drug discovery using a reductionist approach, the current strategy employs the principles of *Jun-Chen-Zuo-Shi* and *Qiqing* in devising a *Jun-Shi* medicinal compatibility model to reduce the number of targets for the identification of active ingredients. This can reduce the arbitrary nature of the drug discovery process and also improve its efficiency. Significantly, it can further enrich TCM theory and offer new perspectives in the research of complex biomedical questions.

Future perspectives

Based on the successful work with IDHP, we have further synthesized a series of *Jun-Shi* compounds including 1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl 3-(3,4-dihydroxyphenyl)-2-hydroxypropanoate by incorporating borneol in the structures of active compounds of *Danshen* such as *Danshensu*, caffeic acid, and rosmarinic acid, according to our drug discovery strategy. Patents on these compounds have been approved in 34 countries or regions, including the United States (No. 8017786), Canada (No. 2652299), Russia (No. 2421443), and the European Union (No. 2019090). Preliminary pharmacological investigations have shown that some of these compounds are effective against the formation of atherosclerotic plaques in ApoE^{-/-} mice (Yonggong Zhai, unpublished observations). Results indicate that these *Jun-Shi* compounds may be potential candidates for constructing a database of combinational TCM-derived molecules for drug discovery.

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Deciphering ancient combinatorial formulas: The *Shexiang Baoxin* pill

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An ancient combinatorial formula (*Fufang*) carries similarities to the polypill used in Western medicine. A polypill is a single medication that contains multiple pharmaceutical therapies, and may be intended for the treatment of a single or even multiple diseases. An example of a polypill is Polycap—comprising aspirin, simvastatin, ramipril, atenolol, and hydrochlorothiazide—used to treat hypertension and prevent heart attack and stroke (1). In traditional Chinese medicine (TCM), *Fufang* refers to a group of therapeutic compounds derived from multiple plant, mineral, or occasionally animal sources. For example, the formulation Realgar-Indigo naturalis—containing tetra-arsenic tetra-sulfide (As₄S₄), indirubin, and tanshione IIA—has been demonstrated to be an effective treatment for promyelocytic leukemia (2). In Europe, a five-herb formulation of cowslip (*Primula veris/elatior*), yellow gentian (*Gentiana lutea*), black elder (*Sambucus nigra*), common sorrel (*Rumex* species), and vervain (*Verbena officinalis*) has been approved by the European Medicines Agency Committee on Herbal Medicinal Products for sinusitis and bronchitis.

To study the efficacy and bioactivity of combinatorial formulas, the *Shexiang Baoxin* Pill (SBP) was chosen as a test case. SBP originates from a classical ancient prescription *Suhexiang* Pill recorded in *Prescriptions of the Bureau of Taiping People's Welfare Pharmacy*, which was used for the treatment of chest pain with dyspnea in the Southern Song Dynasty (10th to 12th centuries C.E.). The modern form of SBP was developed in 1981 under Professor Rui-Hong Dai's leadership (3), and consists of seven Chinese materia medica (medical materials) (Figure 1). Today in China, SBP has become a widely used *Fufang* for the treatment of stable angina pectoris, chest pain or discomfort caused by coronary heart disease (CHD). Several randomized, prospective clinical studies strongly suggest SBP can decrease the frequency of angina due to CHD and daily nitroglycerine use (4, 5) and may even reduce ischemic myocardial changes as measured by electrocardiogram and perfusion imaging (6).

However, as with many *Fufang*, numerous questions must

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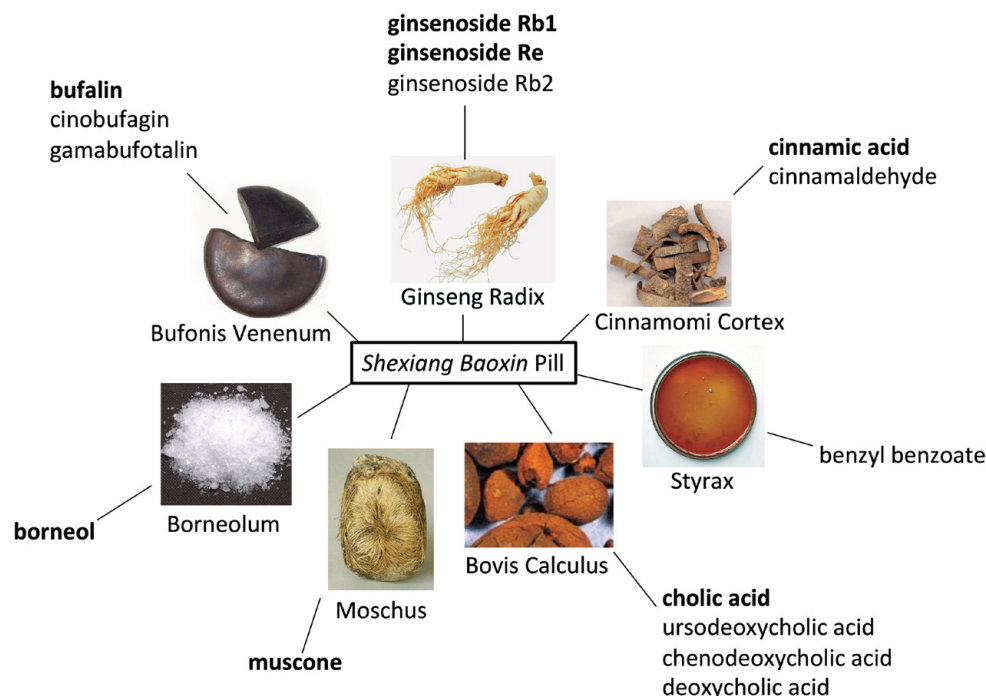


FIGURE 1. Composition of *Shexiang Baixin Pill* (SBP). Fifteen of the more abundant compounds that comprise SBP and the materia medica from which they are derived are shown. Pure compounds were identified by high-performance liquid chromatography-mass spectrometry of SBP (7, 11). (Images courtesy of www.tcmwiki.com.)

be answered if SBP is to reach patient populations in countries with stringent food and drug regulations. In contrast to modern Western pharmaceuticals, where the active ingredients are synthesized and examined independently in clinical trials before being co-administered, the process in TCM is reversed. The current challenge is to define SBP's composition, pharmacodynamics, pharmacokinetics, and, since it is a formulation, any pharmacodynamic synergies that may be present.

A reductionist approach: Breaking down SBP

Advanced separation and analysis techniques such as gas and liquid chromatography, coupled with mass spectrometry, have made it possible to identify the exact chemical species that comprise *Fufang*-based therapies. To date, over 70 non-volatile and over 40 volatile chemical species have been identified in SBP (7, 8). Following oral administration of SBP in rats, as many as 22 of these pure compounds and eight metabolites could be observed in blood plasma (9, 10). These analytical techniques help to establish a "chemical fingerprint" for SBP, which allows for batch-to-batch comparison of individual materia medica and the resultant drug, an important step towards quality control in manufacturing (11, 12), and reproducible safety and efficacy.

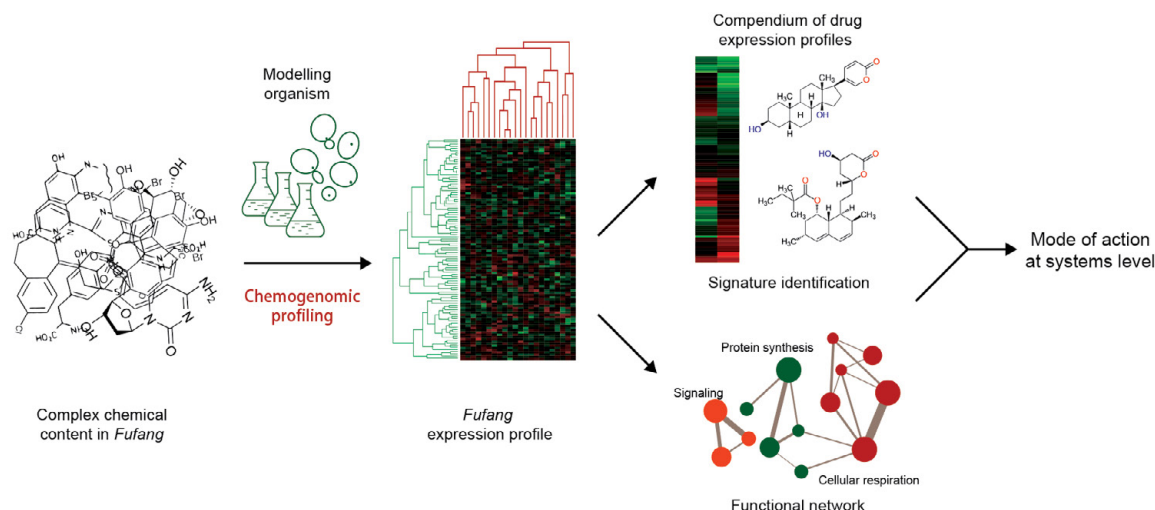
Such a chemical fingerprint is also vital for identifying active ingredients. For instance, several of the major classes of compounds that have been identified in SBP include bufadienolides, ginsenosides, and bile acids (Figure 1). Two bile acids identified, ursodeoxycholic acid and chenodeoxycholic acid, have been approved by the U.S. Food and Drug Administration (FDA) and marketed as chenodiol and ursodiol, respectively. Prescribed for the prevention and management of gallstones, these bile

acids decrease the production of cholesterol and reduce hypertriglyceridemia, a strong risk factor for CHD (13). The bufadienolides that are abundant in *V. bufonis* and SBP (11) are cardiac glycosides, and carry similarities to digoxin, a plant-derived cardiac glycoside, FDA-approved class V anti-arrhythmic, and positive inotropic agent (14). The main bufadienolide in *V. bufonis*, bufalin, is also a Na^+, K^+ -ATPase inhibitor that increases cardiac contractility (15) and may protectively downregulate the renin-angiotensin system during heart failure (16). The small molecule cinnamaldehyde, abundant in *Cortex cinnamomi* (7), has been shown to be a strong vasodilator (17) and activates transient receptor potential channels (TRPV1 and TRPA1) involved in nociception (18, 19). Active reduction of pain perception, cholesterol and triglyceride levels, and effects on contractility may represent a few of the ways the bioactive ingredients in SBP exert a cardioprotective effect. As part of a reductionist approach for any *Fufang*, a deeper examination of how identified bioactive ingredients affect disease processes may prove vital in characterizing the formulas' mechanism of action.

A systems biology approach: Keeping SBP intact

Systems biology provides an additional strategy for exploring *Fufang* mechanisms of action and already has contributed to the successful development of two antianginal therapeutics, ranolazine and ivabradine (20). In contrast to a reductionist approach, which studies complex systems by investigating its individual components, systems biology focuses on understanding biological networks in a broader context by integrating transcriptomics, proteomics, metabolomics, and bioinformatics data and analyses (20,

FIGURE 2.
Chemogenomic
profiling.



21). Recently, we initiated a more comprehensive study of the in vivo mechanisms of *Fufang* using a chemogenomic approach (Figure 2). For example, using drug-induced haploinsufficiency profiling in *Saccharomyces cerevisiae* (22), functional information can be obtained from loss-of-function assays to systematically investigate the cellular response to either individual bioactive entities or combined subsets of SBP. Heterozygous strains that show hypersensitivity to SBP can reveal pathways and targets that respond to the drug, thereby providing clues about its mode of action in a cellular context. A previous compendium of cellular responses to small molecules allows mechanisms of novel compounds to be inferred on the basis of profile similarity to established drugs (23).

Metabolomic methods have already proven useful in characterizing SBP. In a rat model of acute myocardial infarction (MI), numerous plasma and urinary biomarkers involved in oxidative injury, dysfunction of energy and amino acid metabolism, and inflammation have been identified using partial least squares discriminant analysis plots (24, 25). SBP given orally before MI can significantly reverse the changes in a number of these identified biomarkers—including lactic acid, homocysteine, and prostaglandin E_2 —nearly returning their levels to normal (25). Analysis of the chemogenomic and metabolomic response signatures of SBP and other *Fufang* can clarify their impact on broader cellular processes and identify potential targets.

From complexity to simplicity—A new development strategy for *Fufang*

As not all components in *Fufang* are active, a combination of its active components may provide a simplified *Fufang* that facilitates easier identification of therapeutic targets and mechanisms of action. Early attempts to develop a simplified formulation of SBP (Figure 1, molecules in bold) have shown some promising results in rat models of MI (26), illustrating the potential of integrating reductionist and systems biology approaches in the development of *Fufang*. Ultimately, by

conducting rigorous quality control and purification, and removing extraneous compounds, such an approach would be equally applicable to create a new generation of polypills for other diseases.

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Lessons from the development of the traditional Chinese medicine formula PHY906

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Traditional Chinese medicine (TCM) has been practiced for thousands of years. While the historical usage of TCM is well documented, it is not broadly accepted by mainstream

physicians who question the quality and consistency of TCM products, the scientific basis for usage, and the lack of evidence-based clinical studies. Nonetheless, TCM formulas are currently being used to relieve the side effects of nonhematological toxicities caused by chemotherapy, including diarrhea, nausea, vomiting, and fatigue. We decided to further explore the mechanisms of action of TCM in chemotherapy, laying the groundwork for its potential use as an adjuvant treatment.

We selected several TCM formulas composed of common herbs for the treatment of the abovementioned symptoms. Considering their long history of usage, these formulas should be relatively safe. Simple formulas consisting of a limited number of herbs were used to facilitate quality control and simplify analysis of the mechanisms of action. Among these formulas, we found that *Huang-Qin Tang* could enhance the therapeutic index of irinotecan, a chemotherapeutic agent for the treatment of metastatic colon and rectal cancer. This four-herb formula (*Glycyrrhiza uralensis* Fisch, *Paeonia lactiflora* Pall, *Scutellaria baicalensis* Georgi, and *Ziziphus jujube* Mill) has been used to treat gastrointestinal disorders for approximately 1,800 years. The formula, named PHY906, was manufactured using standard operation procedures and following current good manufacturing practice standards.

Quality control for TCM

Since the sites of action and bioactive compounds found in TCMs are not always known, it is not sufficient to rely on either chemistry (1, 2) or biological analysis (3) alone for quality control (QC) purposes. We therefore developed Phytomics QC, an analysis system that integrates both chemical and biological data (from in vitro and in vivo studies) to assess the consistency of TCM using a novel statistical methodology (4). Using this system, we demonstrated that batches of PHY906 formulations spanning over a decade had a Phytomics Similarity Index (PSI) > 0.9 (1, perfectly identical; 0, no similarities). Interestingly, commercial *Huang-Qin Tang* products had a wider PSI range and showed inconsistent in vivo biological activity when compared to PHY906.

Are all herbs in a TCM formula required?

Based on TCM principles, a formulation should have the proper ratio of the “imperial” herb (the main ingredient), the

“ministerial” herb (ancillary to the imperial herb), the “assistant” herb (reduces side effects of the main herb), and the “servant” herb (aids in harmonizing the other herbs) to achieve the best therapeutic effect (5). By comparing formulations in which one herb has been removed, as well as single-herb preparations, a formulation for PHY906 was found that achieved the optimal therapeutic effect in combination with irinotecan (6). Different herbs appear to play different roles in the enhancement of antitumor activity and in protection against weight loss and mortality (6). These findings support the theory that each herb might play a specific role in a TCM formulation and suggests that multiple targets, and multiple active compounds for each target, are involved in the PHY906’s action. TJ14, a seven-herb TCM formula, has the potential to reduce diarrhea and oral mucositis due to chemotherapy (7–9). While TJ14 shares three herbs with PHY906, it did not increase the therapeutic index of irinotecan in our preliminary studies (unpublished data).

Scientific validation of TCM

In preclinical studies, PHY906 was found to reduce diarrhea and intestinal damage following irinotecan or irradiation treatment by inhibiting multiple inflammatory processes and/or by promoting intestinal recovery (10, 11). These preclinical studies of PHY906 may help to explain why *Huang-Qin Tang* is effective in treating diarrhea. Based on these findings, the use of PHY906 in treating inflammatory bowel disease and stimulating stem/progenitor cell growth is being explored.

Preclinical studies indicate that PHY906 could potentiate the antitumor activity of a broad spectrum of anticancer agents in vivo (12). PHY906 plus irinotecan may increase tumor cell apoptosis associated with strong macrophage infiltration and induce an acute inflammation in tumors (13). Chemicals and metabolites of PHY906 varied based on the particular tissue being studied, which could partly explain why intestine and tumor tissue had different inflammation responses to PHY906 (13).

Like PHY906, many other herbal formulas have been reported to have multiple effects. TJ14 is thought to inhibit multiple targets of the cyclooxygenase pathway through a range of bioactive compounds (14). TJ48 improves patient host-immunity and quality of life following chemotherapy (15), and was shown to have anti-angiogenic activity in tumors (16) as well as stimulating hematopoietic stem cell proliferation (17).

TCM provides an opportunity to treat patients in a holistic manner. The polychemical nature of TCMs allows them to target multiple organs in which absorption and metabolism could be quite different. Additionally, the in vivo impact of PHY906 on RNA expression across different tissues (liver, spleen, and tumor) was variable (13). A more detailed analysis of the responses of different organs and tissues to herbal

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formulations could support the TCM theory that there may be a preferential response in certain organs to certain herbs. This work could lead to new applications for TCM.

Challenges of TCM clinical trials

Traditionally, TCM is prescribed as a decoction based on the diagnosis of a practitioner and the individual patient's needs. It is therefore challenging to design randomized, placebo-controlled, dose-escalation studies. Herbal formulas have been administered in capsule form for many years, so it made sense to initiate clinical studies using encapsulated products. Phase 1/2 or phase 2 clinical trials in the United States have suggested that an encapsulated form of PHY906 could have beneficial effects for cancer patients treated with irinotecan or capecitabine for advanced colorectal cancer, hepatocellular carcinoma, and pancreatic cancer (18–22). Recently, clinical trials of PHY906 with irinotecan, sorafenib, or radiation for colorectal cancer, liver cancer, or rectal cancer have been initiated in the United States. They are using a comprehensive systems biology approach to identify predictive pharmacodynamics biomarkers associated with PHY906 treatment, including immunocytokines, metabolomic profiles, herbal metabolites, and circulating tumor DNA. These results may aid in the identification of active compounds and in stratifying patient populations prior to treatment.

A number of herbal products are at various clinical development stages, including Dantonix (T89) (23), Selected Vegetable and Herb Mix (NCT00246727), *Rhodiola rosea* extract (NCT01098318), *Fuzheng Huayu* (NCT00854087), and HMPL-004 (NCT01805791). Thus far, the U.S. Food and Drug Administration (FDA) has approved only two highly purified botanical drugs with defined polychemicals: Veregen Ointment, a green tea extract for the topical treatment of genital warts, in 2006, and Fulyzaq (crofelemer), purified oligomeric proanthocyanidin from *Croton lechleri*, for the treatment of diarrhea in HIV patients, in 2012 (see page S32). FDA does not provide specific quality control guidance for orally administered herbal mixtures. However, they advise that multiple batches of an herbal product go through phase 3 clinical trials to demonstrate consistent efficacy. Although it is currently not required that the active ingredient from the herbal mixture be identified, it is anticipated that FDA will require information from more in-depth studies in the future.

In summary, although TCM formulas often vary, it is possible to make consistent preparations, as exemplified by PHY906. TCMs often have multiple sites of action and the active compounds acting at each site may be different. Systems biology and modern bioinformatics technologies are needed to fully explore the value of TCM for future medical applications.

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The potential role of Chinese herbal medicines in cancer management

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The management of cancer involves multiple disciplines, including surgery, chemotherapy, radiation therapy, targeted therapy, biological therapy, and systemic therapy. In spite of scientific advances and the evidence-based practice of these treatments, limitations in their benefits still exist, resulting in the increasing use of complementary and alternative medicine (CAM) by cancer patients and survivors (1). Numerous preclinical and clinical studies of CAM have been documented over the past decade (1, 2). Recent surveys revealed that the overall prevalence of CAM use among cancer patients in Germany and Ireland was 77% (3) and 32.5% (4), respectively. Other prospective and multicenter studies in the United States have shown that CAM usage was reported in 52–54% of cancer patients (5, 6). One of the modalities commonly used in Chinese cancer patients is Chinese herbal medicines (CHMs). A similar proportion (53%) of cancer patients in southwestern China using CHMs was reported (7). However, the potential benefits of CHMs as a cancer therapy have been less well studied. This article aims to illustrate the potential role of CHMs in cancer management and their adjuvant value in conventional cancer therapy.

Possible CHM targets in cancer management

Although various active antitumor compounds have been isolated from CHMs (8), the therapeutic rationale for the treatment of cancer using CHMs is not limited to only cytotoxicity. Other therapeutic principles include boosting the natural host immune response, improving quality of life, and preventing relapse after surgery (8–10).

In the last decade, scientists (including our research group) have carried out a series of investigations using different experimental systems examining four possible targets of CHM in cancer treatment, namely cytotoxicity (including reversal of multidrug resistance), immunomodulation, anti-angiogenesis, and antimetastasis (Table 1) (11–24).

Despite of the fact that hundreds of CHMs have been used in clinics to treat cancer patients in China (25), only a handful are undergoing clinical trials that meet international quality standards (double-blinded and placebo-controlled). One example is our own clinical trial demonstrating that oral consumption of the *Yunzhi-Danshen* capsule was beneficial in promoting immunological function after conventional

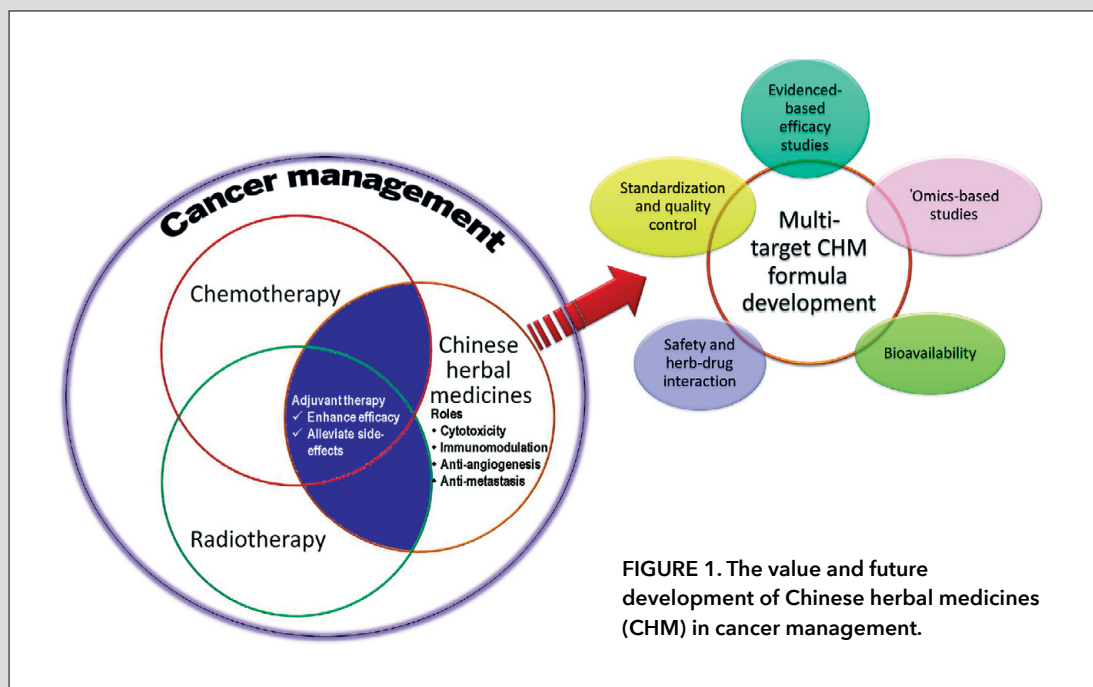


FIGURE 1. The value and future development of Chinese herbal medicines (CHM) in cancer management.

treatment of nasopharyngeal carcinoma patients (26). In the United States, PHY906 (*Huang Qin Tang*) has recently passed a phase 1 trial in colorectal cancer patients (27) and a phase 2 trial in pancreatic cancer patients (28). Furthermore, a Kampō formula (TJ14, *hangeshashinto*) is undergoing a phase 2 trial for gastric cancer patients in Japan (29).

Single mode of action vs. multitarget approach

An herb can serve as a CAM for the treatment of cancer when it is used alone or in combination with other herbs.

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TABLE 1. Possible targets of Chinese herbal medicines (CHMs) in experimental systems and their implications.

Possible targets	Examples of CHM or isolated compounds	Experimental systems	Principal parameters measured	Implications
Cytotoxicity	• Andrographolide from <i>Andrographis paniculata</i> (11) • Curcumin from <i>Curcuma longa</i> (12) • Eriocalyxin B from <i>Isodon eriocalyx</i> (13)	➤ Cancer cell lines ➤ Tumor-bearing animals	◇ Inhibition of proliferation ◇ Induction of apoptosis	Inhibit tumor growth at primary site
	• <i>Hedyotis corymbosa</i> (14) • <i>Scutellaria barbata</i> (15)	➤ Multidrug resistance hepatoma cells	◇ Reverse P-glycoprotein-mediated multidrug resistance ◇ Induction of apoptosis	Improve chemotherapy efficacy
Immunomodulation	• <i>Astragalus</i> species (16) • <i>Coriolus versicolor</i> (17) • <i>Ganoderma sinense</i> (18)	➤ Immune cells (e.g., lymphocytes, dendritic cells) ➤ Tumor-bearing animals	◇ Production of cytokines and/or chemokines ◇ Population changes of certain immune cell types	Improve immune response; strengthen attack system of immune cells against cancer cells
Anti-angiogenesis	• Bigelovin from <i>Inula helianthus-aquatica</i> (19) • n-Butylidenephthalide from <i>Angelica sinensis</i> (20) • Cyclopeptide RA-V from <i>Rubia yunnanensis</i> (21)	➤ Endothelial cells	◇ Inhibition of ✓ proliferation ✓ tube formation ✓ migration	Inhibit new blood vessels formation towards and inside the tumor
		➤ Zebrafish embryos ➤ Tumor-bearing animals	◇ Inhibition of blood vessels growth	
Antimetastasis	• <i>Andrographis paniculata</i> (22) • <i>Camellia sinensis</i> (23) • <i>Ganoderma lucidum</i> (24)	➤ Invasive cancer cell lines	◇ Inhibition of ✓ migration ✓ invasion	Prevent migration of tumor cells from primary site to other organs
		➤ Tumor-bearing animals	◇ Inhibition of metastasis	

Active compounds isolated from herbs can also be developed as anticancer drugs, often aimed at specific targets (8). Certain extracts from single herbs, containing a complex array of constituent molecules, have been shown to exhibit direct or indirect antitumor activities (30, 31). In this case, the extract could be considered to be a multitarget combination therapy. CHMs are generally administered as combinations of multiple herbs, emphasizing one of the CAM principles of treating multiple targets while protecting internal harmony. The multicomponent, multitarget, and synergistic nature of CHMs can thus be fully utilized, particularly in multifactor metastasis management, an active area of research in our laboratory.

Adjuvant therapy

CHM is increasingly being used in conjunction with

chemotherapy and radiotherapy, with the hope that it can alleviate or even eliminate the adverse effects of the treatments, as well as improve their overall efficacy (1, 32). Previous meta-analyses have suggested that *Astragalus*-based CHM may increase the effectiveness of platinum-based chemotherapy (33). Recent studies showed enhanced anti-tumor effects (27, 28), improved prognosis (34), and beneficial effects on the survival of cancer patients (35) receiving chemotherapy in combination with CHM, emphasizing the potentially positive adjuvant role of CHMs in cancer therapy.

The way forward

The potential roles of CHM in cancer management are summarized in Figure 1. Future development of CHMs will require extensive further research such as evidenced-based efficacy studies, standardization and quality control, 'omics-

based studies into the mechanisms of action, bioavailability studies, and careful examination of safety and herb-drug interactions. We suggest that future research should focus on developing CHM formulae for multitarget therapy approaches to improve prognosis and survival outcomes. Further research on interactions between CHM and chemotherapeutics can provide more information on the safety and/or potential benefit of adjuvant therapies. In this way, the full holistic benefits of Chinese herbal medicines for cancer management can be realized.

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Evaluating the safety of herbal medicines: Integrated toxicological approaches[‡]

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Many complex herbal mixtures are already commonly used worldwide, either for primary health care or as complementary or alternative medicines (1). Ancient traditional remedies—notably traditional Chinese medicine (TCM) and Ayurveda—have been passed down and refined over their long history of clinical use. Often perceived as innocuous, some herbs exhibit delayed or cumulative toxicity that may not be obviously attributed to the herbs, but instead identified by serendipity or unfortunate clinical findings. Given the large number

of herbal products on the market and the relatively low budgets available for research to date, safety assessment in accordance with modern guidelines has been carried out on relatively few herbs (2). Despite these concerns, a recent survey of practitioners in Europe and China, although limited in scope, provides some reassurance that the vast majority of herbs in regular use are known to be relatively safe (3). Reports of serious adverse events regarding TCM mainly concern those that are used very rarely in Europe and extremely carefully in China (4). It is also important to differentiate between intrinsic herbal toxicity and malpractice: A recent Hong Kong study found that, of 52

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clinical case reports of aconite poisoning, the majority were actually related to poor-quality herbs, poor prescribing practices, or dispensing errors (5). In Europe, adverse events have mainly resulted from contaminated products and a practitioner's incompetence, rather than herbal medicines being inherently risky. The reasons why the safety of herbal products for clinical use may be compromised are summarized in Figure 1. Pharmacovigilance systems have only recently been established for herbal medicines, thus the true incidence of adverse events may be under-reported; nevertheless the available data indicate that their overall safety is better than would be suggested by widely publicized incidents involving adulterated products and herbs already known to be toxic.

Preclinical and clinical toxicology research on herbal medicines is in most cases inadequate or insufficient to fulfil official medicine registration requirements. Safety has primarily relied on rational clinical use, avoiding drug interactions, ensuring correct botanical identification and labeling, quality control for adulterants and contaminants (mycotoxins and heavy metals, among others), mastery of sometimes complex processing methods, detection of new structural alerts, and avoidance of known toxicophore-bearing species. These measures are, however, inadequate for evaluating complex mixtures of incompletely known composition and where adverse events may take months or even years to manifest (2, 4).

Preclinical toxicology assessments for medicinal herbs

There is a need for realistic evaluation of the toxicity of herbal drugs as they are prepared traditionally and used clinically. Special attention must be paid to insidious toxicities which are not easily detected by pharmacovigilance, including genotoxicity, carcinogenicity, and developmental toxicity (6).

The complexity of herbal medicines is a major issue for safety assessments since the often large number of components have variable potencies and affinities for various targets. Toxicity, as with efficacy, may be the result of a mixture of active compounds rather than a single chemical entity, therefore different methods of testing are required to replace or supplement those routinely used in classical medicine risk assessment. Toxicological and pharmacokinetic studies of individual constituents of herbal materials must be compared with qualitative

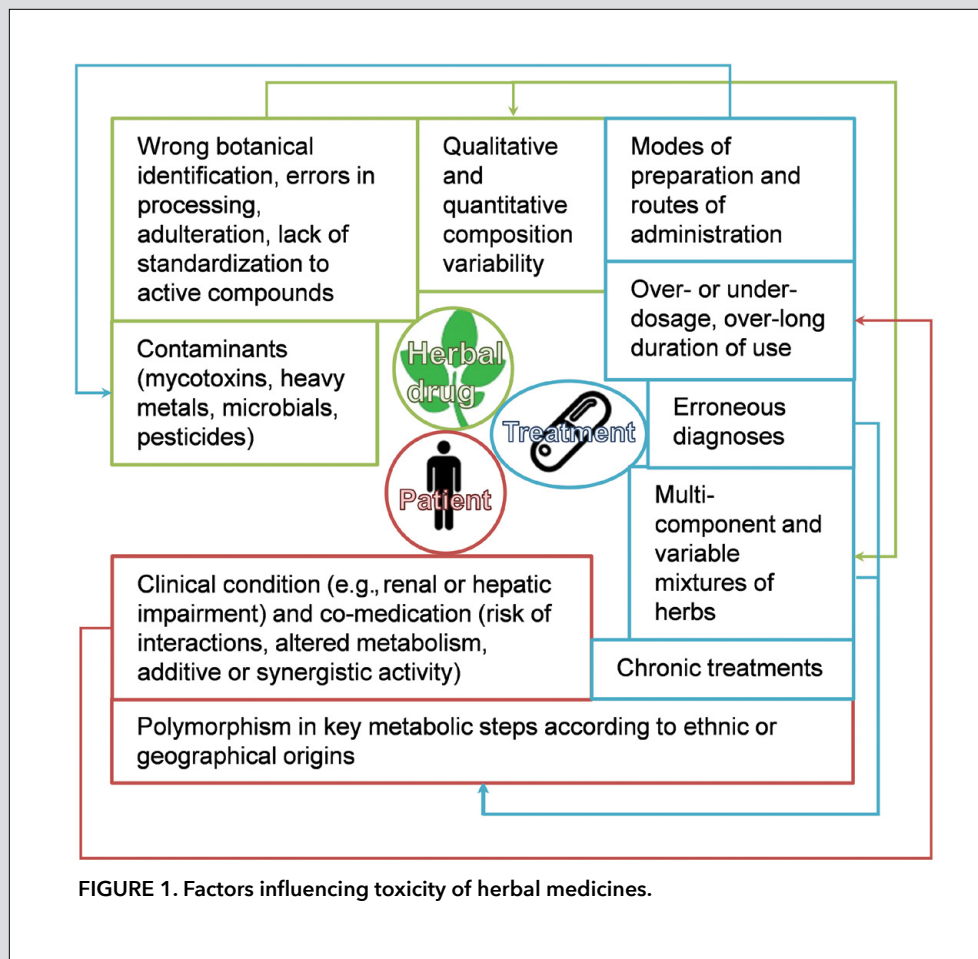


FIGURE 1. Factors influencing toxicity of herbal medicines.

and quantitative profiling data for the total extract, using appropriate analytical tools (7), in vitro and in silico methods (2), and modern screening techniques (8). In vitro models using subcellular organelles and human-derived cellular systems form the basis of pharmacological and toxicological screenings and are generally low-cost, efficient, and easy-to-handle. However, they are reductionist techniques, which focus on nonspecific phenomena at the cellular level and neglect pharmacokinetic and pharmacodynamic aspects relevant to clinical conditions that affect the nature and concentration of active moieties. Therefore, the recently developed in vitro 3-D tissue and organ-on-a-chip models—which simulate the in vivo cell microenvironment—should also be considered for toxicological and pharmacological herbal assessment. Herbal matrix effects must also be considered in the experimental design of toxicity screens. In vitro and in silico models should therefore be used only for comparison of toxicity profiles or deciphering toxic activities at the molecular level rather than for direct estimation of toxicological risks. In silico methods linking components with potential targets can provide an indication of adverse effects that may result from having multiple constituents and signal the need for a closer investigation of relevant toxicological and pharmacological issues. Measuring the kinetic characteristics of metabolism, transport, disposition of active components, and matrix effects should provide

TABLE 1. Integrated strategies for the toxicological assessment of herbal drugs.

Strategy	Information yielded
Analytical chemistry	• Chemical profiles, identification of components of interest or concern
In vitro, in silico methods and nonmammalian animal models, eventually combined with 'omics-based methods	• Identification of main pharmacological and toxicological issues • Comparison of toxicity profiles • Deciphering toxic activities at a molecular level
Mammalian models, eventually combined with 'omics-based methods	• Acute and repeated dose toxicities (animal models only) • Pharmacokinetic data, metabolism
Clinical studies, eventually combined with 'omics-based, personalized methods	• Identification of pharmacological and toxicological issues • Identification of exposure or toxicity biomarkers • Genotoxicity, carcinogenicity; reproductive and developmental toxicity
Pharmacoepidemiology and pharmacovigilance	• Evaluation of safety in clinical use • Identification of clinically relevant adverse effects

the necessary information to ensure that in vivo studies effectively integrate these critical parameters, and offer the opportunity to consider the realistic exposure levels in various organs and the biological activities at concentrations relevant to human consumption (9, 10). In vivo models such as zebrafish, *Drosophila*, and *Caenorhabditis elegans* offer convenient methods for high throughput toxicity assessment and inform further toxicological considerations, but must be cautiously interpreted due to their differences from human physiology.

Preclinical pharmacological and toxicological assessment of herbal medicines therefore needs to combine 'omics, bioinformatics, and network-centred systems biology with in vitro and in vivo assays, targeting studies on biomarkers identified from animal experiments (11–13), as summarized in Table 1. At all stages, it is imperative to remember the multicomponent nature and variability of herbal remedies, and that studies using wrongly identified or processed, contaminated, or adulterated preparations are not only useless but misleading for toxicological evaluation (14).

Pharmacovigilance and the clinical assessment of herbal medicines

To compensate for the lack of safety evaluations of herbal drugs, empirical clinical assessment must be carried out through pharmacovigilance. This can only happen if herbal products and practitioners are regulated in some way so that adverse events can be traced to a particular medicine or practice. Continuing professional development should be mandatory to ensure that practitioners and other healthcare workers are able to provide safe and appropriate advice to patients.

New techniques are being developed for evaluating the toxicological risk of herbal drugs in a clinical context. Urinary metabolomics appears to be a promising noninvasive tool for the prediction of specific organ toxicity (15). This type of active surveillance allows better assessment of causality and detection of possibly harmful components of the treatment, and can be adapted for other herbal medicines.

In most herbal traditions, certain combinations of herbal drugs are believed to reduce toxicity and others to enhance it. To rationalize formulations and enhance safety, it is crucial to take into account the complex chemical interactions taking

place in herbal mixtures (and mixtures of mixtures) using systems-based approaches.

Conclusions

Herbal safety is compromised when any element of the herbal medicine-practitioner-patient triangle is flawed. To meet the challenge, integrating emerging systems-based technologies with conventional means is essential. There remains a clear and urgent need for novel methods able to rapidly pinpoint indicators of major mid-term and long-term toxicities, to yield warning signals, and identify those herbal drugs and formulae that need further toxicological investigation. Recent advances in 'omics and bioinformatics techniques have made it possible to investigate efficacy and toxicity at the organism level and in an individual manner. When further developed and validated, these methods should enhance the detection of insidious toxicities, provide the necessary background information for effective pharmacovigilance, and aid mechanistic studies of specific herbal medicines.

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Combining 'omics and comparative effectiveness research: Evidence-based clinical research decision-making for Chinese medicine

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Systematic reviews and meta-analyses of Chinese medicine trials have demonstrated issues with consistent quality, and an evidence gap between the practice of, and research on, traditional treatments. Clinical practice is built on knowledge, clinical experience, and patient preferences, all of which can be influenced by values and belief systems. A current movement in clinical medicine research, known as comparative effectiveness research, supports the development of evidence-based recommendations to enable more informed decision-making in the clinic and more valid health policies that also meet the criteria for practicing “P4” (predictive, preventive, personalized, and participatory) medicine. Creating a modern, strategic research framework for Chinese medicine that takes into account the stakeholders’ perspectives, follows a patient-centered approach, uses mixed methods research methodologies, and combines modern scientific techniques such as systems-biology-based ‘omics technologies would be beneficial for bridging the gap between Chinese medicine theory and modern clinical research methodologies.

Background

The most prominent medical research model compares “one disease, one treatment;” however, this strategy often does not have comparable clinical practices. Further, clinical trials based on such research models are usually performed in a standardized setting with a carefully selected patient group, and often produce results that are neither generalizable nor able to guide and inform clinical care. Systematic reviews and meta-analyses summarizing such trials might even be misleading for various chronic diseases, but especially for complex conditions such as diabetes, cardiovascular disease, and pain, which often occur in patients with multiple comorbid diseases who are receiving a number of different treatments. Decision makers—clinicians, patients, and funders—require studies that are comparable with actual treatment options in real life settings (1). Comparative effectiveness research (CER) is intended to provide real-world evidence that helps clinicians and patients choose the options that best fit the individual’s needs and preferences (2). CER involves the stakeholders’ needs at all relevant steps and includes a number of different types of research designs, clinical trials being one of them. These so called pragmatic trials are characterized by including more “real-life” patients presenting in routine clinical care including

those that have comorbidities and use comedication, providing more individualized treatments, using patient-relevant outcomes, and being performed in a setting that is “in line” with routine clinical care (3).

Chinese medicine has been historically based on a descriptive and phenomenological approach and has relied on complex mixtures of herbal medicines as well as nonpharmacological interventions such as acupuncture and lifestyle advice. Research on some of the individual treatment components of Chinese medicine (e.g., acupuncture) have already made relevant contributions to CER evidence (4) and provided guidance for the design of further acupuncture CER (5). However, in clinical practice Chinese medicine is a complex intervention that focuses on the whole system’s organization, and not on physiological pathways or single targets. Treatments are built on knowledge accumulated from ancient texts, experts, clinical experiences, and patient preferences, which are influenced by values and belief systems (6). Chinese medicine has a fundamental patient participation element, including general lifestyle aspects (e.g., diet and exercise) in the complex intervention strategies. Traditional Chinese diagnoses (or “syndrome differentiation”), a comprehensive analysis of clinical information from a Chinese medicine perspective (e.g., information derived from case taking, examining the patient’s pulse and tongue), is used to guide personalized treatment options (7). Each syndrome consists of symptoms that determine their own unique treatment protocol.

Integrating syndrome differentiation with the biomedical techniques of modern clinical practice would be helpful for determining personalized treatments (8). The beta version of the International Classification of Diseases (ICD) 11 already allows Chinese syndrome coding in addition to Western diagnoses (9). Currently, these Chinese syndromes are considered important tools for predicting disease (10, 11) and ongoing efforts are correlating them with measurable biomarkers. Recently, a systems-biology-based approach has been utilized for Chinese medicine syndrome differentiation studies enabling the stratification of patient populations (8). This strategy may help researchers optimize their clinical trial design by having the ability to determine which patients are most appropriate for a specific intervention. One advantage of a systems biology approach is that it aims to understand both the connectivity and interdependence of individual components within a dynamic and nonlinear system, such

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as traditional medicine, as well as the properties that emerge at different organizational levels. In addition, the use of 'omics techniques (including genomics, proteomics, metabolomics, transcriptomics, and lipidomics) align very closely with the concepts and practices of Chinese medicine (8). Using 'omics-based techniques in Chinese medicine presents the unique opportunity to better understand both personalized diagnoses and the systems-based interventions of Chinese medicine (12). Two recent studies on rheumatoid arthritis have demonstrated the potential of this approach for conventional medicine. By combining Chinese syndrome diagnoses with the identification of biomarkers and the use of genomics to determine the diagnostic subgroups, opportunities for better treatment outcomes were provided (13, 14).

In the future, it is hoped that P4 medicine will enable the prediction and prevention of diseases rather than reactive health care (15). Understanding how genomic differences in individuals, along with an individual's environmental exposures, influence biological systems has the potential to enable medical professionals to make patient-specific predictions followed by personalized treatments or, even better, preventive interventions. In the future, health care consumers will be increasingly equipped with their personal health information, including genome sequences, molecular profiles of diseased tissues, and biomarker panels (16). Participation from all major stakeholders will be needed to provide clinical and health policy guidance for this new medical era.

Moreover, one strategy CER could benefit from is incorporating at least genomics as part of a future research approach (17). Including 'omics techniques into CER would be a new area for Chinese medicine—one yet to be incorporated into methodological CER guidance (18). It could directly bridge the gap between the personalized approach of Chinese medicine theory and Western science. The trend toward P4 medicine in CER also creates an ideal setting to provide information from large, real-life populations. Furthermore, the characteristics of P4 medicine dovetails well with the foundations of Chinese medicine.

Below, we have proposed some recommendations that combine the underlying concepts of CER with systems biology based 'omics technologies in order to collect scientific evidence for Chinese medicine that can be used to broaden the use of traditional medicine and optimize clinical decision-making.

Recommendations

When there is insufficient evidence for a treatment, a combination of both existing data from trials and systematic reviews of the literature should be used to inform future research and support clinical decision-making. This requires:

- Tools for systematic reviews and meta-analyses that provide comprehensive information about both the context of the studies and the extent to which the results are generalizable
- Secondary data-analyses of existing studies that have utilized a systems biology approach to identify possible associations between syndrome differentiation in Chinese medicine, other patient characteristics, and disease progression.

Future clinical research on Chinese medicine would benefit from combining the evolving CER methodology, modern systems biology 'omics approaches, and patients' needs during routine care. In practice, this would require:

1. Strategic clinical trial designs:

- Trials that include heterogeneous and "realistic" patient samples, are performed in settings reflective of a patient's routine care, and have sample sizes that facilitate further subgroup analyses
- Trial designs that balance multiple factors, including the type of study and context of the relevant diagnostic scenarios with both qualitative information (e.g., Chinese syndrome differentiation, patient preferences, and expectations) and quantitative parameters (e.g., systems biology including 'omics analysis)
 - The development of guidance on the appropriate outcome measures for future research
 - Realistic treatment protocols that shift patient treatments toward a personalized care model that allows the use of complex interventions, including lifestyle factors, and reflects the changes patients experience during routine clinical practice; 'omics-based analyses should be used to answer open questions about the complex pharmacological networks that are activated by complex herbal preparations (12)
 - More studies with a diagnostic focus are needed to learn how predictive factors from Chinese medicine correlate with modern quantitative parameters.

2. Stakeholder involvement:

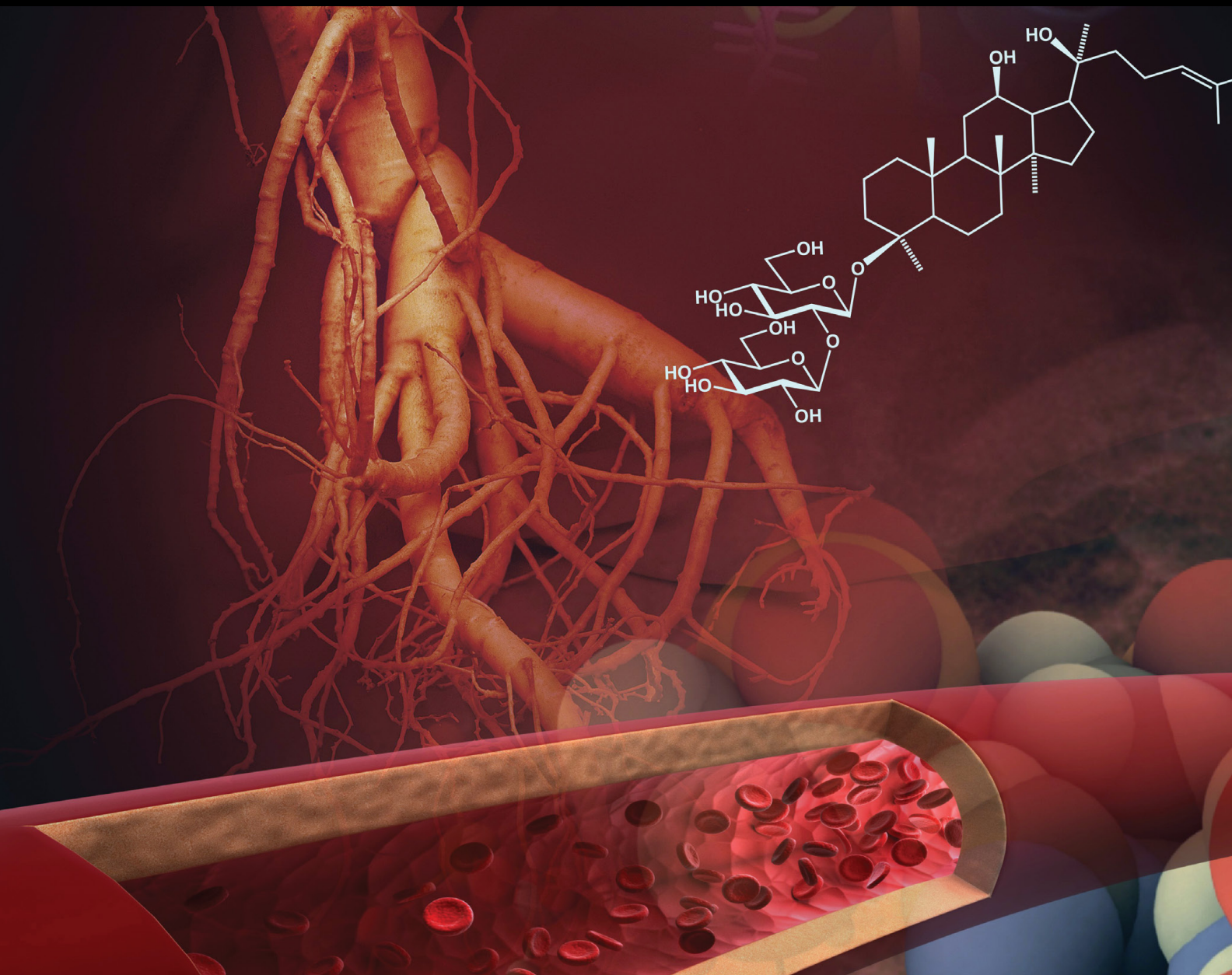
- Each stakeholders' needs (patients, clinicians, government, and payers) should be taken into account when identifying a study's focus, planning clinical trial designs, and interpreting results; to allow a high level and systematic inclusion of stakeholder viewpoints, both qualitative and quantitative research methodologies would need to be applied (19).

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