

# Anticancer potential of *Salvia miltiorrhiza* and its tanshinones: an efficacy perspective

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**Abstract:** *Salvia miltiorrhiza* Bunge (Danshen in Chinese or Tanshen in Anglicized literature) is a well-known Traditional Chinese Medicine herbal remedy for treating cardiovascular- and cerebrovascular-related disorders. To date, >40 hydrophobic tanshinones and structurally related compounds have been isolated from the Danshen root, as have some 50 hydrophilic phenolics and other minor components. In the past 2 decades, a large quantity of literature has reported inhibitory activities of tanshinones against cancers of various organ sites in cell culture models, and in some cases with efficacy confirmation in preclinical animal cancer models. This study follows up on a 2012 review we published on the sources, pharmacokinetics, and anticancer activities of tanshinones. Here, we update on the recent progress in understanding the anticancer potential of tanshinones and derivatives and critically assess merits of these entities for future research and development. Overall, potency data from in vivo efficacy assessment experiments in preclinical models varied from nil for chemoprevention of a prostate carcinogenesis model to strong inhibition of some xenograft or allograft models. Lack of uniformity of excipients, doses, and routes of administration aside, we caution that the reviewed data should be appreciated in balance of publication bias exemplified by our own data from primary carcinogenesis study and false positivity. Novel formulations and chemical modifications had been made to improve the poor solubility and bioavailability of tanshinones. Human clinical studies so far dealt with case reports of tanshinone IIA use and small-scale trials on Danshen-containing formulas with chemotherapy for cancers of multiple organ sites in People's Republic of China. Available human data are not sufficient for supporting any anticancer indication of tanshinones.

**Keywords:** human studies, cancer therapy, cancer chemoprevention, preclinical efficacy

## Introduction

The dried roots of Danshen (in Chinese) or Tanshen (in Anglicized literature) *Salvia miltiorrhiza* Bunge have been used in Traditional Chinese Medicine (TCM) in People's Republic of China and Asian countries as preventive or therapeutic remedies for coronary heart diseases, vascular diseases, stroke, hyperlipidemia, endangiitis, arthritis, and hepatitis.<sup>1,2</sup> Fufang Danshen, a composite multi-herbal TCM formula containing Danshen as the major ingredient, has been officially listed in the Chinese Pharmacopoeia for many indications. Recently, Danshen has been recorded as a monograph in United States Pharmacopoeia USP 37/NF 32 ed.; 2014). In the USA, a Phase II clinical trial has been completed with Fufang Danshen Dripping Pills (best-known commercialized forms of mixture of Danshen, *Radix notoginseng* [San Qi in Chinese] and *borneolum* [Bing Pian in Chinese]) for evaluating the efficacy and safety in patients with chronic stable angina pectoris (Clinicaltrials.gov NCT00797953). A large-scale Phase III trial

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with the Dripping Pills (NCT01659580) is being conducted in the USA, Canada, Mexico, and several European countries for the same indication. Three Phase I trials (NCT01473888, NCT01475279, NCT01679028) have been completed to study safety, interaction with P450, or Warfarin use.

To date, >90 chemicals have been identified from Danshen. They belong to two major groups: >40 hydrophobic<sup>2,3</sup> and >50 hydrophilic compounds.<sup>1</sup> Tanshinones are hydrophobic abietane diterpenes. First isolated and named in the 1930s by Nakao and Fukushima,<sup>4</sup> they now include (Figure 1A) cryptotanshinone (CT, **1**), tanshinone IIA (TIIA, **2**), tanshinone IIB (TIIB, **3**), tanshinone I (TI, **4**), dihydrotanshinone I (DH-TI, **5**), tanshindiols B and C (**6**, **7**), methyltanshinone (**8**), isotanshinone I, isocryptotanshinone I, and isocryptotanshinone II.<sup>1,3,5-9</sup> Tanshinlactone (**9**) and neo-tanshinlactone (**10**) are structurally related to tanshinone backbone and have been reported to possess anticancer activities as well.<sup>2</sup>

The hydrophilic chemicals isolated from Danshen include danshensu (also known as salvianic acid A or salvianic acid B), protocatechuic acid, protocatechuic aldehyde, rosmarinic acid, and salvianolic acids A, B, and C, with the last three also known as lithospermic acids A–C, or magnesium lith-

ospermates A–C, or tanshinoates A–C.<sup>1,5,10,11</sup> Other minor compounds include baicalin, 5,3'-dihydroxy-7,4'-dimethoxy flavanone, ursolic acid,  $\beta$ -sitosterol, daucosterol, vitamin E, and tannin.<sup>12</sup> Tanshinones and the hydrophilic Danshen compounds have been extensively investigated for their cardiovascular activities.<sup>13-16</sup>

The chemistry, biosynthesis, and total chemical synthesis of tanshinones have been comprehensively reviewed.<sup>2,3</sup> Figure 1B presents a summary scheme of current knowledge of the botanical chemical relationship among the major tanshinones. In our previous paper,<sup>17</sup> we reviewed the natural and alternative sources of tanshinones and their pharmacokinetic (PK) characteristics, and provided an in-depth analysis of their anticancer activities *in vitro*.

The two most extensively studied tanshinones for anticancer activities are TIIA (**2**) and CT (**1**), followed by TI (**4**) and DH-TI (**5**). Some recently identified tanshinone-related compounds<sup>18-21</sup> including tanshinlactone (**9**) and neo-tanshinlactone (**10**, Figure 1A) appear to have greater cytotoxic potency and better selectivity than corresponding tanshinones.<sup>22</sup> In addition to our 2012 paper,<sup>17</sup> others have also reviewed potential anticancer properties of

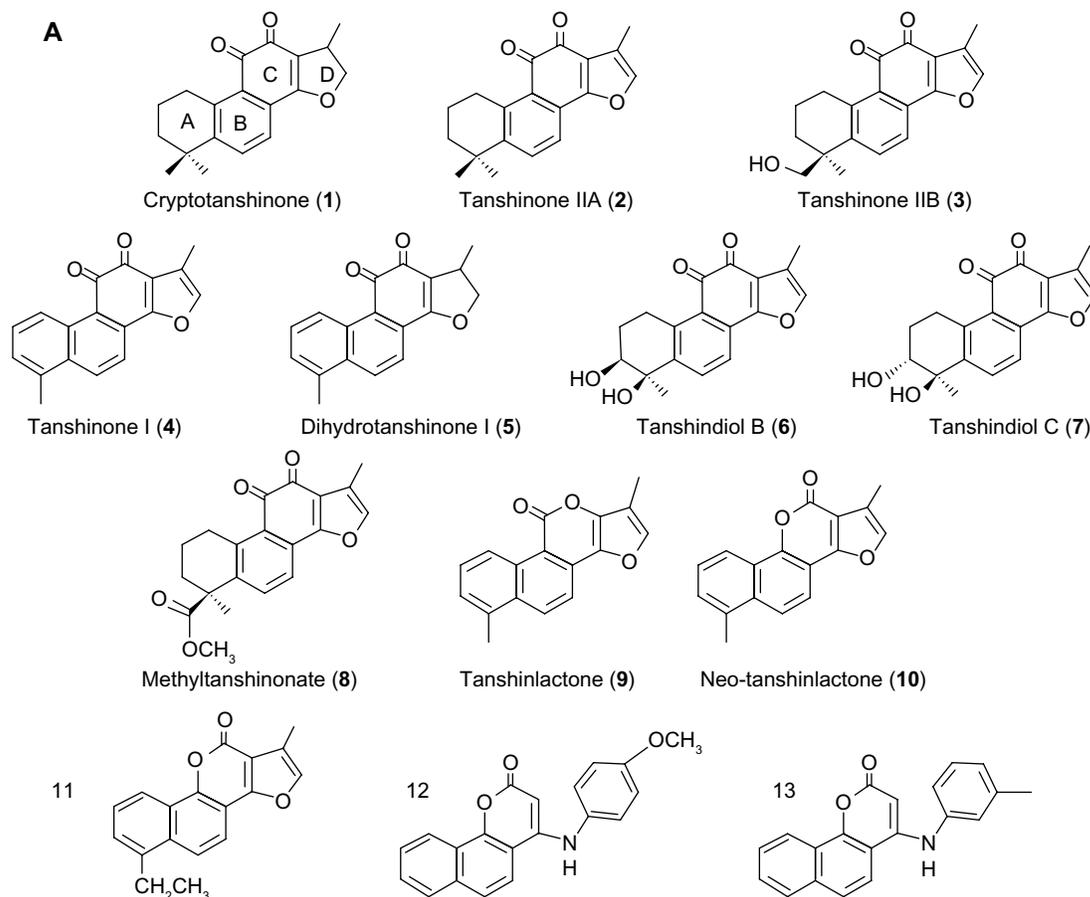
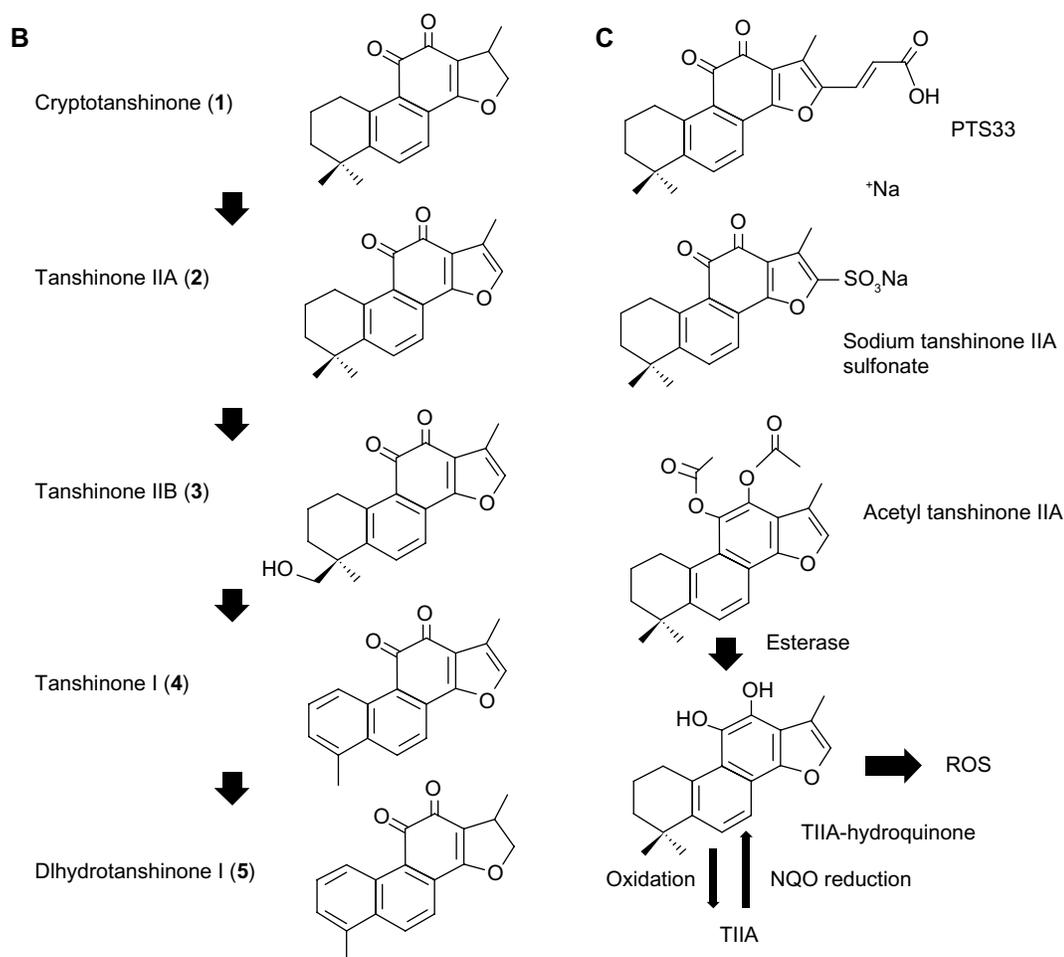


Figure 1 (Continued)



**Figure 1** Structure of Danshen tanshinones, their likely botanical synthesis pathway, and select synthetic derivatives.

**Notes:** (A) Chemical structures of major tanshinones, tanshindiols, and tanshinolactones. (B) Botanical relationship among the major tanshinones in Danshen *Salvia miltiorrhiza*. (C) Reported structurally modified tanshinones.

**Abbreviations:** ROS, reactive oxygen species; TIIA, tanshinone IIA.

tanshinones from various perspectives.<sup>2,23</sup> In the current paper, we focus on anticancer efficacy evaluation outcomes in preclinical animal models and human clinical use experiences with tanshinones and Danshen-containing TCM preparations, aiming to provide an objective assessment of the merit of these entities for future research and development.

## PK characteristics of tanshinones and herbal interactions

### The PK characteristics of singly administered tanshinones or herbal mixtures

The PK parameters of major tanshinones (CT, TIIA, TI, DH-TI) have been studied in animal models such as rats, rabbits, and pigs, almost exclusively by scientists in the People's Republic of China. Readers are referred to our previous review for specifics.<sup>17</sup> Some general conclusions are summarized here.

We note upfront that there were great variations among the analytical methods of extraction and detection, tanshinone dosages, and excipient solvents/vehicles used for administration that made generalization challenging.

First, being essentially insoluble in aqueous media, tanshinones are poorly bioavailable with conventional delivery formulations through oral administration (po). For example, when CT was dosed at 100 mg/kg body weight, the po and intraperitoneal (ip) bioavailability in rats was estimated as 2.1% and 10.6%, respectively.<sup>24</sup> Maximal/peak concentration ( $C_{max}$ ) values were reported in the nanomolar to sub-micromolar range after po administration in most studies on tanshinones.

Corroborating the reported concentration ranges, we found, in a single-dose PK experiment with male C57BL/6 mice fed a AIN93M purified diet gavage-dosed 1.5 mg TIIA/mouse (standardized to body weight of 20 g; ~75 mg/kg body weight) in 200  $\mu$ L corn oil, that the plasma TIIA  $C_{max}$  is 81.9 ng/mL (~0.28  $\mu$ M) at time to peak concentration = 2.4 hours (Figure 2; Wu et al, unpublished data). The mouse PK data are important

because anticancer efficacy studies have been done almost exclusively in murine models.

Second, the plasma PK curves of most intravenous (iv)-administered tanshinones follow two-compartment or multiple-compartment models in model animals such as rats, rabbits, and pigs; that is, a very fast component with a half-life of shorter than 10 minutes that likely represents rapid uptake by organs and tissues from the blood, and a slower component with half-life ranging 1–3 hours that likely represents systemic clearance balanced by tissue–blood redistribution. In the mice, we estimated that the slower clearance half-life  $T_{1/2}$  was 3.4 hours (Figure 2B).

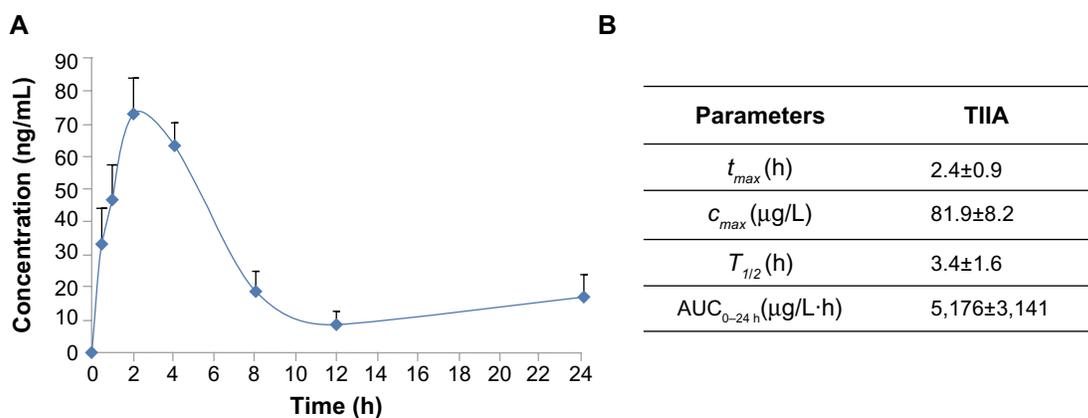
As with other herbal preparations, interactions among Danshen chemicals will invariably occur after administration of tanshinone mixtures or Danshen extracts to affect their PK parameters. For example, Guo et al<sup>25</sup> studied the interactions between tanshinones and Danshen polyphenolic extract after iv administration in the rat model. They showed that plasma TIIA concentration at 5 minutes post-iv ( $C_{5\text{ min}}$ ) was significantly increased by including the salvianolic acid B extract in the dosing emulsion (up to 28-fold). Another study reported that such interactions did not appear to be as dramatic when examined by oral delivery,<sup>26</sup> perhaps a reflection of the involvement of liver first-pass metabolism of these compounds to attenuate the magnitude of their interactions.

Third, in a number of species including pigs and rats, there were reports of in vivo metabolism or biotransformation of tanshinones, especially CT to TIIA.<sup>27–29</sup> Liu et al reported in 2013 that the glucuronidation enzyme UGT1A, in particular UGT1A9, can compromise TIIA

cytotoxicity via reducing its intracellular exposure and switching the NQO1-mediated redox cycle for reactive oxygen species (ROS) generation to metabolic elimination of TIIA.<sup>30</sup> The expression and activity of UGT1A affected the intracellular accumulation of TIIA and the severity of the apoptosis in colon cancer cells by ROS.<sup>30</sup> Therefore, interpretation of animal efficacy studies should be made with knowledge of not only the administered tanshinone but also its in vivo metabolites and the metabolic flux.

## Novel formulations to improve solubility and PKs

To overcome poor aqueous solubility of tanshinones, some groups have experimented with novel pharmaceutical formulations. Solid lipid nanoparticles (SLNs) can improve bioavailability of poorly water-soluble drugs.<sup>31</sup> TIIA-loaded SLNs coated with poloxamer 188 extended plasma elimination time and mean residence time of TIIA in rats and reduced opsonization by serum proteins and macrophage uptake.<sup>32</sup> Polylactic acid nanoparticles containing TIIA exhibited better pharmaceutical effects than neat compound in mice.<sup>33</sup> Similarly, CT administered in an SLN formulation significantly improved its absorption and decreased its metabolism to TIIA in a rat model.<sup>34</sup> Nanoparticles made with methoxy polyethylene glycol, polylactic-co-glycolic acid, poly-L-lysine, and cyclic arginine-glycine-aspartic acid with TIIA payload demonstrated extended TIIA releasing time, and improved hepatocellular carcinoma (HCC)-inhibitory activity.<sup>35</sup> Solid inclusion complexes of TIIA and TI with naturally occurring, water-soluble cyclic oligosaccharide  $\beta$ -cyclodextrins



**Figure 2** Tanshinone IIA pharmacokinetic study in mice.

**Notes:** (A) Concentration–time profile of TIIA in mouse plasma after gavage administration of 75 mg TIIA/kg body weight (mean  $\pm$  SD, n=5). Liquid chromatography–tandem mass spectrometry method was used for quantitation. (B) Pharmacokinetic parameters of TIIA in mice. Values are mean  $\pm$  SD (n=5).

**Abbreviations:** TIIA, tanshinone IIA; SD, standard deviation; AUC, area under the curve;  $t_{max}$ , time to peak concentration;  $C_{max}$ , peak concentration;  $T_{1/2}$ , time for concentration to decrease by half.

have been made and tested as well.<sup>36</sup> An article published in Chinese reported a self-microemulsifying drug delivery system (SMEDDS) to increase solubility and intestinal absorption in situ of tanshinones.

Several papers since the publication of our 2012 review<sup>17</sup> added further novelty and refinement. For example, Ma et al encapsulated TIIA or tanshinone mixture containing TI, TIIA, TIIB, DH-TI, and CT into a microemulsion composed of phospholipid, ethyloleate, glycerol, and Pluronic F68 and found enhanced apoptosis in vitro with murine HCC models.<sup>37,38</sup> The microemulsion exerted greater antitumor effects of both TIIA and tanshinone mixture than respective free forms in murine allograft models.<sup>37,38</sup> In 2014, Zhang et al reported a mixed micelle system of D- $\alpha$ -tocopheryl polyethylene glycol succinate-graft-poly (D, L-lactide-co-glycolide) copolymer and Pluronic F68 to encapsulate TIIA.<sup>39</sup> They showed that TIIA-loaded mixed micelles exerted higher in vitro cytotoxicity and pro-apoptotic effect than free TIIA against HCC HepG2 cells. Their PK data revealed that TIIA delivered by mixed micelles significantly prolonged the circulation time and improved its bioavailability in rats.<sup>39</sup>

In addition, chemical structural modifications were made to improve the bioavailability or bioactivity of tanshinones such as sodium tanshinone TIIA sulfonate (STS) and acetyl tanshinone IIA (ATA) (Figure 1C) (described in the “Structural modifications of tanshinones” section). Liang et al obtained two new glycosylated derivatives of TIIA by microbial transformation of TIIA using *Cunninghamella elegans* AS 3.2028, namely hydroquinone TIIA 11-O- $\beta$ -D-glucopyranoside (TIIA 11-glu) and hydroquinone TIIA 12-O- $\beta$ -D-glucopyranoside.<sup>40</sup> The solubility of TIIA 11-glu in 50% methanol was ~50-fold that of TIIA (84.6 vs 1.7  $\mu$ g/mL). PK study in mice with TIIA 11-glu after gavage administration showed detectable TIIA 11-glu and TIIA, whereas TIIA gavaged similarly did not reach the detectable level. Therefore, in addition to chemical modification approaches, biotransformation through glycosylation could increase the oral absorption of TIIA.

## Anticancer efficacy of tanshinones in preclinical animal models

### Cancer therapy or adjuvant chemoprevention models

To date, more than 30 papers reported the evaluation of in vivo efficacy of tanshinones in preclinical animal models of cancer. Table 1 summarizes these studies. These studies used xenograft tumors or allograft tumors in mice. Routes of delivery of tanshinones varied among studies, including gavage,

ip injection, iv, or even subcutaneous (sc) injection close to the tumor site. The excipient solvent vehicles involved corn oil, Tween-20:ethanol, dimethyl sulfoxide, saline, conditioned medium, and special formulations (nanoparticles, emulsions, mixed micelles), or were not described in some studies. We would caution readers to be aware of publication bias against negative efficacy outcomes that were not published and of false positive outcomes in published studies. The poor solubility of tanshinones in organic solvents, let alone aqueous media, presents tough challenges to ensure consistent and reliable delivery, and therefore, the comparability among studies from different papers is low. One should be very suspicious of studies reporting delivery of tanshinone(s) in aqueous vehicles such as saline unless special formulation was done to first disperse the tanshinone(s) into a water-miscible form.

A number of recently published studies are highlighted here. Li et al<sup>41</sup> found that TIIA inhibited the angiogenesis and growth of MDA-MB-231 human breast cancer xenograft in athymic nude mice along with a suppression of HIF-1 $\alpha$  and VEGF. Their study suggested the mTOR/p70S6K/4E-BP1 signaling pathway as a potential target for TIIA. Lin et al<sup>42</sup> reported that TIIA decreased cell proliferation and mammosphere formation by enriched human MCF-7 breast cancer stem cells (CSCs) in vitro. In xenograft model generated with CSC-rich MCF-7 mammospheres, treating mice with TIIA of 10, 20, and 40 mg/kg doses by ip injection three times a week for 4 weeks inhibited tumor growth by 39%, 48%, and 58%, respectively. Similarly, by sc injection every other day, Chiu et al<sup>43</sup> showed that TIIA 60 or 90 mg/kg dissolved in corn oil suppressed LNCaP prostate cancer (PCa) xenograft growth by 57% and 86% after 13 days of treatment, respectively. Upregulation of GADD153/CHOP and caspase-3 activation were observed in TIIA-treated tumors, supporting endoplasmic reticulum stress induction and apoptosis in PCa in vivo. Munagala et al<sup>44</sup> demonstrated a significant repression of the HPV oncogenes by TIIA in human Ca Ski epidermoid cervical cancer by ip injection of TIIA, 30 mg/kg body weight, every other day for 8 weeks, and tumor volume was inhibited by 66% in athymic nude mice. Chen et al<sup>45</sup> used patient-derived lung cancer tissues (PDX) to engraft (~3 $\times$ 3 $\times$ 3 mm<sup>3</sup> pieces) the right hind limbs of male athymic nude mice and treated them daily with CT (100 mg/kg body weight) by sc injection around the xenotransplantation area for 20 days. They found that CT not only inhibited the growth of xenografted human lung cancer in vivo but also improved the physical and mental status of tumor-bearing mice.

**Table 1** Summary of in vivo efficacy studies in preclinical animal models of cancer

Tanshinone	Route/frequency	Dose, range	Vehicle	Cancer model	Efficacy
TIIA	Gavage/daily	20 mg/kg	Corn oil	Colo205 colon Ca xenograft	Yes <sup>46</sup>
TIIA (plus 5F)	Gavage/twice per week	20 mg/kg	Corn oil	Colo205 colon Ca xenograft	Yes <sup>47</sup>
TIIA	Gavage/daily	25 mg/kg	Corn oil	LNCaP prostate Ca xenograft	Yes <sup>48</sup>
TIIA	Gavage/every other day	20, 60 mg/kg	Corn oil	MDA-MB-231 breast Ca xenograft	Yes <sup>49</sup>
TIIA	Gavage/daily	20, 80 mg/kg	ND	Liver metastasis of SW480 colon Ca	Yes <sup>50</sup>
TIIA	Gavage/daily	150, 450, 1,350 mg/kg	ND	Lung metastasis of HepG2 xenograft	Yes <sup>51</sup>
TIIA	Gavage/daily	100 mg/kg	Saline	HSV-tk and WT B16 cells(1:9) murine melanoma	No <sup>52</sup>
TIIA	ip/4 times per week	30 mg/kg	1% Tween 20/ethanol	MDA-MB-231 and MCF-7 breast Ca xenograft	Yes <sup>53</sup>
TIIA	ip/4 times per week	30 mg/kg	ND	MDA-MB-231 and MCF-7 breast Ca xenograft	Yes <sup>54</sup>
TIIA	ip/3 times per week	10–40 mg/kg	ND	MCF-7M (CSC) breast Ca xenograft	Yes <sup>42</sup>
TIIA	ip/daily	50 mg/kg	Saline	MDA-MB-231 breast Ca xenograft	Yes <sup>41</sup>
TIIA	ip/every other day	30 mg/kg	ND	HPV+ Ca Ski cervical Ca xenograft	Yes <sup>44</sup>
TIIA	ip/3 times per week	10, 20, 40 mg/kg	ND	GBMS (CSC) glioma xenograft	Yes <sup>55</sup>
TIIA	ip/twice per week	30 mg/kg	Corn oil	J5 Liver HCC xenograft	Yes <sup>56</sup>
TIIA	ip/every other day	20, 40 mg/kg	0.5% CMC	Ascitic-type hepatic Ca H22 allograft	Yes <sup>57</sup>
TIIA	ip/every other day	20 mg/kg	ND	A549 lung Ca xenografts	Yes <sup>58</sup>
TIIA	ip/daily	0.3 mg/kg	TPA-CM	CL1-5 lung Ca xenograft and metastases	No <sup>59</sup>
TIIA	ip/daily	15 mg/kg	Saline	S180 sarcoma xenograft	Yes <sup>60</sup>
STS	ip/daily	0.2–50 mg/kg	ND	Lewis lung Ca allograft	No <sup>61</sup>
STS	ip/daily	~50 mg/kg	Aqueous injectable	HCCLM3 liver cancer metastasis	Yes <sup>62</sup>
ATA	ip/3 times per week	30 mg/kg	ND	MDA-MB-435 melanoma Ca xenograft	Yes <sup>63</sup>
ATA	ip/3 times per week	35 mg/kg	ND	MDA-MB-435 melanoma Ca xenograft	Yes <sup>64</sup>
TIIA	iv/daily	0.5–2 mg/kg	Nanoparticle	Murine hepatoma allograft	Yes <sup>33</sup>
TIIA	iv/every other day	1 mg/kg	Nanoparticle	Murine hepatoma allograft	Yes <sup>35</sup>
TIIA	iv/daily–1 week only	0.5–2 mg/kg	ND	Murine C26 colorectal Ca allograft	Yes <sup>65</sup>
TIIA	sc/3 times per week	30 mg/kg	0.5% CMC	Breast IDC F35 xenotransplant	Yes <sup>66</sup>
TIIA	sc/daily	10, 30 mg/kg	0.5% CMC	MKN45, SGC7901 gastric Ca xenograft	Yes <sup>67</sup>
TIIA	sc/every other day	60, 90 mg/kg	Corn oil	LNCaP prostate Ca xenograft	Yes <sup>43</sup>
CT	Gavage/daily	150 mg/kg	0.1% SDS	MCF-7 breast Ca xenograft	Yes <sup>68</sup>
CT	ip/every other day	2.5 mg/kg	3% DMSO, 30% PEG	HCT116 colorectal Ca xenograft	Yes <sup>69</sup>
CT	ip/every other day	5, 25 mg/kg	Corn oil	22Rv1 prostate Ca xenograft	Yes <sup>70</sup>
CT	ip/every other day	25 mg/kg	ND (corn oil <sup>†</sup> )	22Rv1 prostate Ca metastasis	Yes <sup>71</sup>

(Continued)

**Table 1** (Continued)

Tanshinone	Route/frequency	Dose, range	Vehicle	Cancer model	Efficacy
CT	ip/every other day	10 mg/kg	2% Tween 80	PC-3 prostate Ca xenograft	Yes <sup>72</sup>
CT	sc/daily, near tumor	100 mg/kg	ND	Human lung cancer PDX <sup>b</sup>	Yes <sup>45</sup>
TI	Gavage/daily	150 mg/kg	Corn oil	DUI45 prostate Ca xenograft	Yes <sup>73</sup>
TI	Gavage/daily	80, 200 mg/kg	Corn oil	H1299 lung Ca xenograft	Yes <sup>74</sup>
TI	ip/daily	10 mg/kg	ND	Lung metastasis/MDA-MB-231 breast Ca	Yes <sup>75</sup>
TI	ip/daily	0.3 mg/kg	TPA-CM	CL1-5 lung Ca xenograft and metastases	Yes <sup>59</sup>
TI	ip/3 times per week	1 mg/kg	CM	Transgenic lung Ca allograft	Yes <sup>76</sup>
DH-TI	ip/3 times per week	10 mg/kg	DMSO	MDA-MB-231 breast Ca xenograft	Yes <sup>77</sup>
DH-TI	ip before radiation	10 mg/kg	<0.5% DMSO	HeLa cervical Ca xenograft	Yes <sup>78</sup>
DH-TI	ip/3 times per week	10 mg/kg	Liposome	AGS gastric Ca xenograft	Yes <sup>79</sup>
Tanshinone Mix	iv/daily	2–8 mg/kg	Microemulsion	H22 murine hepatoma allograft	Yes <sup>37</sup>
TIIA	ip/daily	24 mg/kg	Corn oil	Rat benign prostatic hyperplasia	Yes <sup>80</sup>
TIIA	Gavage/daily	~60–75 mg/kg	Corn oil	TRAMP mouse carcinogenesis (current study)	No

**Notes:** <sup>a</sup>Likely corn oil per other report from same group.<sup>70</sup> <sup>b</sup>Patient-derived xenograft in athymic nude mice.

**Abbreviations:** TIIA, tanshinone IIA; ND, not described; ip, intraperitoneal; CMC, carboxyl methyl cellulose; TPA-CM, tetradecanoyl phorbol acetate conditioned medium; STS, sodium tanshinone IIA sulfonate; ATA, acetyl tanshinone IIA; iv, intravenous; sc, subcutaneous; CT, cryptotanshinone; SDS, sodium dodecyl sulfate; DMSO, dimethyl sulfoxide; PEG, polyethylene glycol; TI, tanshinone I; CM, conditioned medium; DH-TI, dihydrotanshinone I.

A common feature of these efficacy studies was using parenteral injections to deliver the tanshinones rather than gastrointestinal route. The gastrointestinal route is more practical and desirable for cancer chemopreventive agent administration for the long term.

### Primary cancer chemoprevention model

We have recently used a transgenic prostate carcinogenesis model (transgenic adenocarcinoma of mouse prostate, TRAMP) to evaluate the *in vivo* chemoprevention efficacy of TIIA. In this study, in-house-bred male C57BL/6 TRAMP mice and their wild-type littermates received TIIA (1.5 mg/mouse in corn oil; ~60–75 mg/kg body weight) by gavage once daily, 5 days a week, from 8 to 28 weeks of age. We found that TIIA gavage neither affected the neuroendocrine-carcinoma

lineage burden in the TRAMP mice model nor inhibited the growth of epithelial lesions estimated by prostate weight (Table 2). We detected plasma level of TIIA of 98 ( $\pm 30.9$  standard deviation) ng/mL ( $\sim 0.33 \mu\text{M}$ ) by liquid chromatography–tandem mass spectrometry method at 4 hours after the last dose. These results combined with our PK data of the same dosage (Figure 2) indicated that either the threshold level of TIIA was not achieved through the route and dose/form of delivery to exert *in vivo* efficacy or the TRAMP prostate carcinogenesis model was refractory to TIIA in both the epithelial lesion and neuroendocrine lineages.

Concerning androgen-driven prostate epithelial hyperproliferation, in a study published in 2015, Wang et al reported the efficacy of TIIA in the treatment of a rat model of benign prostatic hyperplasia,<sup>80</sup> a noncancerous epithelial

**Table 2** Gavage administration of 1.5 mg TIIA/mouse (~60–75 mg/kg) daily did not decrease TRAMP prostate weights or NE-Ca burden by 28 weeks

Group	Genotype of the mice	Gavage began at 8 weeks of age	Number of mice sacrificed by 28 weeks of age	Final body weight (g) <sup>a</sup>	Prostate weight (mg) <sup>a</sup>	Number of NE-Ca	Total NE-Ca burden (g) <sup>b</sup>
1	TRAMP	Vehicle – corn oil	19	26.8 $\pm$ 2.31	150 $\pm$ 29	4 (20%)	19.4
2	TRAMP	TIIA, 1.5 mg/mouse	20	27.4 $\pm$ 2.2	161 $\pm$ 38	5 (25%)	19.9
3	Wild type	Vehicle – corn oil	10	27.3 $\pm$ 1.7	78 $\pm$ 13	0	–
4	Wild type	TIIA, 1.5 mg/mouse	5	28.4 $\pm$ 1.5	79 $\pm$ 18	0	–

**Notes:** <sup>a</sup>Mean  $\pm$  SD. <sup>b</sup>Summation of individual tumor weights of each group.

**Abbreviations:** TIIA, tanshinone IIA; TRAMP, transgenic adenocarcinoma of mouse prostate; NE-Ca, neuroendocrine-carcinoma; SD, standard deviation.

hyperproliferative condition driven by dihydrotestosterone in man. In the rat model of estradiol/testosterone-induced benign prostatic hyperplasia, TIIA (24 mg/kg body weight, in corn oil, ip daily for 4 weeks) inhibited the growth of prostate stromal and epithelial cells in vivo by a mechanism that involved downregulating ER $\alpha$  and androgen receptor (AR) expression (Table 1).<sup>80</sup>

## Structural modifications of tanshinones

Researchers have made structural modifications to improve the solubility and intestinal absorption of some tanshinones and/or to enhance selective cytotoxicity against cancer cells. Chinese scientists made STS as a water-soluble derivative of TIIA (Figure 1C). In fact, STS has been widely used to treat patients with cardiovascular diseases, likely through its antioxidant activities, for more than 3 decades.<sup>81–84</sup> STS possesses a broad range of pharmacological functions including protecting the myocardium by attenuating hypertrophy, immune-mediated liver injury via modulating NF- $\kappa$ B and IFN- $\gamma$ /STAT1 pathways, and exhibiting a strong vasodilating effect against vasoconstriction by activating conductance Ca<sup>2+</sup>-sensitive K<sup>+</sup> channels.<sup>85</sup>

However, STS was not active to induce apoptosis in several cancer cell lines.<sup>61,86</sup> Therefore, ATA (Figure 1C) was synthesized and tested.<sup>63,64</sup> Compared to TIIA, ATA exhibited increased water solubility and stronger apoptotic activity on multiple cancer cell lines, including HER2/Neu-positive cancer cells. The stronger apoptosis effect of ATA was attributed to mitochondrial redox-ROS generation.<sup>63</sup> Its in vivo efficacy against MDA-MB-435 (now a confirmed melanoma cell line) xenograft growth in mice was demonstrated at a dose of 30 mg/kg body weight by ip injection, three times per week.

Xu et al synthesized a new sodium derivative of CT, PTS33, to target AR pathway.<sup>87</sup> PTS33 selectively inhibited AR activities but did not repress the activities of other nuclear receptors, including ER $\alpha$ , glucocorticoid receptor, and progesterone receptor. PTS33 suppressed the growth of AR-positive PCa cells, and had little effect on AR-negative PCa cells. PTS33 also modulated AR transactivation and suppressed AR target genes PSA, TMPRSS2, and TMEPA1 in castration-resistant LNCaP C4-2 cells. In addition, PTS33 inhibited estrogen/ $\Delta$ 5-androstenediol-induced AR activities. Further mechanistic studies indicated that PTS33 inhibited AR function by suppression of AR protein expression, AR N–C interaction, and AR co-regulator interaction.

## Cell culture-based studies: potential mechanisms

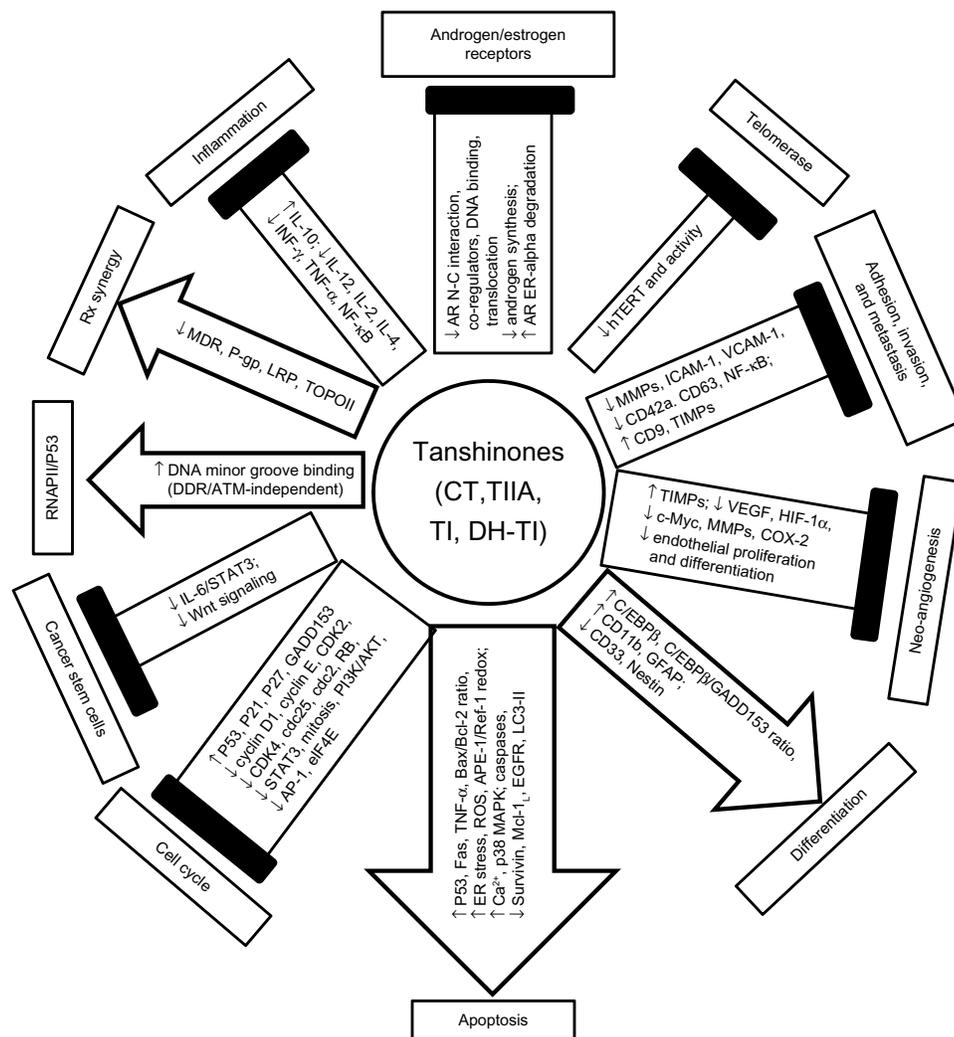
Most cell culture models used concentrations without regard to in vivo achievable tanshinone levels, and therefore, the mechanistic relevance of such studies should be cautiously appreciated. As discussed in the tanshinone PK section, plasma levels of nanomolar to sub-micromolar ranges were commonly observed with oral or ip dosing. One should regard “mechanisms” derived from cell culture studies with exposure levels of >10  $\mu$ M with suspicion. However, be cognizant that localized targeted delivery through novel formulations may make such exposure levels possible. We discussed the reported cellular and molecular activities of tanshinones in nine impact categories (updated in Figure 3) in our 2012 review,<sup>17</sup> and we refer readers to that paper for “mechanistic” details. Here we discuss only a new impact category of recent findings of effects of TIIA on CSCs.

## Effect on CSCs

Lin et al<sup>42</sup> found that TIIA decreased mammosphere formation by human breast CSCs enriched from MCF-7 cell line in vitro. The CSC growth suppression was associated with decreased expression of IL-6, STAT3, phospho-STAT3 (Tyr705), NF- $\kappa$ Bp65 in nucleus, and cyclin D1 protein.<sup>42</sup> In a xenograft model generated with MCF-7 mammosphere cells, treating mice with TIIA of 10, 20, and 40 mg/kg doses by ip injection three times a week for 4 weeks inhibited tumor growth by 39%, 48%, and 58%, respectively.<sup>42</sup> In glioblastoma multiforme model, TIIA exerted a significant inhibitory effect on human glioma stem cells (GSCs) in vitro and in vivo.<sup>55</sup> TIIA increased the expression of differentiation and neural lineage markers including GFAP and  $\beta$ -tubulin, decreased expression of GSC markers including CD133 and nestin, and induced GSC apoptosis. The IL-6/STAT3 signaling axis was likely targeted by TIIA to mediate the growth inhibition of GSCs. In contrast to CSCs, TIIA promotes stem cell functions in cardiovascular injury and other epithelial skin cells.<sup>88,89</sup>

## Cancer-related clinical studies

Table 3 summarizes reported clinical experience with TIIA or Danshen-containing TCM formula for cancer. The clinical use of TIIA was described in two single-case reports. The first case was published in 2006 and involved a 30-year-old man diagnosed with acute promyelocytic leukemia.<sup>90</sup> All-trans retinoic acid differentiation therapy (20 mg, three times per day) was administered for 14 days but did not achieve complete remission (CR). He was given oral TIIA, 30 mg,



**Figure 3** Summary of reported cellular and molecular effects of tanshinones in cell culture models related to cancer or its microenvironments.

**Notes:** Arrows indicate induction effects, and block T (⊥) indicates suppression effects. Upward arrow indicates upregulation or activation, and downward arrow indicates downregulation or inactivation. Adapted from Zhang Y, Jiang P, Ye M, Kim SH, Jiang C, Lu J. Tanshinones: sources, pharmacokinetics and anti-cancer activities. *International Journal of Molecular Sciences*. 2012;13(10):13621–13666.<sup>17</sup>

**Abbreviations:** CT, cryptotanshinone; TIIA, tanshinone IIA; TI, tanshinone I; DH-TI, dihydrotanshinone I; ROS, reactive oxygen species; CSC, cancer stem cells.

twice per day. His blood cell count at 8 weeks and bone marrow cell counts at 12 weeks were restored to normal levels, indicating a CR. Another case reported a 21-year-old man with relapsed acute promyelocytic leukemia after 1 year of all-trans retinoic acid, arsenic trioxide, 6-mercaptopurine, and methotrexate treatments.<sup>91</sup> After 54 days of TIIA iv infusion, 80 mg once per day, he achieved completely morphological remission without obvious side effects.<sup>91</sup> Well-designed clinical trials with more patients and randomized placebo-controlled design are needed to further validate the clinical efficacy of TIIA and other tanshinones.

Small-scale trials found improved quality of life and overall response rates (CR + partial remission + stable disease) of therapeutic modalities when combined with Fufang Danshen (composite) Dripping Pills (oral intake) against

gastric carcinoma,<sup>92</sup> colorectal carcinoma,<sup>93</sup> esophageal carcinoma,<sup>94</sup> pancreatic carcinoma,<sup>95</sup> liver carcinoma,<sup>96</sup> and non-small-cell lung carcinoma<sup>97</sup> (Table 3). Combining these trials for a meta-analysis would suggest a statistical and meaningful health benefit on enhancing objective response (CR + partial remission) by the Dripping Pill use. By liquid chromatography–tandem mass spectrometry analyses,<sup>101</sup> Dripping Pills contained none to barely detectable TIIA and DH-TI and a small amount of CT and TI (2.7–5.5 μg per pill).

Compared to chemotherapy alone, Fufang Danshen Injection (injectable formula made from aqueous extracts of Danshen and one or more medicinal herbs) attenuated chemotherapy-induced complications and increased the CR rate for acute leukemia.<sup>98</sup> Because of water extraction

**Table 3** Cancer-related clinical studies of TIIA and Danshen-containing formulas

Tanshinone tested/ tanshinone- containing formula	Cancers	Treatment(s)	Number of patients	Clinical benefit	References
TIIA	Leukemia	TIIA (30 mg, po, twice per day for 3 months)	Single-case report	CR	90
TIIA	Leukemia	TIIA (80 mg, iv once per day for 54 days)	Single-case report	CR	91
Fufang (composite) Danshen Dripping Pills	Gastric carcinoma	Control: chemotherapy only; treatment: chemotherapy plus Fufang Danshen Dripping Pill (250 mg, po 3 times per day for 3 weeks)	Control: 44 Treatment: 46	CR + PR rate (control vs treatment): 36.4% vs 41.3% ( $P>0.05$ ) CR + PR + SD rate 43.2% vs 67.4% ( $P<0.05$ )	92
Fufang (composite) Danshen Dripping Pills	Colorectal carcinoma	Control: chemotherapy only; treatment: chemotherapy plus Fufang Danshen Dripping Pill (250 mg, po 3 times per day for 3 weeks)	Control: 46 Treatment: 47	CR + PR rate (control vs treatment): 41.3% vs 51.1% ( $P>0.05$ ) CR + PR + SD rate 58.7% vs 80.8% ( $P<0.05$ )	93
Fufang (composite) Danshen Dripping Pills	Esophageal carcinoma	Control: chemotherapy only; treatment: chemotherapy plus Fufang Danshen Dripping Pill (250 mg, po 3 times per day for 3 weeks)	Control: 34 Treatment: 36	CR + PR rate (control vs treatment): 38.2% vs 52.8% ( $P>0.05$ ) CR + PR + SD rate 52.9% vs 80.6% ( $P<0.05$ )	94
Fufang (composite) Danshen Dripping Pills	Pancreatic carcinoma	Control: chemotherapy only; treatment: chemotherapy plus Fufang Danshen Dripping Pill (250 mg, po 3 times per day for 2 weeks)	Control: 40 Treatment: 41	CR + PR rate (control vs treatment): 35.0% vs 46.3% ( $P>0.05$ ) CR + PR + SD rate 50.0% vs 73.2% ( $P<0.05$ )	95
Fufang (composite) Danshen Dripping Pills	Liver carcinoma	Control: chemotherapy only; treatment: chemotherapy plus Fufang Danshen Dripping Pill (250 mg, po 3 times per day for 3 weeks)	Control: 47 Treatment: 49	CR + PR rate (control vs treatment): 61.7% vs 69.4% ( $P>0.05$ ) CR + PR + SD rate 70.2% vs 89.8% ( $P<0.05$ )	96
Fufang (composite) Danshen Dripping Pills	Non-small-cell lung carcinoma	Control: chemotherapy only; treatment: chemotherapy plus Fufang Danshen Dripping Pill (250 mg, po 3 times per day for 2 weeks)	Control: 43 Treatment: 45	CR + PR rate (control vs treatment): 34.9% vs 46.7% ( $P>0.05$ ) CR + PR + SD rate 62.8% vs 84.4% ( $P<0.05$ )	97
Fufang (composite) Danshen Injection	Leukemia	Control: chemotherapy only; treatment: chemotherapy plus Fufang Danshen Injection (20–30 mL, iv, once per day for 28 days)	Control: 46 Treatment: 86	Fufang Danshen slightly increased CR rate but significantly attenuated the side effects of chemotherapy	98
Fufang (composite) Danshen Injection	Liver carcinoma	Control: surgical resection only; treatment: surgical resection plus chemotherapy and Fufang Danshen Injection (250 mL, TUV perfusion, once per day for 7 days, repeat every 3–4 weeks)	Control: 30 Treatment: 30	1- and 2-year recurrence rates (control vs treatment): 60.7% vs 15.3% ( $P<0.05$ ) and 75.1% vs 30.0% ( $P<0.05$ )	99
Fufang (composite) Danshen Injection	Liver carcinoma	Control: TACE only; treatment: TACE plus Fufang Danshen Injection (16 mL, iv once per day for 7 days)	Control: 37 Treatment: 53	1-, 2-, and 3-year survival rate (control vs treatment): 73.0% vs 79.3% ( $P>0.05$ ), 43.2% vs 66.0% ( $P<0.05$ ) and 24.3% vs 45.3% ( $P<0.05$ )	100

**Abbreviations:** TIIA, tanshinone IIA; TCM, Traditional Chinese Medicine; po, oral administration; CR, complete remission; iv, intravenous; PR, partial remission; SD, stable disease; TUV, trans-umbilical-portal vein; TACE, transcatheter arterial chemoembolization.

procedures, such injectables were essentially free of hydrophobic tanshinones. Fufang Danshen Injection plus chemotherapeutic drug mitomycin and adriamycin through trans-umbilical-portal vein perfusion after surgical resection of the primary liver carcinomas significantly delayed the 1- and 2-year recurrence rates compared to the surgical resection alone.<sup>99</sup> Furthermore, combination of hepatic artery perfusion of Fufang Danshen Injection with liver transcatheter arterial chemoembolization improved the survival and life quality of patients with HCC compared to transcatheter arterial chemoembolization alone.<sup>100</sup>

Cancer-related clinical studies in People's Republic of China suggested potential benefit of TIIA and Danshen-containing TCM formulas for cancer patients and improvement of side effect profiles and quality of life; yet, most of these studies had serious limitations, such as single-case reports, small sample size for Phase II trials ( $N \leq 40$ ), lack of randomization and placebo control and blinding in trial designs, and poorly defined dose-formulation information and content of tanshinones used.

## Conclusion and future directions

In cell culture models, tanshinones exhibit broad-range anticancer activities including anti-proliferation, pro-apoptosis, anti-angiogenesis, induction of differentiation, and inhibition of adhesion, migration, invasion, and metastasis, and may sensitize cancer cells to apoptosis by current therapeutic modalities<sup>17</sup> and inhibit CSCs (Figure 3).<sup>42,55</sup> Tanshinones may also modulate inflammatory and immune responses, inhibit telomerase, interact with DNA minor groove to activate P53 tumor suppressor, or regulate specific pathways such as AR (eg, TIIA, CT) or STAT3 (eg, CT), and endoplasmic reticulum stress and cancer cellular energetics<sup>17</sup> (Figure 3). However, in vivo potency evaluation outcomes so far varied greatly due to lack of uniformity of excipients, doses, and routes of administration. One major challenge for efficacy evaluation is the poor water solubility and oral bioavailability of natural tanshinones. The ongoing pharmaceutical approach through nanoparticle- or lipid-based delivery formulations can help to improve the delivery and bioavailability of tanshinones and consequently efficacy evaluation as demonstrated in a few studies already. The chemical modification approaches have resulted in improved water solubility in all cases and improved anticancer efficacy in some instances such as ATA<sup>63,64</sup> and PTS33<sup>87</sup> but inactivated apoptosis potency in others such as STS.<sup>86</sup> In the future, formulation optimization

combined with animal PK modeling followed by rigorous safety and efficacy testing in relevant animal cancer models will improve decision making on the clinical translation worthiness of tanshinones.

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## Disclosure

The authors report no conflicts of interest in this work.

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