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REVIEW

Combinational Antitumor Strategies Based on the Active Ingredients of Toad Skin and Toad Venom

Huan Tian[1], Feng Zhao[1], Bao-Sen Yue[1], Bing-Tao Zhai[2–5]

[1]Department of Pharmacy, Xi'an Hospital of Traditional Chinese Medicine, Xi'an, People's Republic of China; [2]College of Pharmacy, Shaanxi University of Chinese Medicine, Xi'an, People's Republic of China; [3]State Key Laboratory of Research & Development of Characteristic Qin Medicine Resources (Cultivation), Xi'an, People's Republic of China; [4]Shaanxi Key Laboratory of Chinese Medicine Fundamentals and New Drugs Research, Xi'an, People's Republic of China; [5]Shaanxi Collaborative Innovation Center of Chinese Medicinal Resources Industrialization, Xi'an, People's Republic of China

Correspondence: Bing-Tao Zhai, Email zbp@sntcm.edu.cn

Abstract: A multidrug combination strategy is an important mean to improve the treatment of cancer and is the mainstream scheme of clinical cancer treatment. The active ingredients of traditional Chinese medicine, represented by toad skin and toad venom, have the advantages of high efficiency, low toxicity, wide action and multiple targets and have become ideal targets in combined treatment strategies for tumors in recent years. Toad skin and toad venom are traditional Chinese animal medicines derived from *Bufo bufo gargarizans* Cantor or *Bufo melanostictus* Schneider that have shown excellent therapeutic effects on the treatment of various cancers and cancer pain as adjuvant antitumor drugs in clinical practice. The involved mechanisms include inducing apoptosis, arresting the cell cycle, inhibiting cell proliferation, migration and invasion, inhibiting tumor angiogenesis, reversing the multidrug resistance of tumor cells, and regulating multiple signaling pathways and targets. Moreover, a multidrug combination strategy based on a nanodelivery system can realize the precise loading of the active ingredients of toad skin or toad venom and other antitumor drugs and carry drugs to overcome physiological and pathological barriers, complete efficient enrichment in tumor tissues, and achieve targeted delivery to tumor cells and the controlled release of drugs, thus enhancing antitumor efficacy and reducing toxicity and side effects. This article reviewed the clinical efficacy and safety of the combination of toad skin and toad venom with chemotherapeutic drugs, targeted drugs, analgesics and other drugs; evaluated the effects and mechanisms of the combination of toad skin and toad venom with chemotherapy, targeted therapy, radiotherapy or hyperthermia, traditional Chinese medicine, signaling pathway inhibitors and other therapies in cell and animal models; and summarized the codelivery strategies for the active ingredients of toad skin and toad venom with chemotherapeutic drugs, small-molecule targeted drugs, monoclonal antibodies, active ingredients of traditional Chinese medicine, and photodynamic and photothermal therapeutic drugs to provide a basis for the rational drug use of toad skin and toad venom in the clinic and the development of novel drug delivery systems.

Keywords: toad skin, toad venom, combinational antitumor strategies, codelivery strategies, cancer, cancer pain

Introduction

For decades, cancer has been a leading cause of death.[1] For many years, chemotherapy has been regarded as the main method of tumor treatment. However, the long-term use of chemotherapy drugs, which are based on a single antitumor mechanism, easily destroys the human immune system, causing multidrug resistance (MDR) and serious adverse reactions.[2] In recent years, with the rise of targeted therapy, immunotherapy, photodynamic therapy (PDT), traditional Chinese medicine (TCM), and interventional therapy, cancer patients have an increasing number of treatment options, and combination therapy has become the standard strategy for cancer treatment. Combination therapy with anticancer drugs targeting different targets can achieve synergistic effects by regulating multiple pathways of abnormal cells and has many advantages, such as reducing adverse drug reactions, delaying the occurrence of MDR, and improving the antitumor immune response.[3–5] At present, diverse combined antitumor drug strategies include combinations of chemotherapy drugs and targeted drugs, combinations of chemotherapy drugs and immunotherapy drugs, combinations of targeted drugs and immunotherapy drugs, combinations of active ingredients of TCM and chemotherapy drugs, and

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combinations of active ingredients of TCM and targeted drugs.[6–9] Among them, the active ingredients of TCM have a wide range of sources, low toxicity and high safety, can exert an antitumor effect through multiple targets and multiple pathways, and show unique advantages in reducing drug resistance, regulating the tumor microenvironment, promoting immune function recovery, reducing recurrence and metastasis, and improving the long-term prognosis. Therefore, in recent years, an increasing number of researchers have focused on the active ingredients of TCM for combined application to prolong the survival of cancer patients.[10]

The toad is the whole body of *Bufo bufo gargarizans* Cantor or *Bufo melanostictus* Schneider. The head, tongue, skin, secretions of the posterior ear glands and skin glands, liver and gallbladder are all medicinal parts. The head is the Chinese medicine toad head, which has the effects of eliminating malnutrition and digesting the retained food and can be used to treat infantile malnutrition. The tongue is the Chinese medicine toad tongue, which detoxifies and removes furuncles and can be used to treat furuncles. The dried skin lacking the viscera is the Chinese medicine toad skin, which removes heat and toxic material, induces diuresis to remove edema, and can be used to treat ulcers, pyogenic infections, scrofula, eczema, infantile malnutrition, abdominal distension, and chronic tracheitis. The dry secretions of the gland behind the ear and the gland of the skin constitute the traditional Chinese medicine toad venom, which has the effects of detoxifying, relieving pain and inducing resuscitation and can be used for carbuncles and furuncles, sore throat, heat stroke, dizziness, diarrhea, acute filthy disease, and vomiting. The liver is used in the Chinese medicine toad liver, which detoxifies and disperses knots, removes furuncles and relieves swelling, and can be used to treat ulcers, furuncles, sores, snake bites and measles. The gallbladder is the Chinese medicine toad bile, which relieves cough and reduces sputum, detoxifies and disperses knots, and can be used to treat tracheitis, infantile aphonia, early lymph node tuberculosis and nose furuncle.[11,12] Currently, dried toad skin has been developed as capsule (Huachansu capsule), tablet (Huachansu tablet), oral liquid (Huachansu oral liquid) and injection (Huachansu injection) for treating intermediate and advanced tumors, chronic hepatitis B and other diseases.[13–15] Toad venom was also developed as an injection (Toad venom injection) for treating acute and chronic suppurative infections and is used as an adjuvant antitumor drug.[16] Moreover, various preparations containing dried toad skin and toad venom, including Tianchan capsule, Kang Ai Ping pill, Xianchan tablet, Shenchan Xiaojie capsule, Chanwu Babu ointment, Chanwu gel ointment, Delisheng injection, Hupo Zhitong ointment, Hechan tablet, Jinpu capsule, Compound toad venom ointment, Jiawei Xihuang pill, Xiaojin pill, Tianfoshen oral liquid, Toad venom Zhentong gel ointment, Toad venom ingot, Toad venom Zhentong ointment and Compound toad venom pill, have also shown excellent therapeutic effects on the treatment of lung, liver, stomach and other cancers, as well as various types of cancer pain, as adjuvant antitumor drugs in clinical practice. Table 1 summarizes the marketed antitumor products containing toad skin or toad venom in China.

In addition to traditional preparations of toad skin and toad venom, the advantages of the active ingredients of toad skin and toad venom in the treatment of cancer are increasingly prominent with the advancement of separation technology and the study of drug action mechanisms. The chemical components of toad skin and toad venom are mainly bufodienolides, indole alkaloids, steroids and other compounds. Among them, the monomeric components of bufadienolides, such as bufalin, cinobufagin, bufotalin, cinobufotalin, gamabufotalin, and arenobufagin, are the main active antitumor components (Figure 1). A number of studies have confirmed that these compounds can exert antitumor effects through multiple targets and multiple pathways and have the advantages of enhancing efficacy, reducing toxicity, reversing tumor MDR, reducing cancer pain, etc., when combined with chemotherapy, radiotherapy, targeted therapy and other treatment methods.[17–20] Although combination therapeutic strategies are helpful for better treating cancer to a certain extent, the abovementioned active ingredients have the disadvantages of low bioavailability and poor solubility in water, and free drug combinations cannot solve problems such as the low drug-targeting ability, significant harmful and adverse off-target effects, and the inconsistent pharmacokinetic characteristics and tissue distributions of different drugs.[21,22] With the rapid development of nanomedicine, nanodelivery systems have shown great potential in overcoming the shortcomings of existing antitumor multidrug delivery strategies. Nanodrug delivery systems can accurately regulate the flexible loading of multiple components of drugs and deliver drugs to specific tissues, organs, and even cells and intracellular structures, thereby enhancing the therapeutic effects and reducing adverse reactions. In addition, by constructing biomimetic drug delivery systems or introducing targeted molecules and stimulus-responsive groups into the codelivery vector, the duration of the drug delivery system in the blood circulation can be extended, and the

Table 1 Marketed Antitumor Products Containing Toad Skin or Toad Venom in China

Marketed Product	Composition	Function	Indications	Source (Drug Standards of China)
Huachansu capsule (华蟾素胶囊)	Dried toad skin	Detoxification, reduce swelling, relief of pain	For intermediate and advanced tumors, chronic hepatitis B and other diseases	Drug Standards of the National Medical Products Administration, YBZ30992005-2011Z-2021
Huachansu tablet (华蟾素片)	Dried toad skin extract	Detoxification, reduce swelling, relief of pain	For intermediate and advanced tumors, chronic hepatitis B and other diseases	Drug Standards of Ministry of Health, Prescription preparation of Chinese medicine, Volume 14, WS_3-B-2687-97
Huachansu oral liquid (华蟾素口服液)	Dried toad skin	Detoxification, reduce swelling, relief of pain	For intermediate and advanced tumors, chronic hepatitis B and other diseases	Drug Standards of Ministry of Health, Prescription preparation of Chinese medicine, Volume 11, WS_3-B-2132-96
Huachansu injection (华蟾素注射液)	Dried toad skin extract	Detoxification, reduce swelling, relief of pain	For intermediate and advanced tumors, chronic hepatitis B and other diseases	Drug Standards of Ministry of Health, Prescription preparation of Chinese medicine, Volume 16, WS_3-B-3045-98
Toad venom injection (蟾酥注射液)	Bufonis Venenum	Clearing heat and detoxifying	For acute and chronic suppurative infection; it can also be used as an adjuvant antitumor drug	National Drug Standards of the National State Food and Drug Administration, WS_3-B-3345-98-2013
Tianchan capsule (天蟾胶囊)	Corydalis Decumbentis Rhizoma, Aconiti Radix Cocta, Bufonis Venenum, Cirald Daphne Bark, Chelidonii Herba, Gentianae Macrophyllae Radix, Angelicae Dahuricae Radix, Chuanxiong Rhizoma, Paeoniae Radix Alba, Glycyrrhizae Radix Et Rhizoma	Activating Qi and blood, channeling meridians and collaterals, relieving pain	It is used for mild to moderate cancer pain caused by lung cancer, gastric cancer, liver cancer and so on with qi stagnation and blood stasis	National Drug Standards of the National State Food and Drug Administration, WS-114(Z-022)-2002-2012Z
Kang Ai Ping pill (抗癌平丸)	Lysimachia clethroides, Actinidia Chinensis Root, Isodon amethystoides, Sarcandre Herba, Duchesnea indica, Scutellariae Barbatae Herba, Caryopteris incana, Hedyotis diffusa Willd, Selaginella doederleinii Hieron, Bufonis Venenum	Clearing heat and detoxifying, dispersing stasis and relieving pain	It is used for gastric cancer, esophageal cancer, cardiac cancer, rectal cancer and other digestive tract tumors caused by heat toxicity and blood stasis obstructing the gastrointestinal tract	Drug Standards of Ministry of Health, Prescription preparation of Chinese medicine, Volume 20, WS_3-B-3844-98
Xianchan tablet (仙蟾片)	Strychni Semen Pulveratum, Processed Pinelliae Rhizoma, Ginseng Radix Et Rhizoma, Astragali Radix, Agrimoniae Herba, Psoraleae Fructus, Curcumae Radix, Bufonis Venenum, Angelicae Sinensis Radix	Dissipate blood stasis and disperse knots, tonifying qi and relieving pain	For esophageal cancer, stomach cancer, lung cancer	Drug Standards of the National State Food and Drug Administration, WS-10076(ZD-0076)-2002-2015Z
Shenchan Xiaojie capsule (参蟾消解胶囊)	Ginseng Radix Et Rhizoma, Realgar, Wine Processed Bufonis Venenum, Croci Stigma, Bovis Calculus Artifactus, Musk, Borneolum Syntheticum, Notoginseng Radix Et Rhizoma, Bambusae Concretio Silicea, Aloe	Dispersing stasis and detoxifying, dispelling phlegm and reducing swelling	It is used for the adjuvant treatment of lung and gastric adenocarcinoma	Drug Standards of the National Medical Products Administration, WS-10119(ZD-0119)-2002-2012Z

(*Continued*)

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Table 1 (Continued).

Marketed Product	Composition	Function	Indications	Source (Drug Standards of China)
Chanwu Babu ointment (蟾乌巴布膏)	Bufonis Venenum, Aconiti Radix, Zanthoxyli Radix, Paridis Rhizoma, Aconitum Coreanum, Piperis Longi Fructus, Asari Radix Et Rhizoma, Caryophylli Flos, Cinnamomi Cortex, Olibanum, Myrrha, Camphor, Menthol, Borneolum Syntheticum, Methyl Salicylate, and so on	Promoting blood circulation and removing blood stasis, relieving swelling and pain	It is used for pain caused by lung, liver, stomach and other cancers	National Standard Collection of Chinese Proprietary Medicine, Oral tumors, Pediatric volume, WS-11431(ZD-1431)-2002
Chanwu gel ointment (蟾乌凝胶膏)	Bufonis Venenum, Aconiti Radix, Zanthoxyli Radix, Paridis Rhizoma, Aconitum Coreanum, Hibisci Mutabilis Folium, Sparganii Rhizoma, Curcumae Rhizoma, Carthami Flos, Caryophylli Flos, Asari Radix Et Rhizoma, Cinnamomi Cortex, Rhododendri Fructus, Piperis Longi Fructus, Nardostachyos Radix Et Rhizoma, Kaempferia Galanga, Olibanum, Myrrha, Camphor, Menthol, Borneolum Syntheticum, Methyl Salicylate	Promoting blood circulation and removing blood stasis, relieving swelling and pain	It is used for pain caused by lung, liver, stomach and other cancers	Promulgation documents of National Drug Standards, WS-11431(ZD-1431)-2002-2012Z
Delisheng injection (得力生注射液)	Red ginseng, Astragali Radix, Bufonis Venenum, Mylabris	Nourish Qi and strengthen the body, relieve swelling and loose knots	For middle and advanced primary liver cancer with Qi deficiency and blood stasis syndrome, symptoms include mass in the right flank, persistent pain, abdominal distension, lack of appetite, and fatigue	National Drug Standards of the National State Food and Drug Administration, WS_3-134(Z-019)-2006(Z)
Hupo Zhitong ointment (琥珀止痛膏)	Kaempferia Galanga, Acori Tatarinowii Rhizoma, Coptidis Rhizoma, Strychni Semen, Mylabris, Clematidis Radix Et Rhizoma, Arisaematis Rhizoma, Bufonis Venenum, Hupo Oil, Ocimum Gratissiimum Oil, Peppermint Oil, Star Anise Oil, Chinese cinnamon Oil, Borneolum Syntheticum, Camphor	Promoting blood circulation and eliminating phlegm, relieving swelling and loose knots, channeling meridians and collaterals, and relieving pain	It is used for tumor pain, neuropathic pain, rheumatism and stasis pain caused by phlegm and blood stasis	Drug Standards of Ministry of Health, Prescription preparation of Chinese medicine, Volume 14, WS_3-B-2794-97
Hechan tablet (鹤蟾片)	Agrimoniae Herba, Dried toad skin, Ranunculi Ternati Radix, Fritillariae Thunbergii Bulbus, Pinelliae Rhizoma, Houttuyniae Herba, Descurainiae Semen Lepidii Semen, Ginseng Radix Et Rhizoma, Asparagi Radix	Detoxifying and dispelling phlegm, cooling blood and removing stasis, relieving swelling and loose knots	It is used for primary bronchial lung cancer and lung metastatic cancer, which can improve the subjective symptoms of patients and improve the patient's physique	Drug Standards of Ministry of Health, Prescription preparation of Chinese medicine, Volume 5, WS_3-B-1065-91
Jinpu capsule (金蒲胶囊)	Bovis Calculus Artifactus, Lonicerae Japonicae Flos, Scolopendra, Manis Squama, Bufonis Venenum, Taraxaci Herba, Scutellariae Barbatae Herba, Cremastrae Pseudobulbus Pleiones Pseudobulbus, Curcumae Rhizoma, Hedyotidis Herba, Sophorae Flavescentis Radix, Solani Nigri Herba, Margarita, Rhei Radix Et Rhizoma, Dioscoreae Bulbiferae Rhizoma, Processed Olibanum, Processed Myrrha, Processed Corydalis Rhizoma, Carthami Flos, Pinelliae Rhizoma Praeparatum Cum Zingibere Et Alumine, Codonopsis Radix, Astragali Radix, Acanthopanacis Senticosi Radix Et Rhizoma Seu Caulis, Amomi Fructus	Clearing heat and detoxifying, relieving swelling and pain, nourishing qi and eliminating phlegm	It is used for advanced gastric cancer and esophageal cancer patients with phlegm-dampness stasis and qi stagnation and blood stasis syndrome	Chinese Pharmacopoeia, 2020 edition, Part I

Compound toad venom ointment (复方蟾酥膏)	Bufonis Venenum, Aconiti Radix, Zanthoxyli Radix, Paris Polyphylla, Aconitum Coreanum, Hibisci Mutabilis Folium, Sparganii Rhizoma, Curcumae Rhizoma, Carthami Flos, Caryophylli Flos, Asari Radix Et Rhizoma, Cinnamomi Cortex, Sambuci Rhizoma, Piperis Longi Fructus, Nardostachyos Radix Et Rhizoma, Kaempferia Galanga, Olibanum, Myrrha, Menthol, Borneolum Syntheticum, Camphor, Methyl Salicylate, Benzyl Alcohol, Dimethyl Sulfoxide	Promoting blood circulation and removing blood stasis, relieving swelling and pain	It is used for pain caused by lung, liver, stomach and other cancers	National Drug Standards of the National State Food and Drug Administration, WS_3-B-3919-98-1
Jiawei Xihuang pill (加味西黄丸)	Bovis Calculus Artifactus, Myrrha, Artificial Musk, Bufonis Venenum, Olibanum	Detoxifying and dispersing knots, relieving swelling and pain	For carbuncle and sore, multiple abscesses, lymphadenitis, and cold abscesses	Drug Standards of Ministry of Health, Prescription preparation of Chinese medicine, Volume 3, WS_3-B-0532-91
Xiaojin pill (肖金丹)	Musk, Bufonis Venenum, Aconiti Kusnezoffii Radix Cocta, Liquidambaris Resina, Processed Olibanum, Processed Myrrha, Pheretima, Wine Processed Trogopteri Faeces, Wine Processed Angelicae Sinensis Radix, Pini Ink, Momordicae Semen	Dispersing knots and relieving swelling, dispersing stasis and relieving pain	It is used for initial Yin gangrene, unchanged skin color, hard and painful swelling, multiple abscess, thyroid tumor, lymphadenitis, lymph node tuberculosis, chronic cystic mammary disease	Drug Standards of Ministry of Health, Prescription preparation of Chinese medicine, Volume 5, WS_3-B-0945-91
Tianfoshen oral liquid (天佛参口服液)	Panacis Quinquefolii Radix, Bufonis Venenum, Asparagi Radix, Acanthopanax Obouatus, Actinidiae Chinensis Radix, Hippophae Fructus, Bolbostemmatis Rhizoma, Citri Sarcodactylis Fructus	Nourishing Yin and Qi, detoxifying and dispersing knots	Combined with anti-tumor chemicals, it is used for non-small cell lung cancer, which is Qi and Yin deficiency syndrome. The symptoms include fatigue, dry mouth and throat, shortness of breath, bloody phlegm, chest pain, etc. Meanwhile, it can reduce nausea, vomiting, constipation, hair loss and other phenomena caused by chemotherapy	Drug Standards of the National State Food and Drug Administration, YBZ08752008
Toad venom Zhentong gel ointment (蟾酥镇痛凝胶膏)	Bufonis Venenum, Strychni Semen, Arisaematis Rhizoma, Aconiti Radix, Realgar, Angelicae Dahuricae Radix, Curcumae longae Rhizoma, Lobeliae Chinensis Herba, Camphor, Menthol, Borneolum Syntheticum, Musk Xylene, Diphenhydramine Hydrochloride	Relieving swelling and dispersing knots, relieving swelling and pain	It is used for pain relief and dissipation of various lumps, and also for pain caused by muscle strain, bone spurs, arthritis, and so on	Standard promulgation documents of the National State Food and Drug Administration (2012), WS-11312(ZD-1312)-2002-2011Z
Toad venom ingot (蟾酥锭)	Bufonis Venenum, Musk, Borneolum Syntheticum, Realgar, Cinnabaris, Snail	Promoting blood circulation and detoxifying, relieving swelling and pain	For furunculosis and malignant sore, carbuncle caused by poor blood and qi in the back, incipient redness and stiffness, numbness and pain, breast carbuncles and pain, bitten by scorpions and malipedes, burning pain, and so on	Drug Standards of Ministry of Health, Prescription preparation of Chinese medicine, Volume 1, WS_3-B-0169-89
Toad venom Zhentong ointment (蟾酥镇痛膏)	Bufonis Venenum, Strychni Semen, Arisaematis Rhizoma, Aconiti Radix, Realgar, Angelicae Dahuricae Radix, Curcumae longae Rhizoma, Lobeliae Chinensis Herba, Camphor, Menthol, Borneolum Syntheticum, Musk Xylene, Diphenhydramine Hydrochloride	Relieving swelling and dispersing knots, relieving swelling and pain	It is used for pain relief and dissipation of various lumps, and also for pain caused by muscle strain, bone spurs, arthritis, and so on	Drug Standards of Ministry of Health, Prescription preparation of Chinese medicine, Volume 5, WS_3-B-1067-91
Compound toad venom pill (复方蟾酥丸)	Bufonis Venenum, Snail, Musk, Olibanum, Myrrha, Tonglv, Chalcanthite, Alumen, Hanshuishi, Cinnabaris, Realgar, Calomelas	Dispelling sore and swollen toxin	For carbuncle and furunculosis	Drug Standards of Ministry of Health, Prescription preparation of Chinese medicine, Volume 15, WS_3-B-2948-98

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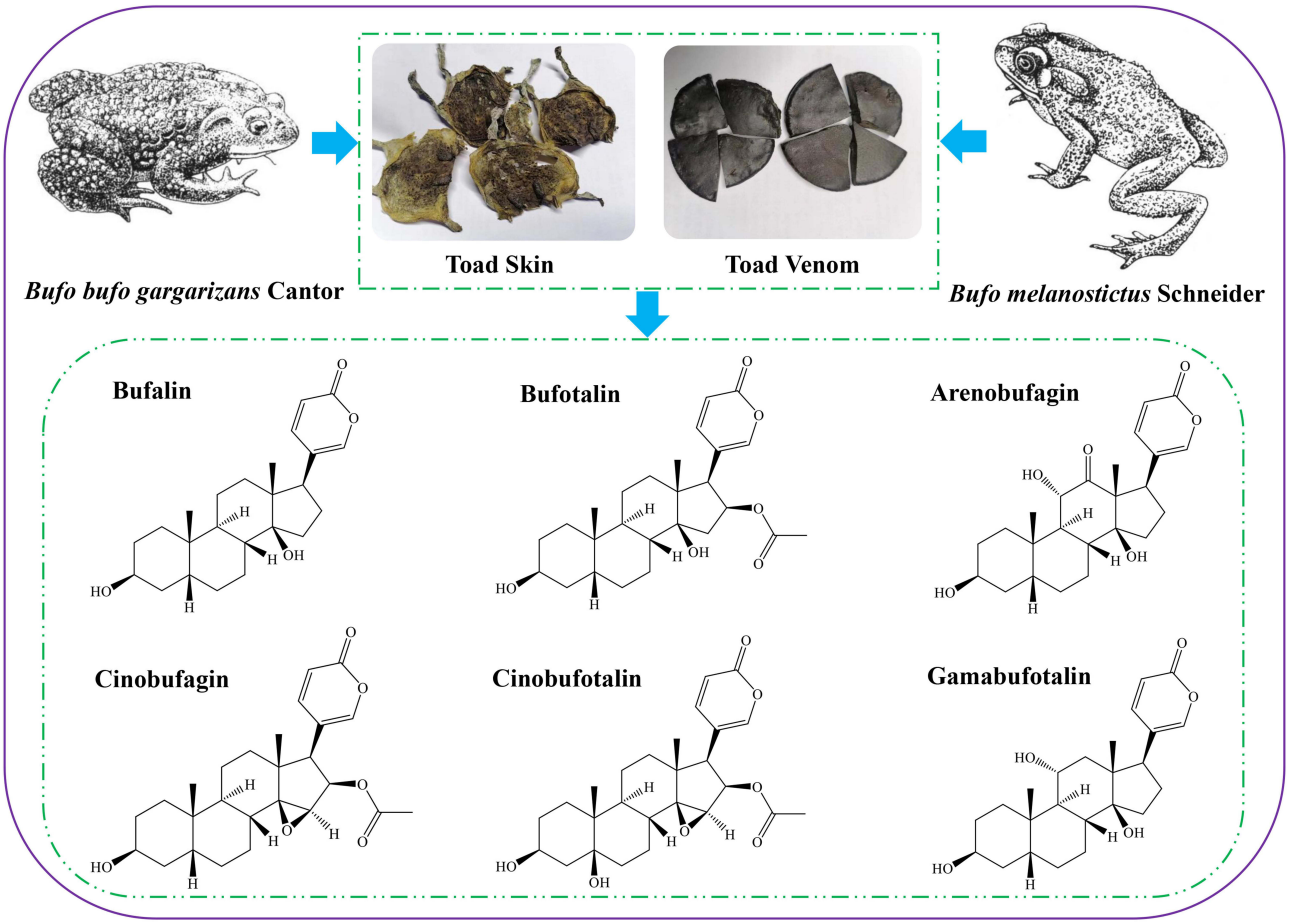

Figure 1 The source of toad skin and toad venom and the structure of their related active ingredients.

enrichment of the drug delivery system at the tumor site and the internalization by specific cells can be improved. Moreover, the drug delivery system can respond to endogenous (pH, hypoxia, and redox) or exogenous (light and heat) stimuli at the tumor site to achieve rapid drug release in the lesion area.[23,24] In recent years, codelivery systems loaded with multiple active ingredients of toad skin or toad venom and other antitumor drugs have been developed to further enhance the antitumor effects, reduce adverse reactions, and overcome MDR.

Therefore, based on the research progress on the use of toad skin and toad venom in combined antitumor therapy, this article first reviews the clinical efficacy and safety of toad skin- and toad venom-related preparations with chemotherapy drugs, targeted drugs, analgesics and transarterial chemoembolization (TACE) interventional therapy and summarizes the meta-analysis results of toad skin- and toad venom-related preparations as adjuvant therapies for cancer and cancer pain. Subsequently, the antitumor effects and mechanisms of toad skin- and toad venom-related preparations and active ingredients with chemotherapy, targeted therapy, radiotherapy, hyperthermia, Chinese herbal therapy, signaling pathway inhibitors, and other therapies in cells and animal models are evaluated. Finally, the combination antitumor strategies of the active ingredients of toad skin and toad venom combined with chemotherapy drugs, small-molecule targeted drugs, monoclonal antibodies, active ingredients of TCM, photodynamic therapy drugs and photothermal therapy drugs based on nanoplatforms are summarized and serve as a foundation for the clinical combination of toad skin and toad venom and the construction of novel drug delivery systems.

Clinical Combination Therapy Strategies

Clinical Efficacy and Safety of Cancer Treatment

Many studies have indicated that the use of toad skin or toad venom-related preparations as adjunct treatments for cancer could enhance therapeutic effects, improve patients' quality of life (QOL) or immunological response, or alleviate the incidence of

side effects. For example, the Huachansu tablet combined with TACE is effective at prolonging progression-free survival (PFS) and overall survival (OS) in patients with unresectable hepatocellular carcinoma (HCC).[25] The use of Huachansu capsule in combination with zoledronic acid therapy can result in a more favorable therapeutic outcome and an improved QOL in the treatment of metastatic bone tumors.[26] Huachansu injection combined with the CEMT regimen (carboplatin, etoposide, methotrexate, and thalidomide) achieved better clinical effects on patients with relapsed or refractory multiple myeloma.[27] Toad venom injection combined with apatinib can improve the disease control rate (DCR), reduce the incidence of neutropenia and fatigue symptoms, decrease the level of a tumor marker (CA199), and increase immune indices ($CD3^+$, $CD4^+$, $CD8^+$, $CD4^+/CD8^+$, IgG, IgA, and IgM) in patients with advanced gastric cancer.[28]

Moreover, when combined with Kang Ai Ping pill, capecitabine tablets have a beneficial therapeutic effect on rectal cancer, and can improve the objective response rate (ORR), DCR and QOL; reduce the levels of tumor markers (CEA, CA242, and CA199); and enhance the immune function of patients.[29] Xianchan tablets combined with chemotherapy (paclitaxel, calcium folinate and cisplatin) have significant efficacy in the care of elderly esophageal cancer patients and can improve the DCR, QOL, serum tumor markers and immune function indicators without increasing adverse reactions.[30] Delisheng injection combined with the XELIRI regimen (irinotecan + capecitabine) has a better clinical curative effect on liver metastases of colon cancer. It can improve patient survival, reduce the blood toxicity of chemotherapy, and protect the body's immune function.[31] Hechan tablets combined with the DP regimen (docetaxel + cisplatin) have good curative effects on non-small cell lung cancer (NSCLC), and can decrease the levels of tumor markers, improve the QOL, PFS and immune function of patients, and reduce side effects (such as decreased white blood cell count, damage to renal function, damage to liver function, and diarrhea).[32] Tianfoshen oral liquid combined with a GP regimen (gemcitabine + cisplatin) can improve the immune functions of elderly patients with advanced NSCLC.[33] In addition, the Toad venom injection + TC regimen, Toad venom injection + TP regimen, Kang Ai Ping pill + mFOLFOX6 regimen + trastuzumab, Xianchan tablet + docetaxel, Xianchan tablet + mFOLFOX6 regimen + trastuzumab, and Xianchan tablet + GP regimen could all enhance the therapeutic effects, improve patients' QOL or immune system, or reduce the risk of side effects.[34–39] The clinical efficacy and safety of toad skin or toad venom-related preparations as adjunct treatments for cancer are shown in Table 2.

Clinical Efficacy and Safety of Treating Cancer Pain

Cancer patients are deeply affected by cancer pain resulting from either cancer therapy or the malignancy itself. Effective pain treatment is an important step in improving the QOL of cancer patients. Presently, the three-step analgesic method is the standard treatment scheme for cancer pain, but it has large adverse impacts and dependence readily occurs.[40] Recently, numerous investigations have revealed that toad skin- and toad venom-related preparations, especially Huachansu preparations, combined with three-step analgesia can improve the total effective rate of cancer pain treatment, reduce the onset time of pain relief, extend the duration of pain relief, reduce the daily dosage of opioids, improve QOL, and lessen the risk of adverse reactions. For example, the combination of Huachansu capsules with sustained-release oxycodone hydrochloride tablets could significantly improve the analgesic effect, reduce the VAS score, increase the KPS score, shorten the onset time, prolong the duration of analgesia, and reduce the dose of oxycodone hydrochloride.[41,42] The Huachansu capsule + fentanyl transdermal patch combination increased the effective rate of analgesia, reduced the VAS score and estazolam dosage, and improved the QOL of patients.[43] The ability of the Huachansu capsule combined with zoledronic acid to relieve osseous metastasis pain in prostate cancer patients is significant and can effectively decrease the levels of prostate-specific antigen (PSA) and free PSA (f-PSA) and improve patients' QOL.[44] In addition, the Huachansu capsule + three-step analgesic ladder could enhance the analgesic effect by increasing the level of β-endorphin in patients' peripheral blood and decreasing the level of 5-hydroxytryptamine.[45] Intervention with Huachansu and opioids in patients with opium intolerance with incomplete analgesia can reduce breakthrough pain, decrease the rate of long-acting opioid increase, and improve sleep and mood.[46] Huachansu injection + acetaminophen oxycodone effectively alleviated pain; decreased the occurrence of negative reactions, including drowsiness, constipation, dysuria, nausea and vomiting; and was effective at treating patients suffering from moderate to severe cancer-related visceral pain.[47] Huachansu capsule + sustained-release morphine hydrochloride tablets could significantly improve the analgesic effect, shorten the onset time, prolong the duration of analgesia, and reduce the incidence of side effects.[48] Huachansu injection + sodium ibandronate injection can significantly improve the treatment effect and QOL of

Table 2 Clinical Efficacy and Safety of Toad Skin or Toad Venom-Related Preparations as Adjunct Treatments for Cancer

Preparation	Object of Clinical Treatment	Treatment Plan	Clinical Efficacy	Ref.
Huachansu tablet (华蟾素片)	From September 2012 to September 2016, 120 patients diagnosed with unresectable hepatocellular carcinoma were divided into combination group (n = 60) and control group (n = 60)	Control group: transarterial chemoembolization (TACE), combination group: Huachansu tablet + TACE	Progression-free survival (PFS) (6.8 months vs 5.3 months), overall survival (OS) (14.8 months vs 10.7 months)	2023[25]
Huachansu capsule (华蟾素胶囊)	From June 2014 to June 2017, 120 patients diagnosed with metastatic bone tumors were divided into combination group (n = 60) and control group (n = 60)	Control group: zoledronic acid, combination group: Huachansu capsule + zoledronic acid	Overall treatment effect (73.33% vs 46.67%), pain numerical rating scale (NRS) scores (1.68±0.55 vs 4.39±0.60), quality of life (QOL) score after 3 months of treatment (84.30±0.60 vs 78.05±0.58), no difference in rate of adverse reactions	2018[26]
Huachansu injection (华蟾素注射液)	40 patients diagnosed with relapsed or refractory multiple myeloma were divided into combination group (n = 20) and control group (n = 20)	Control group: CEMT regimen (carboplatin, etoposide, methotrexate, and thalidomide), combination group: Huachansu injection + CEMT regimen	The total effectiveness (65.0% vs 40.0%)	2013[27]
Toad venom injection (蟾酥注射液)	From January 2019 to December 2019, 50 patients diagnosed with advanced gastric cancer (stage IV) were divided into combination group (n = 25) and control group (n = 25)	Control group: apatinib, combination group: Toad venom injection + apatinib	Objective response rate (ORR) (20% vs 8%), disease control rate (DCR) (76% vs 48%), the incidence of neutropenia and fatigue symptoms ↓, the levels of tumor markers (CA199) ↓, the changes of immune indexes ($CD3^+$, $CD4^+$, $CD8^+$, $CD4^+/CD8^+$, IgG, IgA, IgM) ↑	2020[28]
Kang Ai Ping pill (抗癌平丸)	From June 2017 to June 2018, 110 patients with rectal cancer were divided into combination group (n = 55) and control group (n = 55)	Control group: capecitabine tablets, combination group: Kang Ai Ping pill + capecitabine tablets	ORR (65.45% vs 54.55%), DCR (89.09% vs 76.36%), QOL ↑, the levels of tumor markers (CEA, CA242, CA199) ↓, the immune function indexes ($CD3^+$, $CD4^+$, NK cells, and $CD4^+/CD8^+$) ↑	2019[29]
Xianchan tablet (仙蟾片)	From July 2016 to July 2017, 68 elderly patients with esophageal cancer were divided into combination group (n = 34) and control group (n = 34)	Control group: paclitaxel, calcium folinate and cisplatin, treatment group: Xianchan tablet + paclitaxel, calcium folinate and cisplatin	DCR (91.2% vs 70.6%), immune function indicators (IgA, $CD3^+$) ↑, serum tumor markers (CEA, NSE) ↓, QOL ↑	2020[30]
Delisheng injection (得力生注射液)	From August 2011 to April 2013, 64 patients with liver metastases of colon cancer were divided into combination group (n = 32) and control group (n = 32)	Control group: XELIRI regimen (irinotecan + capecitabine), treatment group: Delisheng injection + XELIRI regimen	ORR (68.75% vs 43.75%), QOL ↑, White blood cells ↑, immune function indicators ($CD3^+$, $CD4^+$, $CD8^+$) ↑	2014[31]
Hechan tablet (鹤蟾片)	From May 2015 to February 2017, 80 patients with NSCLC were divided into combination group (n = 41) and control group (n = 39)	Control group: DP regimen (docetaxel + cisplatin), treatment group: Hechan tablet + DP regimen	ORR (61.54% vs 39.02%), tumor markers (Cyfra21-1, CEA, CA125) ↓, QOL ↑, immune function indicators ($CD3^+$, $CD4^+$, $CD4^+/CD8^+$) ↑, PFS (7.31±2.07 vs 5.45±1.72), the incidences of adverse drug reactions (Decrease of white blood cell count, renal function damage, liver function damage, diarrhoea) ↓	2018[32]
Tianfoshen oral liquid (天佛参口服液)	From January 2010 to October 2014, 152 elderly patients with advanced NSCLC were divided into combination group (n = 76) and control group (n = 76)	Control group: GP regimen (gemcitabine + cisplatin), treatment group: Tianfoshen oral liquid + GP regimen	Immune function indicators (NK, $CD3^+$, $CD4^+$, $CD4^+/CD8^+$) ↑	2017[33]
Toad venom injection (蟾酥注射液)	From May 2016 to May 2019, 102 patients diagnosed with epithelial ovarian cancer (stage III/IV) were divided into combination group (n = 51) and control group (n = 51)	Control group: TC regimen (paclitaxel + carboplatin), combination group: Toad venom injection + TC regimen	ORR (84.31% vs 66.67%), incidence of adverse reactions (15.69% vs 37.25%), the level of serum carbohydrate antigen 125 (CA125) ↓, the score of QOL ↑, $CD3^+$ and $CD4^+$ ↑, $CD8^+$ ↓	2020[34]

Toad venom injection (蟾酥注射液)	From January 2015 to January 2016, 78 patients diagnosed with advanced ovarian cancer (stage III/IV) were divided into combination group (n = 39) and control group (n = 39)	Control group: TP regimen (paclitaxel + cisplatin), combination group: Toad venom injection + TP regimen	ORR (82.1% vs 66. 7%), CA125 ↓, score of QOL ↑, incidence of adverse reactions (Elevated transaminase, leukopenia, neutropenia, nausea and vomiting, diarrhea and abdominal pain) ↓	2017[35]
Kang Ai Ping pill (抗癌平丸)	From May 2013 to May 2014, 98 patients with HER2 positive advanced gastric cancer were divided into combination group (n = 49) and control group (n = 49)	Control group: mFOLFOX6 regimen (oxaliplatin + calcium folinate + 5-fluorouracil) + trastuzumab, treatment group: Kang Ai Ping pill + mFOLFOX6 regimen + trastuzumab	ORR (69.4% vs 42.8%), DCR (85.7% vs 67.3%), PFS (18.90±1.82 vs 15.68 ±1.57), OS (21.36±2.21 vs 17.89±1.71), 1-year survival rate (81.6% vs 65.3%), 3-year survival rate (53.1% vs 38.8%), the rates of cardiac toxicity, gastrointestinal reaction, hepatotoxicity, nephrotoxicity, bone marrow suppression and neurotoxicity ↓	2019[36]
Xianchan tablet (仙蟾片)	From January 2013 to January 2015, 64 patients with advanced NSCLC were divided into combination group (n = 32) and control group (n = 32)	Control group: docetaxel, treatment group: Xianchan tablet + docetaxel	ORR (65.6% vs 46.9%), DCR (87.5% vs 68.7%), immune function indicators ($CD3^+$, $CD4^+$, $CD8^+$, IgG and IgA) ↑, QOL ↑, the incidences of adverse drug reactions (Liver function injury, myelosuppression, cardiovascular toxicity, gastrointestinal reaction and neurotoxicity) ↓, PFS (13.45±1.25 vs 11.36±1.19), OS (16.37±1.59 vs 14.12±1.42), 1-year survival rate (87.5% vs 68.7%), 3-year survival rate (65.6% vs 46.8%)	2019[37]
Xianchan tablet (仙蟾片)	From January 2011 to January 2014, 212 elderly patients with HER2 positive advanced gastric cancer were divided into combination group (n = 106) and control group (n = 106)	Control group: mFOLFOX6 regimen (oxaliplatin + calcium folinate + 5-fluorouracil) + trastuzumab, treatment group: Xianchan tablet + mFOLFOX6 regimen + trastuzumab	ORR (73.5% vs 60.3%), DCR (89.6% vs 78.3%), serum biochemical indexes (FSP-l, MRP-l4, CXCR4 and SDF-1) ↓, PFS (17.2±1.9 vs 13.9±1.3), OS (20.1±2.5 vs 16.5±1.7), the incidences of adverse drug reactions (Thrombocytopenia, neutropenia, diarrhea and abdominal pain, nausea and vomiting, dizziness and headache) ↓	2018[38]
Xianchan tablet (仙蟾片)	From May 2014 to May 2016, 114 elderly patients with advanced NSCLC were divided into combination group (n = 57) and control group (n = 57)	Control group: GP regimen (gemcitabine hydrochloride injection + cisplatin sodium chloride injection), treatment group: Xianchan tablet + GP regimen	ORR (77.19% vs 61.40%), immune function indicators ($CD3^+$, $CD4^+$, $CD8^+$, IgG and IgA) ↑, the serum NSE, CEA, CYFRA21-1, and SCC level ↓, the incidences of adverse drug reactions (Gastrointestinal reaction, leukopenia, thrombocytosis, fever, rash) ↓	2017[39]

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patients with metastatic bone cancer pain.[49] Moreover, the clinical efficacy of Tianchan capsule + sustained-release oxycodone hydrochloride tablets in the management of cancer-related pain is remarkable, as they can not only effectively relieve cancer pain but also improve QOL, with good safety.[50,51] Cancer pain can also be effectively managed with the traditional three-step analgesic ladder + acupoint sticking with Chanwu gel ointment. This treatment can also lower the dosage of three-step analgesics and minimize side effects.[52] In addition, other studies have indicated the therapeutic effectiveness and safety of Huachansu + the three-step analgesic ladder.[53–58] The clinical efficacy and safety of toad skin- or toad venom-related preparations as adjunct treatments for cancer pain are shown in Table 3.

Systematic Review and Meta-Analysis of Cancer Treatment and Pain Relief

Many systematic reviews and meta-analyses have reported that toad skin- or toad venom-related preparations are effective and safe as complementary therapies for cancer and cancer pain. For example, a meta-analysis was conducted on the efficacy and safety of Huachansu injection combined with chemotherapy in the treatment of lung cancer using Review Manager 5.3 software. A total of 21 clinical studies involving 1735 patients with lung cancer were included. The results showed that Huachansu injection did not cause additional side effects in lung cancer patients, such as hematological and gastrointestinal toxicity, cardiotoxicity, hepatotoxicity, or nephrotoxicity, when it was used in combination with chemotherapy. Instead, it could significantly improve the ORR, DCR, and QOL, and alleviate pain.[59] Another meta-analysis of the efficacy and safety of Huachansu capsules combined with first-line platinum-based chemotherapy for advanced NSCLC was conducted using Review Manager 5.3 and Stata 15.1 software. A total of 19 clinical studies involving 1564 patients were included. In patients with stage III/IV NSCLC, Huachansu capsule significantly improved the ORR and 1- and 2-year survival rates, increased the percentages of $CD3^+$ cells and $CD4^+$ cells, increased the ratio of $CD4^+/CD8^+$ cells, and minimized toxicity, including thrombocytopenia, leukopenia and vomiting, when combined with first-line chemotherapy based on platinum.[60] Some researchers have also conducted a meta-analysis of the efficacy and safety of Huachansu injection combined with chemotherapy in the treatment of gastric cancer using Review Manager 5.3 and Stata 13.0 software. A total of 14 randomized controlled trials (RCTs) with 976 participants were included. Compared with traditional chemotherapy alone, Huachansu injection + chemotherapy was related to better outcomes in terms of increasing the ORR, improving the performance status, and alleviating leukopenia in patients diagnosed with gastric cancer.[61] Moreover, some researchers have systematically investigated the safety and efficacy of the combination of TACE and Huachansu injection for patients with advanced HCC using Review Manager 5.3 and Stata 12.0 software. A total of 27 RCTs involving 2079 patients were included in this analysis. Huachansu injection combined with TACE prolonged the 1-, 1.5-, 2-, and 3-year OS; improved the ORR, DCR, and QOL; enhanced immune function and liver function ($CD3^+$, $CD4^+$, $CD4^+/CD8^+$, natural killer cell, total bilirubin, alanine aminotransferase [ALT], aspartate aminotransferase [AST] levels); and did not cause serious adverse events in patients diagnosed with advanced HCC.[62] Furthermore, one meta-analysis was conducted on the efficacy and safety of toad venom injection combined with chemotherapy/radiotherapy for tumor patients using Review Manager 5.0.24 software. Six studies involving 369 participants (192 participants in the treatment group and 177 participants in the control group) were included. Toad venom injection has a certain effect on increasing the KPS functional score, improving the clinical syndrome, reducing the toxicity of chemotherapy and radiotherapy, and enhancing immune function in patients with tumors.[63] In addition, two meta-analyses were conducted on the efficacy and safety of Huachansu combined with a three-step analgesic ladder in the treatment of cancer pain using Review Manager 5.3/5.4 software. One study included 10 RCTs with 1293 patients (648 patients in the test group and 645 patients in the control group), and the other included 18 RCTs with 2088 patients (1046 patients in the test group and 1042 patients in the control group). Huachansu + the three-step analgesic ladder could greatly increase the overall effectiveness of cancer pain management, reduce the onset time and extend the duration of pain relief, reduce the daily dosage of opioids, improve QOL, and reduce the risk of adverse reactions (constipation, nausea and vomiting, dizziness, drowsiness, and anorexia).[64,65] Additionally, other systematic reviews and meta-analyses have shown that Huachansu is effective and safe as a complementary therapy for cancer.[66–72] The meta-analyses of the use of toad skin- or toad venom-related preparations as adjunct treatments for cancer are shown in Table 4.

Table 3 Clinical Efficacy and Safety of Toad Skin or Toad Venom-Related Preparations as Adjunct Treatments for Cancer Pain

Preparation	Object of Clinical Treatment	Treatment Plan	Clinical Efficacy	Ref.
Huachansu capsule (华蟾素胶囊)	From January 2018 to January 2019, 196 patients with advanced cancer pain were divided into combination group (n = 98) and control group (n = 98)	Control group: sustained-release oxycodone hydrochloride tablets, combination group: Huachansu capsule + sustained-release oxycodone hydrochloride tablets	The effective rate of analgesia (97.96% vs 81.63%), the VAS scores ↓, the KPS scores ↑, onset time of analgesia ↓, duration of analgesia ↑, Oxycodone hydrochloride dose ↓	2020[41]
Huachansu capsule (华蟾素胶囊)	From January 2018 to May 2019, 65 patients with moderate and severe cancer pain were divided into combination group (n = 33) and control group (n = 32)	Control group: sustained-release oxycodone hydrochloride tablets, combination group: Huachansu capsule + sustained-release oxycodone hydrochloride tablets	The effective rate of analgesia (96.9% vs 81.2%), the VAS scores ↓, the KPS scores ↑, onset time of analgesia ↓, duration of analgesia ↑, Oxycodone hydrochloride dose ↓	2019[42]
Huachansu capsule (华蟾素胶囊)	From April 2016 to June 2018, 298 patients with bone metastasis with moderate and severe cancer pain were divided into combination group (n = 147) and control group (n = 151)	Control group: fentanyl transdermal patch, combination group: Huachansu capsule + fentanyl transdermal patch	The effective rate of analgesia (72.1% vs 49.7%), the VAS scores ↓, Estazolam dosage ↓, improve the QOL of patients	2019[43]
Huachansu capsule (华蟾素胶囊)	From January 2014 to December 2016, 60 prostatic cancer patients with osseous metastasis pain were divided into combination group (n = 30) and control group (n = 30)	Control group: androgen deprivation therapy + zoledronic acid, combination group: Huachansu capsule + androgen deprivation therapy + zoledronic acid	The total effective rate of pain relief (100% vs 90%), prostate specific antigen (PSA) ↓, free PSA (f-PSA) ↓, improve the QOL of patients	2017[44]
Huachansu capsule (华蟾素胶囊)	From May 2019 to January 2020, 60 patients with cancer pain were divided into combination group (n = 30) and control group (n = 30)	Control group: three-step analgesic ladder, combination group: Huachansu capsule + three-step analgesic ladder	The pain relief rates (83.33% vs 56.66%), the NRS scores ↓, improve the QOL of patients, improve constipation, β-endorphin ↑, 5-hydroxytryptamine ↓, total incidence of adverse reactions ↓	2020[45]
Huachansu capsule (华蟾素胶囊)	From February 2017 to February 2019, 66 patients with opioid tolerance with cancer pain were divided into combination group (n = 33) and control group (n = 33)	Control group: opioids, combination group: Huachansu capsule + opioids	Average daily frequency of breakthrough pain ↓, long-acting opioid increase rate ↓, improve sleep and mood, the proportion of constipation (69.6% vs 90.9%)	2019[46]
Huachansu injection (华蟾素注射液)	From May 2013 to April 2016, 82 patients with moderate to severe cancerous visceral pain were divided into combination group (n = 41) and control group (n = 41)	Control group: acetaminophen oxycodone, combination group: Huachansu injection + acetaminophen oxycodone	The pain relief rates (78.1% vs 63.4%), pain intensity ↓, the KPS scores ↑, incidence of adverse reactions (Drowsiness, constipation, dysuria, nausea and vomiting, stun) ↓	2018[47]
Huachansu capsule (华蟾素胶囊)	From September 2019 to September 2020, 60 patients with cancer pain were divided into combination group (n = 30) and control group (n = 30)	Control group: sustained-release morphine hydrochloride tablets, combination group: Huachansu capsule + sustained-release morphine hydrochloride tablets	The effective rate of analgesia (80% vs 53.3%), onset time of analgesia ↓, duration of analgesia ↑, incidence of adverse reactions (26.7% vs 40%) ↓	2021[48]
Huachansu injection (华蟾素注射液)	From January 2011 to September 2017, 240 patients with bone metastases with cancer pain were divided into combination group (n = 120) and control group (n = 120)	Control group: sodium ibandronate injection, combination group: Huachansu injection + sodium ibandronate injection	The effective rate of analgesia (81.7% vs 65%), the improvement rate of QOL (63.3% vs 40%)	2018[49]
Tianchan capsule (天蟾胶囊)	From February 2017 to February 2021, 113 patients with advanced gastric cancer with cancer pain were divided into combination group (n = 56) and control group (n = 57)	Control group: sustained-release oxycodone hydrochloride tablets, combination group: Tianchan capsule + sustained-release oxycodone hydrochloride tablets	The effective rate of analgesia (94.64% vs 77.19%), the VAS scores ↓, the scores of physical function, mental function, social function and material life ↑, the incidences of adverse drug reactions (gastrointestinal reaction, myelosuppression, liver function damage and peripheral neurotoxicity) ↓	2022[50]

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Table 3 (Continued).

Preparation	Object of Clinical Treatment	Treatment Plan	Clinical Efficacy	Ref.
Tianchan capsule (天蟾胶囊)	From June 2015 to December 2017, 167 patients with various types of cancer with moderate cancer pain were divided into combination group (n = 84) and control group (n = 83)	Control group: sustained-release oxycodone hydrochloride tablets, combination group: Tianchan capsule + sustained-release oxycodone hydrochloride tablets	The pain relief rates (94.05% vs 79.52%), the onset time of pain relief ↓, the VAS scores ↓, improve the QOL of patients	2020[51]
Chanwu gel ointment (蟾乌凝胶膏)	From June 2014 to July 2016, 100 patients with advanced cancer pain were divided into combination group (n = 50) and control group (n = 50)	Control group: three-step analgesic ladder, treatment group: three-step analgesic ladder + acupoint sticking with Chanwu gel ointment	Pain relief rate (86% vs 68%), onset time of analgesia ↓, duration of analgesia ↑, total incidence of adverse reactions ↓	2017[52]
Huachansu capsule (华蟾素胶囊)	120 patients with gastrointestinal malignancy with cancer pain were divided into combination group (n = 60) and control group (n = 60)	Control group: three-step analgesic ladder, combination group: Huachansu capsule + three-step analgesic ladder	Mild and moderate pain relief rates (86.7% vs 44.4%), incidence of constipation (35% vs 53.3%), improve the QOL of patients	2017[53]
Huachansu capsule (华蟾素胶囊)	From January 2019 to January 2020, 100 patients with moderate and severe cancer pain were divided into combination group (n = 50) and control group (n = 50)	Control group: sustained-release oxycodone hydrochloride tablets, combination group: Huachansu capsule + sustained-release oxycodone hydrochloride tablets	The pain relief rates (88.0% vs 70.0%), the NRS scores ↓, the KPS scores ↑, improve the QOL of patients, total incidence of adverse reactions ↓	2021[54]
Huachansu tablet (华蟾素片)	From September 2012 to July 2014, 160 patients with cancer pain were divided into combination group (n = 80) and control group (n = 80)	Control group: three-step analgesic ladder, combination group: Huachansu tablet + three-step analgesic ladder	The pain relief rates (91% vs 80%), improve the QOL of patients, incidence of constipation and nausea ↓	2015[55]
Huachansu tablet (华蟾素片)	From January 2014 to December 2018, 120 elderly patients with advanced liver cancer with cancer pain were divided into combination group (n = 60) and control group (n = 60)	Control group: sustained-release oxycodone hydrochloride tablets, combination group: Huachansu tablet + sustained-release oxycodone hydrochloride tablets	The pain relief rates (88.3% vs 68.3%), improve the QOL of patients	2019[56]
Huachansu capsule (华蟾素胶囊)	From January 2014 to December 2017, 240 patients with cancer pain were divided into combination group (n = 120) and control group (n = 120)	Control group: sustained-release morphine sulfate tablets, combination group: Huachansu capsule + sustained-release morphine sulfate tablets	The effective rate of analgesia (80% vs 65%), the NRS scores ↓, the KPS scores ↑, onset time of analgesia ↓, duration of analgesia ↑, daily dosage of Morphine sulfate sustained release tablets ↓	2018[57]
Huachansu tablet (华蟾素片)	From June 2011 to June 2014, 120 patients with cancer pain were divided into combination group (n = 60) and control group (n = 60)	Control group: sustained-release oxycodone hydrochloride tablets, combination group: Huachansu tablet + sustained-release oxycodone hydrochloride tablets	The effective rate of analgesia (95% vs 78.2%), improve the QOL of patients	2019[58]

Table 4 Meta-Analyses of the Use of Toad Skin or Toad Venom-Related Preparations as Adjunct Treatments for Cancer

Preparation	Group	Patient	Meta-Analysis Results	Ref.
Huachansu injection (华蟾素注射液)	Huachansu injection combined with chemotherapy	21 studies, 1735 cases of lung cancer patients	Increase ORR, DCR, QOL, and the effect of pain relief, and do not bring additional adverse events such as hematological toxicity, gastrointestinal toxicity, cardiotoxicity, hepatotoxicity, and nephrotoxicity	2021[59]
Huachansu capsule (华蟾素胶囊)	Huachansu capsule combined with first-line platinum-based chemotherapy	19 RCTs, 1564 cases of patients diagnosed with stage III/IV NSCLC (796 patients in the test group and 768 patients in the control group)	Show significant effects in improving ORR, 1-year and 2-year survival rate, raising the percentages of $CD3^+$ cells, $CD4^+$ cells, and ratio of $CD4^+/CD8^+$, and reducing chemotherapy toxicity including leukopenia, thrombocytopenia, and vomiting	2021[60]
Huachansu injection (华蟾素注射液)	Huachansu injection combined with chemotherapy	14 RCTs, 976 cases of patients diagnosed with gastric cancer (478 patients in the test group and 498 patients in the control group)	Improve the ORR, performance status, and alleviate leukopenia	2020[61]
Huachansu injection (华蟾素注射液)	Huachansu injection combined with TACE	27 RCTs, 2079 cases of patients diagnosed with advanced hepatocellular carcinoma (1045 patients in the test group and 1034 patients in the control group)	Prolong the 1-, 1.5-, 2-, and 3-year OS, improve the ORR, DCR, and QOL, enhance the immune function and liver function ($CD3^+$, $CD4^+$, $CD4^+/CD8^+$, natural killer cell, total bilirubin, alanine aminotransferase [ALT], aspartate aminotransferase [AST]), and no serious adverse events occurred	2018[62]
Toad venom injection (蟾酥注射液)	Toad venom injection combined with chemotherapy or radiotherapy	6 RCTs, 369 cases of patients diagnosed with advanced NSCLC (192 patients in the test group and 177 patients in the control group)	Toad venom has a certain effect on increasing the KPS functional state score, improving clinical syndrome, reducing the toxicity of chemotherapy and radiotherapy, and enhancing immune function	2013[63]
Huachansu	Huachansu combined with three-step analgesic ladder	10 RCTs, 1293 cases of patients diagnosed with cancer pain (648 patients in the test group and 645 patients in the control group)	A significant improvement in pain relief, quality of life and alleviation of adverse reactions (constipation, nausea and vomiting, dizziness, drowsiness, anorexia)	2019[64]
Huachansu	Huachansu combined with three-step analgesic ladder	18 RCTs, 2088 cases of patients diagnosed with cancer pain (1046 patients in the test group and 1042 patients in the control group)	Improve the total effective rate of cancer pain treatment, reduce the NRS and VAS scores, reduce the onset time of pain relief, extend the duration of pain relief, reduce the daily dosage of opioids, improve the quality of life, and reduce the incidence of adverse reactions (constipation, nausea and vomiting, dizziness, drowsiness, anorexia)	2023[65]
Huachansu injection (华蟾素注射液)	Huachansu injection combined with platinum-based chemotherapy	32 RCTs, 2753 cases of patients diagnosed with stage III/IV NSCLC	Improve the ORR, DCR, 1- and 2-year survival rates, and QOL and alleviate neutropenia, thrombocytopenia, nausea, vomiting, anemia, liver injury, renal injury, and alopecia	2021[66]
Huachansu injection (华蟾素注射液)	Huachansu injection combined with first-line platinum-based chemotherapy	27 RCTs, 2125 cases of patients diagnosed with NSCLC (1082 patients in the test group and 1043 patients in the control group)	Enhance the ORR, 1- and 2-year survival rates, improve the QOL, and reduce the incidences of WBC toxicity, platelet toxicity, gastrointestinal reactions, pain, and hair loss reaction	2019[67]

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Table 4 (Continued).

Preparation	Group	Patient	Meta-Analysis Results	Ref.
Huachansu injection (华蟾素注射液)	Huachansu injection combined with chemotherapy	29 RCTs, 2300 cases of patients diagnosed with advanced NSCLC (1164 patients in the test group and 1136 patients in the control group)	Prolong the 1-, 2- and 3-year OS, improve the ORR, DCR, QOL and karnofsky performance score (KPS), and display lower incidences of leukopenia, thrombocytopenia, nausea and vomiting, hepatotoxicity, nephrotoxicity, gastrointestinal side effects, diarrhea, peripheral neurotoxicity, granulopenia, alopecia, myelosuppression, constipation, hemoglobin reduction and anemia	2019[68]
Huachansu injection (华蟾素注射液)	Huachansu injection combined with chemotherapy	12 RCTs, 853 cases of patients diagnosed with stage III/IV gastric cancer (423 patients in the test group and 430 patients in the control group)	Huachansu injection could increase response rate and DCR of chemotherapy, improve the QOL, increase leukocytes, improve anemia, improve hand-foot syndrome induced by chemotherapy, and relieve cancer pain	2018[69]
Huachansu	Huachansu combined with chemotherapy	17 RCTs, 1142 cases of patients diagnosed with stage III/IV NSCLC (578 patients in the test group and 564 patients in the control group)	A significant improvement in ORR, 1-year survival, Karnofsky performance status, pain relief, and alleviation of severe side-effects (nausea and vomiting, leukocytopenia)	2015[70]
Huachansu	Huachansu combined with TACE	9 RCTs, 659 cases of patients diagnosed with advanced hepatocellular carcinoma (333 patients in the test group and 326 patients in the control group)	Increase the ORR and 2-year survival rate	2014[71]
Huachansu	Huachansu combined with chemotherapy	15 RCTs, 1008 cases of patients diagnosed with stage III/IV gastric cancer (489 patients in the test group and 519 patients in the control group)	Increase the total response rate and Karnofsky score, and reduce the gastrointestinal side effects and leucocytopenia	2013[72]

Combined Antitumor Strategies Based on in vitro and in vivo Models

Combined with Chemotherapy

Enhancing the Effect of Chemotherapy

Toad skin, toad venom and its main active ingredients, such as gamabufotalin, arenobufagin, bufalin and cinobufagin, combined with chemotherapy drugs, such as cisplatin, arsenite (As^{III}), gemcitabine, paclitaxel, temozolomide, doxorubicin (DOX) and hydroxycampothecin, could strengthen the therapeutic effect on tumors by arresting the cell cycle, triggering apoptosis and autophagic death in cells, and preventing the growth, migration, and invasion of cells in different types of cancer.

The combination of 100 μg/mL Huachansu injection and 1 μg/mL cisplatin increased the apoptosis of OS732 cells by upregulating Fas expression.[73] The combination of Huachansu (aqueous extract) and DOX could increase HCC cell apoptosis via Fas- and mitochondria-mediated pathways.[74] In pancreatic cancer, gemcitabine combined with bufalin suppressed cell proliferation and promoted cell apoptosis by triggering the apoptosis signal-regulating kinase 1 (ASK1)/c-Jun N-terminal kinase (JNK) pathway.[75] By suppressing the integrin α2β5/focal adhesion kinase (FAK) pathway, the combination of bufalin and paclitaxel more effectively inhibited the growth of xenograft tumors and inhibited the proliferation of cervical cancer cells. The tumor weight inhibition rate of the combined group was 75.2%, which was significantly higher than that of the groups treated with bufalin alone (46.8%) or paclitaxel alone (41.3%).[76] Additionally, bufalin increased the apoptosis of glioma stem-like cells induced by temozolomide by triggering the mitochondrial pathway.[77] In castration-resistant prostate cancer, bufalin (0.6 or 0.8 mg/kg) + hydroxycampothecin (2 mg/kg) greatly reduced the tumor volume (tumor inhibition rate: 81.26±9.19% or 92.99±3.96%) and induced higher levels of cell apoptosis by increasing the expression of Bcl-2-like protein 4 (Bax), p53, programmed cell death 4 (PDCD4) and glycogen synthase kinase 3beta (GSK-3β) and reducing the expression of B-cell lymphoma-extra large (Bcl-xl) and phosphorylated protein kinase B (p-Akt) compared with bufalin or hydroxycampothecin alone.[78] When combined with cisplatin, bufalin can reduce the cisplatin-induced activation of AKT under both normoxic and hypoxic conditions, which can limit cell growth and promote apoptosis in gastric cancer cells. Downstream molecules of AKT such as glycogen synthase kinase, mechanistic target of rapamycin (mTOR), ribosomal protein S6 kinase and eukaryotic translation initiation factor-4E-binding protein-1 are likewise inhibited by bufalin.[79] In addition, compared with cinobufagin or cisplatin treatment alone, the combination of these two agents significantly reduced cell migration and invasion, enhanced apoptosis and S-phase arrest in vitro, inhibited the growth and metastasis of xenograft tumors, and prolonged the survival rate in vivo by blocking the Notch pathway.[80] Arsenite (As^{III}) and gamabufotalin together had synergistic cytotoxic effects on U-87 cells by triggering necrosis, autophagic cell death, and G2/M arrest.[81] Arenobufagin also sensitized U-87 cells to As^{III}-mediated cytotoxicity. The synergistic effect was partially attributed to the induction of apoptosis, necrosis, and G2/M-phase arrest, as well as autophagic cell death and the regulation of the Jagged1/Notch pathway.[82]

Reversing Chemotherapy Resistance

The active ingredients of toad skin and toad venom, such as bufalin, cinobufagin and bufotalin, can also reverse MDR by affecting the cell cycle and cell apoptosis, regulating cancer cell stemness and the polarization of M2 macrophages, inhibiting drug efflux pump activity, and decreasing the expression of drug resistance genes and proteins, among others.

In methotrexate (MTX)-resistant human osteosarcoma U-2OS/MTX300 cells, bufalin promotes apoptosis and G2/M phase arrest via a p53-dependent pathway without influencing or being impacted by the expression of dihydrofolate reductase (DHFR).[83] The chemosensitivity of BEL-7402/5-Fluorouracil (5-FU) cells to 5-FU was also increased by bufalin at a dose of 1 nM, with a reversal of 3.8-fold, which was comparable to that of 1 μM verapamil. This reversal of drug resistance was associated with suppressing drug efflux pump activity by downregulating multidrug resistance-associated protein 1 (MRP1), inducing cell G0/G1 phase arrest, increasing the Bax/Bcl-xl ratio to trigger apoptosis, and decreasing TS expression.[84] In the vincristine (VCR)-resistant leukemia cell line K562/VCR, bufalin has the ability to reverse MDR, with reversal index values of 4.85, 6.94 and 14.77 at doses of 0.0002, 0.001 and 0.005 μM, respectively, by suppressing the expression of MRP1 and triggering apoptosis signaling through changes in the Bcl-xl/Bax ratio.[85] By increasing p53 and p21 expression and decreasing the expression of Aurora A, cell division cycle 25 (CDC25), cyclin-dependent kinase 1 (CDK1), cyclin A, and cyclin B1, bufotalin causes G2/M phase arrest in multidrug-resistant HepG2 cells. Additionally, bufotalin causes apoptosis by regulating B-cell lymphoma (Bcl-2) and Bax, activating caspase-9/-3, inducing the cleavage of poly ADP-ribose polymerase (PARP), decreasing the mitochondrial membrane potential ($\Delta\Psi m$), and increasing reactive oxygen species (ROS) and intracellular calcium levels. Moreover, bufotalin-induced apoptosis can be amplified in a synergistic manner by the Akt inhibitor LY294002 or an siRNA targeting Akt. Additionally, an in

vivo test revealed that bufotalin markedly suppresses the proliferation of a xenograft R-HepG2 tumor model without causing splenic toxicity.[86]

Cinobufagin had no effect on the parental HCT116/oxaliplatin (L-OHP) (HCT116/L), LoVo/adriamycin (ADR), or Caco-2/ADR cells, but it greatly increased the sensitivity of these cells to L-OHP and DOX. With no appreciable in vivo toxicity, cinobufagin also increased the anticancer efficacy of DOX in a P-glycoprotein (P-gp)-overexpressing LoVo/ADR xenograft tumor model. Subsequent investigations revealed that cinobufagin reversed MDR by increasing apoptosis and the intracellular accumulation of DOX via the inhibition of P-gp-mediated efflux through the noncompetitive suppression of P-gp ATPase activity.[87] In docetaxel (DCT)-resistant MCF-7 and MDA-MB-231 cell lines (MCF-7/DCT^R and MDA-MB-231/DCT^R), bufalin has the ability to overcome ATP-binding cassette subfamily B member 1 (ABCB1)-mediated DCT resistance by increasing DCT accumulation through a decrease in ABCB1 ATPase function and the inhibition of ABCB1 protein expression. Additionally, in an MDA-MB-231/DCT^R xenograft tumor model, bufalin + DCT greatly reduced tumor growth and decreased ABCB1 expression.[88] By suppressing nuclear factor erythroid 2-related factor 2 (Nrf2) and reducing the expression of heme oxygenase-1 (HO-1) and P-gp, bufalin can reverse MDR in K562/A02 cells by limiting DOX efflux. Furthermore, by activating the inositol-requiring enzyme 1a (IRE1a)/tumor necrosis factor receptor associated factor 2 (TRAF2)/JNK/caspase-12 pathway, bufalin can trigger endoplasmic reticulum (ER) stress and apoptosis in drug-resistant cells.[89] In ABCB1-overexpressing HCT8/ADR, LoVo/ADR and HCT8/ABCB1 colorectal cancer cells, bufalin increased intracellular drug accumulation to increase chemosensitivity by limiting the transport function of ABCB1, suppressing the expression of the ABCB1 protein and regulating ABCB1 ATPase activity. In an HCT8/ADR xenograft tumor model, the efficacy of DOX was also significantly strengthened by bufalin, but no observable toxicity was induced.[90] CD133-overexpressing cells exhibit increased chemoresistance and levels of Akt/nuclear factor-kappa B (NF-κB) signaling mediators and MDR1. By controlling cancer cell stemness by lowering multidrug resistance gene 1 (MDR1)/P-gp expression through the inhibition of CD133 expression, AKT phosphorylation, NF-κB/p65 nuclear translocation, and MDR1 translation in vitro and in vivo, bufalin could reverse MDR in colorectal cancer.[91] By suppressing the stemness of colorectal cancer cells and decreasing the expression of stemness markers such as CD133, CD44, octamer-binding transcription factor 4 (OCT4), sex-determining region Y-box 2 (SOX2), and NANOG, as well as the ATP-binding cassette transporter G2 (ABCG2) protein, bufalin can overcome acquired cisplatin resistance.[92] M2 macrophage polarization is one of the key factors of chemoresistance. According to Chen et al, bufalin inhibits macrophage migration inhibitory factor (MIF) release by targeting the steroid receptor coactivator 3 (SRC-3) protein, which in turn controls M2 macrophage polarization in chemoresistant cells. Moreover, they reported that the Huachansu capsule improved the antitumor effect of L-OHP both in vivo and in the clinic by controlling the polarization of M2 macrophages via the SRC-3/MIF pathway.[93]

Moreover, in lung adenocarcinomas, cinobufotalin reduces cisplatin resistance, migration and invasion. Mechanistically, cinobufotalin induces the expression of ENKUR by inactivating the phosphoinositide 3-kinase (PI3K)/AKT/c-Jun pathway. In addition to its interaction with myosin heavy chain 9 (MYH9), increased ENKUR also recruits β-catenin, which reduces MYH9 transcription induced by c-Jun. Downregulating MYH9 reduces ubiquitin-specific protease 7 (USP7) recruitment, ubiquitination-mediated c-Myc degradation, and epithelial–mesenchymal transition (EMT) signaling, thus attenuating cisplatin resistance.[94] Bufalin may mitigate cisplatin resistance in gastric cancer cells by decreasing AKT, GSK-3β, mTOR, S6 kinase (S6K), and eIF4E-binding protein 1 (4EBP1) activation.[79] In addition, cinobufagin could increase the sensitivity of MNNG/HOS and U2OS cells to ADR, which was mediated by increased forkhead box O1 (FOXO1)-mediated transcription of the Fc fragment of the IgG binding protein (FCGBP) in osteosarcoma.[95]

Relieving Chemotherapy-Induced Cancer Pain

Approximately 80% of patients who receive cytostatic pharmacotherapy may experience chemotherapy-induced peripheral neuropathic pain (CIPNP), a serious side effect.[96] As the number of cancer survivors quickly increases, avoiding and treating this adverse reaction becomes increasingly important. Some evidence has demonstrated that Huachansu + chemotherapy not only enhances anticancer effects but also alleviates cancer-related pain symptoms.

Paclitaxel, a commonly used chemotherapy drug, often results in significant PNP with treatment. Ba et al investigated the effects of Huachansu on a rat model of paclitaxel-induced peripheral neuropathic pain (PIPNP). Compared with paclitaxel, a single intraperitoneal injection of Huachansu (2.5 g/kg) reduced mechanical and thermal hypersensitivity, hence alleviating preestablished PIPNP. Frequent intraperitoneal injections of Huachansu (1.25 and 2.5 g/kg) during PIPNP induction inhibited

the progression of mechanical and thermal hypersensitivity induced by paclitaxel. This protective effect was linked to reduced spinal tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β) production, as well as the inhibition of transient receptor potential vanilloid 1 (TRPV1) upregulation and spinal astrocyte activation induced by paclitaxel. These results suggest that Huachansu may have therapeutic value in treating and preventing PIPNP.[97]

Colorectal carcinomas are commonly treated with L-OHP as the first-line treatment. Pain and sensory abnormalities may occur in up to 40% of cancer patients treated with platinum drugs. Hao et al studied the effects of Huachansu on a rat model of L-OHP-induced CIPNP. The findings indicated that, in contrast to L-OHP-treated rats, a single injection of 2.5 g/kg Huachansu had a short-term analgesic effect on preestablished CIPNP induced by L-OHP after 60 minutes, as evidenced by reduced mechanical and thermal hypersensitivity, whereas repeated Huachansu administration during CIPNP induction further inhibited CIPNP progression. The protective effect of Huachansu was linked to reduced activation of spinal astrocytes and TRPV1 upregulation produced by L-OHP in the dorsal root ganglia.[98]

Combined with Targeted Therapy

Enhancing the Effect of Targeted Therapy

The active ingredients of toad skin and toad venom, such as bufalin and cinobufagin, could produce sensitive therapeutic effects on HCC and lung cancer via targeted drugs such as gefitinib and sorafenib by inhibiting cell proliferation and migration, arresting the cell cycle, triggering cell apoptosis, and preventing tumor angiogenesis.

In PLC/PRF/5 and HepG-2 cells, bufalin can increase AKT phosphorylation to augment the sorafenib-mediated reduction in extracellular signal-regulated kinase (ERK) phosphorylation, thereby increasing the antitumor activity of sorafenib.[99] Sorafenib + bufalin synergistically induced the apoptosis of PLC/PRF/5 and SMMC-7721 cells by increasing the expression of Bax, Caspase 7 and PARP.[100] Furthermore, by downregulating Akt/NF-κB signaling, bufalin and cinobufagin combined with sorafenib might synergistically suppress proliferation and trigger apoptosis and S phase arrest in HepG2 cells. Compared with single therapy, the expression levels of IκB, Bax, and Caspase 3/8 greatly increased, while the expression levels of p-Akt (Ser473), p-NF-κB p65, Bcl-2, proliferating cell nuclear antigen (PCNA), cyclin-dependent kinase 2 (CDK2) and cyclin A were obviously decreased in response to combination therapy.[101,102]

Bufalin + sorafenib could exert synergistic antiangiogenic effects on an intradermal HCC tumor model. In human umbilical vein endothelial cells (HUVECs), bufalin + sorafenib also increased apoptosis and suppressed proliferation, migration and blood vessel formation by decreasing the levels of angiogenin, platelet-derived growth factor-BB (PDGF-BB) and vascular endothelial growth factor (VEGF). Additional research has shown that bufalin can increase the antiangiogenic activity of sorafenib by modifying the AKT/VEGF pathway.[103] Some researchers have also studied the synergistic anticancer activity of bufalin + sorafenib through the effects of the tumor vascular microenvironment on HCC cells and HUVECs. Bufalin + sorafenib strongly inhibited the migration and tubule formation of HUVECs and reduced the secretion of VEGF by HCC cells, and VEGF incubation reversed the suppression of HUVEC tube formation. In vivo tests also revealed that the mice administered bufalin + sorafenib had the lowest expression of VEGF in the subcutaneous HCC tumor model. Furthermore, HUVECs pretreated with the PI3K inhibitor PI103 presented lower levels of p-mTOR. Moreover, PI103 pretreatment inhibited HCC cell motility and HUVEC tube formation. The results showed that bufalin + sorafenib synergistically influence the tumor vascular milieu by modulating the mTOR/VEGF pathway, reducing VEGF release. A reduction in VEGF–vascular endothelial growth factor receptor (VEGFR) binding on vascular endothelial cells inhibited tumor angiogenesis.[104]

Bufalin + gefitinib markedly inhibited proliferation and induced apoptosis in H1975 cells by downregulating p-epidermal growth factor receptor (EGFR), p-Met, p-Akt and p-mTOR, indicating that the potential antitumor mechanism involves the inhibition of EGFR-Pl3K/Akt signaling.[105] Cinobufotalin + gefitinib suppressed A549 cell viability, facilitated apoptosis, increased the generation of ROS and caused S phase arrest, as evidenced by the reductions in CDK2, cyclin A, and cyclin E levels and the increase in p21 levels. Furthermore, the downregulation of hepatocyte growth factor (HGF) and c-Met levels suggested that cinobufotalin may postpone the development of gefitinib resistance in lung cancer cells.[106] Compared with sorafenib or bufalin therapy alone, sorafenib + bufalin also increased chromatin condensation and cell apoptosis by triggering increased expression of apoptotic protease activating factor-1 (APAF-1), caspase-3/-9, superoxide dismutase (SOD), Bax, and BCL2-associated agonist of cell death (Bad), decreased expression of Bcl-2 and catalase, and a reduced ΔΨm, thereby displaying stronger cytotoxic effects on NCI-H292 human lung cancer cells.[107]

Reversing Resistance to Targeted Therapy

Several studies have shown that targeted drugs such as gefitinib, sorafenib, afatinib or osimertinib combined with bufalin may help overcome acquired tumor resistance. For example, afatinib + bufalin obviously suppressed the proliferation of afatinib-resistant H1975 cells and prevented H1975 cell invasion. Western blot analysis revealed that the combination treatment significantly decreased the p-cMet, p-EGFR, p-ERK, p-AKT, snail and vimentin protein levels and increased the E-cadherin protein level in afatinib-resistant H1975 cells, indicating that the reversal of afatinib resistance in HGF-induced H1975 lung cancer cells by bufalin may be associated with blocking the EMT process and suppressing cMet/mitogen-activated protein kinase (MAPK)/ERK and cMet/PI3K/AKT signaling.[108] In addition, by inhibiting sorafenib-mediated Akt activation, bufalin was able to overcome the intrinsic and acquired resistance of HCC cells to the drug. This effect might be attributed to bufalin-induced ER stress, which then decreases p-Akt levels, reduces cell proliferation, and triggers apoptosis. Furthermore, its action relies on the IRE1 pathway.[109] Moreover, in gefitinib-resistant H460 cells, bufalin markedly suppressed cell adhesion, migration and invasion at concentrations ranging from 2.5 nM to 10 nM by reducing the expression of son of sevenless homolog 1 (SOS-1), matrix metalloproteinase-2 (MMP-2), Ras homolog gene family, member A (RhoA), urokinase-type plasminogen activator (uPA), p-FAK, p-ERK1/2, Ras, E-cadherin and tissue inhibitor of matrix metalloprotease 1 (TIMP1) after 24 or 48 h of treatment.[110] Patients with EGFR-activating mutations in NSCLC are now treated with osimertinib as the conventional treatment; however, osimertinib-induced acquired resistance may be caused by elevated levels of myeloid cell leukemia-1 (MCL-1). Bufalin can suppress Ku70-mediated MCL-1 overexpression, hence restoring the susceptibility of resistant cells to osimertinib-induced growth inhibition and apoptosis.[111] The mechanism of action of the active ingredients of toad skin and toad venom combined with chemotherapy and targeted therapy is shown in Figure 2.

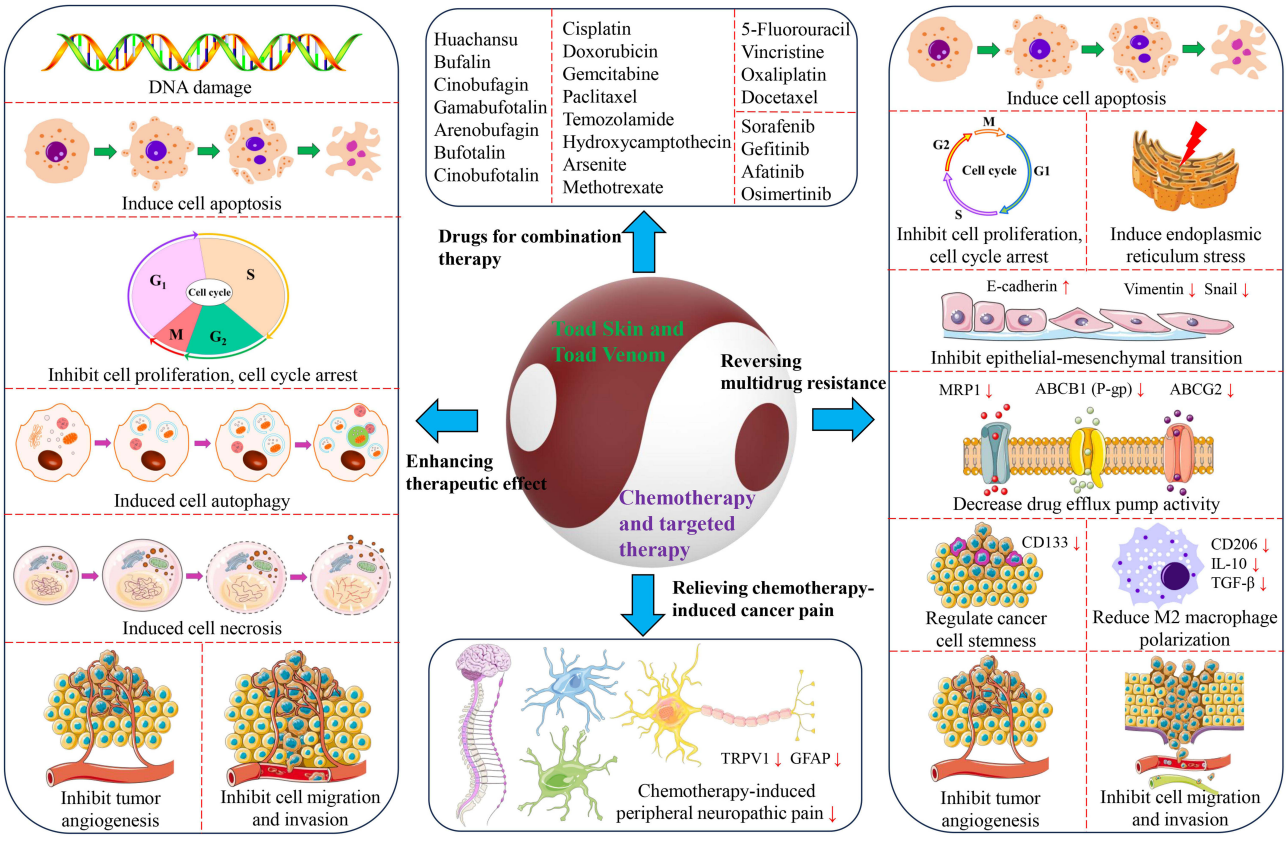

Figure 2 The mechanism of action of the active ingredients of toad skin and toad venom combined with chemotherapy and targeted therapy.
Abbreviations: MRP1, multidrug resistance-associated protein 1; ABCB1, ATP-binding cassette subfamily B member 1; P-gp, P-glycoprotein; ABCG2, ATP-binding cassette transporter G2; TGF-β, Transforming growth factor-beta; TRPV1, Transient receptor potential vanilloid 1; GFAP, Glial fibrillary acidic protein.

Combined with Radiotherapy or Hyperthermia

One of the most important factors influencing the radioresistance of glioblastoma cells is effective DNA damage repair. The Rad51 protein is essential for genomic stability and the regular cell cycle, as well as for homologous recombination (HR). Bufalin could increase the radiosensitivity of glioblastoma via the suppression of DNA damage repair by increasing phosphorylated histone H2AX (γ-H2AX) levels and decreasing Rad51 protein levels and HR efficiency.[112] In addition, Huachansu has the ability to increase the effectiveness of radiotherapy in H460 cells. Huachansu can prolong the existence of radiation-induced γ-H2AX foci to inhibit DNA repair and increase radiation-induced apoptosis by increasing the levels of cleaved caspase-3 and cleaved PARP proteins and reducing the levels of the Bcl-2 and p53 proteins.[113] Moreover, bufalin/cinobufotalin combined with radiotherapy/hyperthermia could significantly increase the degree of DNA fragmentation, decrease the ΔΨm, and increase the level of cleaved caspase-3 to increase cell apoptosis.[114] The EMT is a complex biological mechanism that plays critical roles in tumor migration, invasion, and resistance to radiation. Bufalin was able to abrogate the radiation-induced EMT by suppressing the levels of vimentin, N-cadherin, Snail, and Slug and increasing the expression of E-cadherin through the targeting of Src signaling, thus achieving radiosensitization effects on NSCLC.[115]

Combined with Several Signaling Pathway Inhibitors

The AKT family of kinase enzymes, which includes AKT1, AKT2, and AKT3, is a crucial PI3K partner. In multiple myeloma (MM) plasma cells, AKT kinase is activated, which sensitizes the antiapoptotic pathway and is related to drug resistance. Although bufalin triggers cell apoptosis, it can also induce the phosphorylation of AKT, thereby preventing the drug from killing cells and promoting the development of therapeutic resistance. Some studies have shown that the combination of bufalin with the AKT inhibitor MK2206 can enhance the antitumor effect on MM. For example, bufalin combined with MK2206 increased cell apoptosis by downregulating p-Akt induced by bufalin and increasing the levels of cleaved PARP and cleaved caspase 3 in H929 human MM cells.[116] In addition, MK2206 + bufalin synergistically inhibited the proliferation and triggered the apoptosis of MM cells by suppressing the AKT/mTOR signaling. This phenomenon was also observed after MM cells were cocultured with bone marrow stromal cells (BMSCs) and/or incubated with interleukin-6 (IL-6). The anti-MM effects of bortezomib-resistant cell lines (NCI-H929R and U266R) were comparable to those of the combination therapy. Furthermore, synergistic effects were also observed in MM xenografts in BALB-c mice, NOD-SCID mouse tumor models, and primary MM cells. In summary, MK2206 + bufalin considerably improved antitumor activity by blocking AKT/mTOR signaling, irrespective of MM cell susceptibility to bortezomib.[117]

In HepG2 and Huh-7 cells, cinobufagin triggers cytoprotective autophagy by increasing the levels of the microtubule-associated proteins 1A/1B light chain 3B (LC3B)-II, Beclin1 and autophagy-related gene (Atg)12-Atg5 to counteract its anticancer effects. Cinobufagin + the autophagy inhibitor MRT668921 was able to enhance the antiproliferative and proapoptotic effects by suppressing autophagy signaling through decreased expression of Bcl-2, PCNA, Beclin1, and Atg12-Atg5 and a decrease in the LC3B-II/LC3B-I ratio in vitro and in vivo.[118]

The Hedgehog signaling pathway is a highly conserved cellular signal transduction pathway that controls cell growth and survival in a variety of malignancies. Bufalin + hedgehog signaling inhibitors (GANT61 and cyclopamine) clearly suppressed proliferation; induced apoptosis and G2 + S phase arrest; inhibited adhesion, migration and invasion; and suppressed ECM degradation, the EMT process and tumor angiogenesis by regulating the expression of the hedgehog signaling proteins patched homolog 1 (Ptch1), GLI family zinc finger 1 (Gli1) and GLI family zinc finger 3 (Gli3) in HCC cells. Additionally, the combination treatment had the potential to increase E-cadherin expression by altering the Gli3 protein and decreasing the expression of downstream molecules, including MMP-2/9, β-catenin and VEGF, through effects on the Gli1 and Gli3 proteins.[119]

Combined with the Active Ingredients of TCM

Compared with monotreatment, cotreatment with bufalin and cinobufagin synergistically potentiated the anticancer effect on HepG2 cells. The combination therapy triggered apoptosis and cell cycle arrest by regulating metabolic pathways, including amino acid metabolism, energy metabolism, methionine metabolism and lipid metabolism, as well as glutathione (GSH) biosynthesis, as revealed by metabolomic and lipidomic profiling using liquid chromatography–mass spectrometry (LC–MS).[120] The authors also showed that the intraperitoneal injection of the combination of 2 mg/kg bufalin and 4 mg/kg

cinobufagin synergistically inhibited the growth of a HepG2-xenograft tumor model. A comprehensive approach based on MS-based lipidomics and matrix-assisted laser desorption ionization–mass spectrometry imaging (MALDI–MSI) has shown that cotreatment with bufalin and cinobufagin can regulate sphingolipid metabolism and glycerophospholipid metabolism by increasing the abundance of phosphatidylcholine (PC) and phosphatidylglycerol (PG), and decreasing the abundance of phosphatidylserine (PS) and phosphatidylethanolamine (PE), resulting in cell apoptosis triggered by mitochondria and the breakdown of tumor cell membranes. Additionally, altered lipid markers, which are key components of the tumor framework, are mostly found in nonnecrotic tumor regions.[121]

Combined with Other Therapies

Photodynamic therapy (TiO_2) combined with bufalin enhanced the antitumor effect on human melanoma A375 cells by inhibiting cell proliferation, arresting the cell cycle at S phase, and inducing cell apoptosis by increasing ROS levels and the Bax/Bcl-2 ratio.[122] In addition, high-intensity focused ultrasound (HIFU) combined with bufalin further suppressed proliferation and trigger apoptosis by increasing PARP expression, increasing activated caspase-3 and caspase-8 activation and decreasing Ki-67 expression in MiaPaCa2 and Panc-1 cells and in a $MiaPaCa_2$ tumor model compared with single therapy.[123] c-Myc, a proto-oncogene, is overexpressed in more than 50% of human malignancies and is associated with the formation and progression of several tumor types, including pancreatic cancer. siRNA-c-Myc + bufalin synergistically enhanced apoptosis, cell cycle arrest, migration and invasion by blocking hypoxia-inducible factor-1alpha (HIF-1a)/stromal cell-derived factor 1 (SDF-1)/C-X-C chemokine receptor type 4 (CXCR4) signaling in pancreatic cancer.[124] The expression of microRNA-497 (miR-497) is decreased in colorectal cancer and is linked to increased tumor proliferation and metastasis and a poor prognosis. MiR-497 + bufalin markedly enhanced the anti-colorectal cancer effect by inhibiting tumor proliferation and metastasis.[125] As a member of the TNF superfamily, the type II transmembrane protein known as TNF-related apoptosis-inducing ligand (TRAIL) can trigger programmed cell death. Bufalin + TRAIL greatly improved TRAIL-mediated apoptosis and cell viability inhibition by upregulating death receptor 5 (DR5), downregulating the expression of cellular Fas-associated death domain-like interleukin-1β-converting enzyme inhibitory protein (cFLIP) and X-linked inhibitor of apoptosis protein (XIAP), and activating caspase-3, -8 and -9 in T24 cells.[126] Table 5 summarizes the combination treatment strategies based on toad skin or toad venom-related active ingredients in vitro and in animal models.

Combined Antitumor Strategies Based on Drug Delivery Systems

Compared with the traditional "cocktail" therapy, the multidrug combination strategy for tumors based on a nanodelivery system can not only increase serum stability and biocompatibility, prolong the half-life in vivo, and improve the targeting ability and permeability of drugs at the tumor site but also realize the precise compatibility of combined drugs and specific stimulus-responsive release in the presence of external stimuli or the tumor microenvironment to enhance the therapeutic effect, reduce adverse reactions and resist MDR.[149,150] Codelivery systems based on the active ingredients of toad skin and toad venom include codelivery systems for bufalin, gamabufotalin, and cinobufagin combined with chemotherapy drugs, small-molecule targeted drugs, monoclonal antibodies, the active ingredients of TCM, PDT and photothermal therapy (PTT) (Figure 3 and Table 6).

Codelivery of Active Ingredients of Toad Skin/Toad Venom and Chemotherapy Drugs

Loading bufalin and paclitaxel onto nanocarriers can enhance their antitumor effects. Paclitaxel- and bufalin-loaded mPEG-cholic acid (CA)/D-a-tocopherol polyethylene glycol 1000 succinate (TPGS) polymer micelles (PTX/PCTm and BF/PCTm) were prepared for combined HCC treatment. The circulation time could be extended to 48 h in the liver and tumor by the PEG modification. Moreover, a liver-targeting ability could be achieved by the interaction of cholic acid groups and the Na^+-taurocholate cotransporter polypeptide (NTCP) receptor. PTX/PCTm + BF/PCTm (PB/PCTm) could achieve a synergistic therapeutic effect on HepG2 cells. Moreover, in vivo tests revealed that PB/PCTm (5 mg/kg paclitaxel and 1 mg/kg bufalin) had the highest tumor suppression rate (82.29%) without obvious toxicity, which was significantly greater than that of Taxol® (41.17%), PTX/PCTm (58.26%) and BF/PCTm (73.54%) in a bioluminescence orthotopic HCC model.[127]

Table 5 Combination Treatment Strategies Based on Toad Skin or Toad Venom-Related Active Ingredients in vitro and in Animal Models

Combination Strategies	Drugs	In vitro Model	In vivo Model	Signaling Pathway/Target	Mechanism of Action	Ref.
Combined with chemotherapy-Enhancing the effect of chemotherapy	Huachansu injection + cisplatin	Osteosarcoma cells: OS732 cells	–	Fas ↑	Induce cell apoptosis	[73]
	Huachansu (aqueous extract) + doxorubicin	Hepatocellular carcinoma cell line HepG2 and HLE	–	Mitochondrial membrane potential ↓, Bcl-2 ↓, Bax ↑, Bid ↑, Cytochrome c ↑	Inhibit cell proliferation, induce cell apoptosis	[74]
	Bufalin + gemcitabine	Pancreatic cancer cell lines Bxpc-3, Mia PaCa-2 and Panc-1	Mia PaCa-2 subcutaneous xenograft model	Apoptosis signal-regulating kinase 1 (ASK1)/JNK ↑, cleaved caspase 3 ↑, Bcl-2 ↓, Ki-67 ↓	Induce cell apoptosis	[75]
	Bufalin + paclitaxel	Cervical cancer cell lines Siha and Hela	Siha subcutaneous xenograft model	Integrin α2/β5 ↓, FAK ↓, p-FAK (Tyr397) ↓, GSK3β ↑, p-GSK3β (Ser389) ↓	Inhibit cell proliferation and cell metastasis	[76]
	Bufalin + temozolomide	Human Glioma Stem-Like Cells derived from U87MG and LN-229 cells	–	p-Bad ↑, Bcl-2 ↓, Bcl-xl ↓, cleaved caspase 9 ↑	Induce cell apoptosis	[77]
	Bufalin + hydroxycamptothecin	Human prostate cancer cell line DU 145	DU 145 cells xenograft model	PCNA ↓, Bax ↑, Bcl-xl ↓, p53 ↑, programmed cell death 4 (PDCD4) ↑, glycogen synthase kinase (GSK)-3β ↑, p-Akt ↓	Induce cell apoptosis	[78]
	Bufalin + cisplatin	Human gastric cancer SGC7901, MKN-45 and BGC823 cells	–	p-AKT ↓, p-GSK3β ↓, p-mTOR ↓, p-4EBP1 ↓, p-S6K ↓	Inhibit cell proliferation, induce cell apoptosis	[79]
	Cinobufagin + cisplatin	Human osteosarcoma cell line 143B	143B subcutaneous xenograft model	Ki-67 ↓, PCNA ↓, p21 ↑, cyclinA2 ↓, CDK2 ↓, cdc25A ↓, Bax ↑, Bcl-2 ↓, cleaved caspase 3/9 ↑, VEGF ↓, MMP-2/-9 ↓, Notch1 ↓, Hes1 ↓, Hes5 ↓, Hey-L ↓, NICD1 ↓	Inhibit cell proliferation, S phase arrest, induce cell apoptosis, inhibit cell migration and invasion	[80]
	Gamabufotalin + arsenite	Human glioblastoma cells lines U-87	–	LDH release ↑, cdc25C ↓, cdc2 ↓, Cyclin B1 ↓, Survivin ↓, LC3 ↑	G2/M arrest, necrosis and autophagic cell death induction	[81]
	Arenobufagin + arsenite	Human glioblastoma cells lines U-87	–	Caspase 9/8/3 activity ↑, LDH release ↑, γH2AX ↑, p-AKT ↓, AKT ↓, p-mTOR ↓, mTOR ↓, LC3-I ↑, LC3-II ↑, Jagged1 ↓, Notch1 ↓	Induce cell apoptosis, G2/M arrest, DNA damage, necrosis and autophagic cell death induction	[82]

(*Continued*)

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Table 5 (Continued).

Combination Strategies	Drugs	In vitro Model	In vivo Model	Signaling Pathway/Target	Mechanism of Action	Ref.
Combined with chemotherapy-Reversing chemotherapy resistance	Bufalin → cisplatin	Cisplatin-resistant gastric cancer SGC7901 cells	–	p-AKT ↓, p-GSK3β ↓, p-mTOR ↓, p-4EBP1 ↓, p-S6K ↓	Induce cell apoptosis	[79]
	Bufalin → methotrexate	Human osteosarcoma U-2OS methotrexate (MTX) 300-resistant cell line	–	p53 ↑, p21 ↑, Bax ↑, Bcl-2 ↓, do not affect dihydrofolate reductase (DHFR) expression	Induce cell apoptosis, cell cycle arrest at the G2/M phase	[83]
	Bufalin → 5-Fluorouracil	5-FU-resistant human hepatocellular carcinoma BEL-7402/5-FU cells	–	Thymidylate synthase (TS) ↓, multidrug resistance-associated protein 1 (MRP1) ↓, Bax ↑, Bcl-xl ↓	Cell cycle arrest at the G0/G1 phase, induce cell apoptosis, decrease the drug efflux pump activity	[84]
	Bufalin → vincristine	Human leukemia multidrug resistant cell line K562/VCR	–	MRP1 ↓, Bax ↑, Bcl-xl ↓	Induce cell apoptosis	[85]
	Bufotalin → doxorubicin	Doxorubicin-induced human hepatocellular carcinoma multidrug resistant R-HepG2 cells	R-HepG2 cells xenograft model	cleaved PARP ↑, cyclin A ↓, cyclin B1 ↓, CDK1 ↓, CDC25A ↓, CDC25B ↓, Aurora A ↓, p21 ↑, p53 ↑, pro-caspase 3 ↓, cleaved caspase 3/8/9 ↑, Bax ↑, Bcl-2 ↓, Akt ↓, Akt (Thr^{308}) ↓, Akt (Ser^{473}) ↓	Induce cell apoptosis, cell cycle arrest at the G2/M phase	[86]
	Cinobufagin → oxaliplatin + doxorubicin	P-gp-overexpressing HCT116/L-OHP (HCT116/L), LoVo/ADR and Caco-2/ADR cells	LoVo/ADR cell xenograft model	Ki-67 ↓, cleaved caspase 3/9 ↑, Bcl-2 ↓, Bax ↑, inhibit P-gp-mediated drug efflux and drug transport, inhibit P-gp ATPase activity	Reversal of P-gp-mediated multidrug resistance, induce cell apoptosis	[87]
	Bufalin → docetaxel	Docetaxel-resistant MDA-MB-231 cell line (MDA-MB-231/DCT^R) and MCF-7 cell line (MCF-7/DCT^R)	MDA-MB-231/DCT^R cells xenograft model	Bax ↑, Bcl-xl ↓, Bcl-2 ↓, ABCB1 ↓, Ki-67 ↓	Induce cell apoptosis, reverse ABCB1-mediated docetaxel resistance	[88]
	Bufalin → adriamycin	Adriamycin-resistant K562/A02 cells	–	Nrf2 ↓, MDR1 ↓, heme oxygenase-1 (HO-1) ↓, P-gp ↓, GRP78 ↑, Bax ↑, Bcl-2 ↓, Cytochrome c ↑, cleaved caspase 3 ↑, PAPR ↑, IRE1α ↑, TNF receptor-associated factor 2 (TRAF2) ↑, JNK ↑, p-JNK ↑, caspase 12 ↑	Induce cell apoptosis, induce endoplasmic reticulum stress	[89]
	Bufalin → doxorubicin	DOX-selected ABCB1-overexpression LoVo/ADR, HCT8/ADR, Caco-2/ADR cells, HCT8/ABCB1 cells	HCT8/ADR cells xenograft model	Inhibit ABCB1-mediated drug efflux and transport, ABCB1 ↓, Ki-67 ↓, stimulate ATPase activity of ABCB1	Reverse ABCB1-mediated drug resistance	[90]

	Bufalin → doxorubicin	LoVo/ADR and HCT8/ADR cells, $LoVo^{CD133+}$ and $HCT8^{CD133+}$ cells	LoVo/ADR cells or CD133-OE $LoVo^{CD133+}$ cells xenograft model	MDR1 mRNA expression and MDR1 gene promoter activity ↓, whole cells: CD133, p-Akt, p-NF-κB/p65, and P-gp ↓, nucleus: NF-κB/p65 and p-NF-κB/p65 ↓	Regulate cancer cell stemness, induce cell apoptosis	[91]
	Bufalin → cisplatin	Colorectal cancer HCT116 and LoVo cell lines	HCT116 cells xenograft model	CD133 ↓, CD44 ↓, NANOG ↓, OCT4 ↓, SOX2 ↓, ABCG2 ↓,	Induce cell apoptosis, reverse the high stemness and drug resistance induced by cisplatin	[92]
	Bufalin → oxaliplatin	HCT116/OXA, CT26/OXA, HCT8/OXA cells	CT26/OXA cells xenograft model	CD68 ↓, CD206 ↓, IL-10 ↓, TGF-β ↓, Ki-67 ↓, Bax ↑, P-gp ↓, MIF ↓, SRC-3 ↓	Reduce M2 macrophage polarization induced by chemoresistant cells	[93]
	Cinobufotalin → cisplatin	Lung adenocarcinoma A549 and SPC-A1 cells	ENKUR-overexpressing SPC-A1 cells or control cells xenograft model	E-cadherin ↑, N-cadherin ↓, β-catenin ↓, MYH9 ↓, c-JUN ↓, ENKUR ↑, c-Myc ↓, USP7 ↓, p-PI3K ↓, p-Akt ↓	Reverse multidrug resistance, inhibit cell migration and invasion	[94]
	Cinobufotalin → doxorubicin	Human osteosarcoma MNNG/HOS and U2OS cells	–	Forkhead box O1 (FOXO1) ↑, Fc fragment of IgG binding protein (FCGBP) ↑	Reverse multidrug resistance	[95]
Relieving chemotherapy-induced cancer pain	Huachansu	–	Paclitaxel-induced peripheral neuropathic pain (PIPNP) model in Male Sprague–Dawley rats	TRPV1 ↓, spinal astrocyte activation (GFAP) ↓, TNF-α ↓, IL-1β ↓	Decrease and prevent paclitaxel-induced mechanical allodynia and thermal hyperalgesia	[97]
	Huachansu	–	Oxaliplatin-induced peripheral neuropathic pain model in Male Sprague–Dawley rats	TRPV1 ↓, GFAP ↓	Decrease and prevent oxaliplatin-induced mechanical allodynia and thermal hyperalgesia	[98]

(*Continued*)

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Table 5 (Continued).

Combination Strategies	Drugs	In vitro Model	In vivo Model	Signaling Pathway/Target	Mechanism of Action	Ref.
Combined with targeted therapy-Enhancing the effect of targeted therapy	Bufalin + sorafenib	Human hepatocellular carcinoma cell lines PLC/PRF/5 (p53 mutant) and HepG2 (wild type p53)	–	p-Akt ↑, p-ERK ↓	Inhibit cell proliferation, induce cell apoptosis	[99]
	Bufalin + sorafenib	Human hepatocellular carcinoma PLC/PRF/5 and SMMC-7721 cells	SMMC-7721 cells xenograft model	Bax ↑, Caspase 7 ↑, PARP ↑	Inhibit cell proliferation, induce cell apoptosis	[100]
	Bufalin + sorafenib	HepG2 cells	–	Cyclin A ↓, PCNA ↓, Bcl-2/Bax ↓, p-Akt/Akt ↓, p-NF-κB p65/NF-κB p65 ↓, IκB ↑	Inhibit cell proliferation, induce cell apoptosis, cell cycle arrest at the S phase	[101]
	Cinobufagin + sorafenib	HepG2 cells	–	Cyclin A ↓, PCNA ↓, CDK2 ↓, Bcl-2/Bax ↓, Caspase 3/8 ↑, p-Akt/Akt ↓, p-NF-κB p65/NF-κB p65 ↓, IκB ↑	Inhibit cell proliferation, induce cell apoptosis, cell cycle arrest at the S phase	[101,102]
	Bufalin + sorafenib	Human umbilical vein endothelial cells (HUVEC), SMMC-7721 cells	SMMC-7721 cells xenograft model	Angiogenin ↓, PDGF-BB ↓, VEGF ↓, p-Akt ↓, p-mTOR ↓, p-ERK ↑, CD31 ↓, microvessel density (MVD) ↓	Inhibit tumor angiogenesis, induce cell apoptosis, cell cycle arrest at the G2 phase, inhibit cell migration	[103]
	Bufalin + sorafenib	HUVEC, PLC/PRF/5 and SMMC-7721 cells	SMMC-7721 cells xenograft model	Angiogenin ↓, PDGF-BB ↓, VEGF ↓, p-mTOR ↓	Inhibit cell proliferation and migration, inhibit tumor angiogenesis	[104]
	Bufalin + gefitinib	Lung cancer H1975 cells	–	p-EGFR ↓, p-Met ↓, p-Akt ↓, p-mTOR ↓	Inhibit cell proliferation, induce cell apoptosis	[105]
	Cinobufotalin + gefitinib	Human nonsmall cell lung adenocarcinoma A549 cells	–	Bax ↑, Bcl-2 ↓, Caspase-3 ↑, cyclin A ↓, cyclin E ↓, CDK2 ↓, p21 ↑, ROS ↑, HGF ↓, c-Met ↓	Inhibit cell viability, induce cell apoptosis, cell cycle arrest at the S phase	[106]
	Bufalin + sorafenib	Human lung cancer NCI-H292 cells (non-small cell lung cancer cells)	–	DNA condensation ↑, SOD (Mn) and SOD (Cu/Zn) ↑, Catalase ↓, $\Delta\Psi m$ ↓, Bax ↑, Bak ↑, Bid ↑, Bad ↑, Bcl-2 ↓, Bcl-xl ↓, APAF-1 ↑, Caspase 9 ↑, Caspase 3 ↑	Induce cell apoptosis	[107]
Combined with targeted therapy-Reversing resistance to targeted therapy	Bufalin → afatinib	Hepatocyte growth factor-induced afatinib-resistant H1975 cells	–	p-EGFR ↓, p-cMet ↓, p-Akt ↓, p-ERK ↓, E-cadherin ↑, vimentin ↓, snail ↓	Inhibit cell proliferation and invasion, reverse hepatocyte growth factor-induced resistance to afatinib, inhibition of epithelial-mesenchymal transition	[108]
	Bufalin → sorafenib	Sorafenib-resistant cell lines Huh7-Sora and HepG2-Sora	–	p-Akt ↓, p-eIF2α ↑, CHOP ↑, IRE1 ↑	Induce endoplasmic reticulum (ER) stress, induce cell apoptosis, reversed both inherent and acquired resistance to sorafenib	[109]
	Bufalin → gefitinib	Gefitinib-resistant human lung cancer cell line NCI-H460	–	SOS-1 ↓, MMP-2 ↓, Rho A ↓, p-FAK ↓, p-ERK1/2 ↓, Ras ↓, E-cadherin ↓, uPA ↓, TIMP1 ↓	Inhibit cell adhesion, migration and invasion, reversed resistance to gefitinib	[110]
	Bufalin → osimertinib	Osimertinib-resistant cell lines PC-9/OR and HCC827/OR	PC-9/OR cells xenograft model	cleaved caspase 3 ↑, cleaved PARP ↑, MCL-1 ↓, Ku70 ↓	Inhibit cell proliferation, induce cell apoptosis, reversed resistance to osimertinib	[111]

Combined with radiotherapy or hyperthermia	Bufalin + radiotherapy	Human glioblastoma cell lines U251 and U87MG	–	γ-H2AX ↑, Rad51 ↓, homologous recombination (HR) efficiency ↓, LDH release ↑	Enhance radiosensitivity, inhibit cell proliferation, suppress DNA damage repair	[112]
	Huachansu + radiotherapy	Human lung cancer cell line H460	–	cleaved caspase 3 ↑, cleaved PARP ↑, p53 ↓, Bcl-2 ↓	Enhance radiosensitivity, inhibit DNA repair, increase radiation-induced apoptosis	[113]
	Bufalin/cinobufotalin + radiotherapy/ hyperthermia	Human lymphoma U937 cells	–	DNA fragmentation ↑, ΔΨm ↓, cleaved caspase 3 ↑	Increase cell apoptosis	[114]
	Bufalin + radiotherapy	Human NSCLC cell lines H1299, A549 and HCC827	–	N-cadherin ↓, Vimentin ↓, Snail ↓, Slug ↓, E-cadherin ↑, p-Src ↓, p-STAT3 ↓	Enhance radiosensitivity, inhibit epithelial-mesenchymal transition	[115]
Combined with several signaling pathway inhibitors	Bufalin + AKT inhibitor MK2206	Human multiple myeloma (MM) cell line NCI-H929	–	cleaved caspase 3 ↑, cleaved PARP ↑, p-Akt ↓	Inhibit cell proliferation, induce cell apoptosis, inhibit AKT pathway	[116]
	Bufalin + AKT inhibitor MK2206	Human MM cell lines NCI-H929, U266, LP-1, RPMI8226; murine MM cell line MOPC315; primary myeloma cells, bortezomib-resistant MM H929R and U266R cells	MOPC315 cells and H929 cells xenograft model	cleaved caspase 3/9 ↑, cleaved PARP ↑, p-Akt ↓, p-mTOR ↓, p-P70 ↓, p-4EBP1 ↓, IL-6 ↓, Ki-67 ↓	Inhibit cell proliferation, induce cell apoptosis, reversed resistance to bortezomib, inhibit AKT/ mTOR pathway	[117]
	Cinobufagin + autophagy inhibitor MRT68921	Human liver cancer cell lines HepG2 and Huh-7	HepG2 cells xenograft model	Bcl-2 ↓, PCNA ↓, Beclin1 ↓, Atg12-Atg5 ↓, LC3B-II/ LC3B-I ↓	Inhibit cell proliferation, induce cell apoptosis, inhibit autophagy pathway	[118]
	Bufalin + hedgehog signaling pathway inhibitors (GANT61, cyclopamine)	Human high metastasis potential LM3 hepatoma cells (HCC-LM3)	–	Ptch1 ↓, Gli1 ↓, Gli3 ↓, β-catenin ↓, E-cadherin ↑, MMP-2/9 ↓, VEGF ↓	Inhibit cell proliferation, cell cycle arrest at the G2 + S phase, induce cell apoptosis, inhibit cell adhesion, migration and invasion, inhibit EMT, extracellular matrix (ECM) degradation and angiogenesis, inhibit Hedgehog signaling pathway	[119]

(*Continued*)

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Table 5 (Continued).

Combination Strategies	Drugs	In vitro Model	In vivo Model	Signaling Pathway/Target	Mechanism of Action	Ref.
Combined with the active ingredients of traditional Chinese medicine	Bufalin + cinobufagin	HepG2 cells	–	Glutamine ↓, Glutamic acid ↓, Proline ↓, Aspartic acid ↓, Serine ↓, Isoleucine ↓, Histidine ↓, Phenylalanine ↓, Phenylethylamine ↓, Methionine ↓, PE ↓, LPE ↑, Cer ↑, LPC ↑, PC ↑, S-Adenosylmethionine ↑, S-Adenosylhomocysteine ↑, Spermine ↑, fatty acid (FA) ↓, oxygen consumption rate (OCR) ↓, extracellular acidification rate (ECAR) ↓	Perturbate methionine metabolism, energy metabolism, lipid metabolism, amino acid metabolism and GSH biosynthesis, arrest cell cycle, induce cell apoptosis	[120]
	Bufalin + cinobufagin	–	HepG2 cells xenograft model	α-fetoprotein (AFP) ↓, ceramide synthase (CERS) ↑, sphingomyelinase (SMase) ↓, sphingomyelin synthase (SM synthase) ↓, ceramidase (CDase) ↓, phosphatidylserine decarboxylase (PSD) ↑, phosphatidylethanolamine N-methyltransferase (PEMT) ↑, phosphatidylserine synthase (PSS) 1 ↓, phosphatidylcholines (PC) ↑, phosphatidylglycerol (PG) ↑, phosphatidylserine (PS) ↓, phosphatidylethanolamine (PE) ↓	Modulate sphingolipid metabolism and glycerophospholipid metabolism, induce cell apoptosis, systemic disruption of biomembranes in tumor cells	[121]
Combined with other therapies	Photodynamic therapy (TiO_2) + bufalin	Human melanoma A375 cells	–	Bax/Bcl-2 ↑, ROS ↑	Inhibit cell proliferation, cell cycle arrest at the S phase, induce cell apoptosis	[122]
	High-intensity focused ultrasound (HIFU) + bufalin	Pancreatic cancer $MiaPaCa_2$ and Panc-1 cells	$MiaPaCa_2$ cells xenograft model	cleaved caspase 3 ↑, caspase 8 ↑, PARP ↑, Ki-67 ↓	Inhibit cell proliferation, induce cell apoptosis	[123]
	Bufalin + siRNA-c-Myc	Human pancreatic cancer SW1990 and PANC-1 cells	SW199/PANC-1 cells xenograft model	c-Myc ↓, vimentin ↓, E-cadherin ↑, HIF-1a ↓, CXCR4 ↓, SDF-1 ↓	Cell cycle arrest at the S phase, induce cell apoptosis, inhibit cell migration and invasion	[124]
	MicroRNA-497 + bufalin	HCT116 cells, HUVEC	HCT116-luc cells xenograft model	VEGFA ↓, Ki-67 ↓	Inhibit cell metastasis, inhibit tumor angiogenesis	[125]
	Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) + bufalin	Human bladder carcinoma T24 cells	–	XIAP ↓, cFLIP ↓, DR5 ↑, PARP ↓, cleaved PARP ↑, pro-caspase 3/8/9 ↓, caspase 3/8/9 ↑	Induce cell apoptosis	[126]

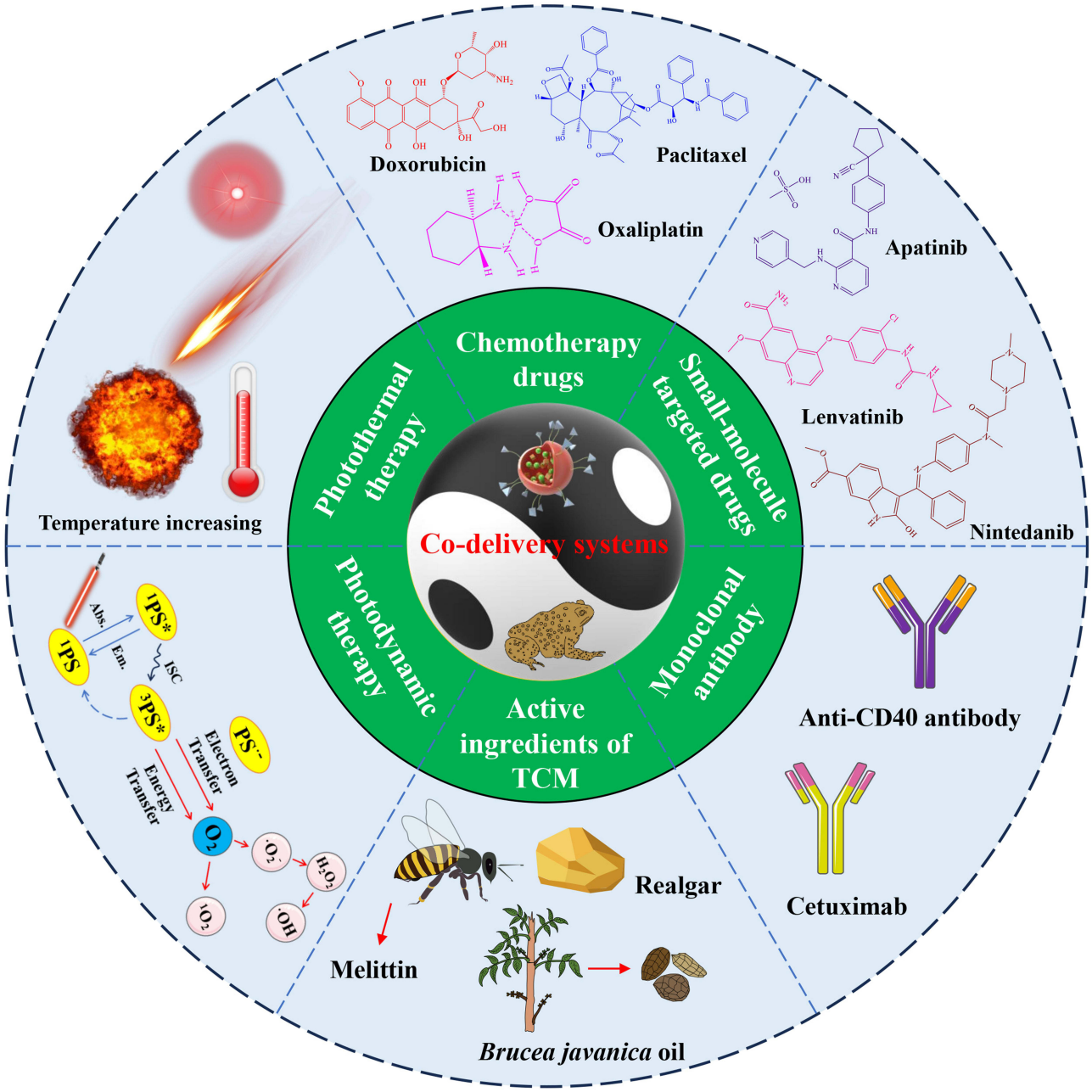

Figure 3 Schematic diagram of the classification of co-delivery systems based on active ingredients of toad skin and toad venom.

DOX + gamabufotalin synergistically inhibited the growth of MDA-MB-231 cells at a molar ratio of 10:1. Based on these findings, a DOX and gamabufotalin coloaded RGD-modified erythrocyte membrane-camouflaged graphene oxide quantum dots (GOQDs)-PEG-TAT@DOX@CS-6 biomimetic nanoplatform (GTDC@M-R NPs) was developed for the treatment of triple-negative breast cancer (TNBC). The tumor cell- and nucleus-targeting abilities of gamabufotalin and DOX were effectively enhanced by the TAT and RGD peptides. Moreover, this nanosystem could achieve the controlled release of drugs through a pH response. Approximately 26.5% of the DOX and 21.4% of the gamabufotalin were released from the GTDC@M-R NPs over 72 h at pH 7.4, while the release rates increased to 59.5% and 56.2% at pH 5.2, which effectively reduced drug release in the blood environment while increasing drug release in the tumor tissue to prevent side effects. In MDA-MB-231 cells, this nanosystem synergistically promoted apoptosis via Bax/Bcl-2 and p53 signaling and inhibited cell metastasis by downregulating VEGF and matrix metalloproteinase 9 (MMP-9) expression

Table 6 Codelivery Systems Based on Toad Skin or Toad Venom-Related Active Ingredients

Drug Delivery System	Drug Combination	In vitro and in vivo Model	Antitumor Effect and Mechanism	Ref.
Mixed micelles of PTX/PCTm and BF/PCTm (PB/PCTm)	Paclitaxel and bufalin	In vitro: HepG2 cells, in vivo: Hepa1-6-luc orthotopic tumor model (5 mg/kg paclitaxel and 1 mg/kg bufalin)	Tumor inhibition rate: 82.29%, no obvious systemic toxicity; apoptosis ↑, Ki-67 ↓	[127]
RGD-modified erythrocyte membrane camouflaged GOQDs-PEG-TAT@DOX@CS-6 nanosystem (GTDC@M-R NPs)	Doxorubicin (DOX) and gamabufotalin (CS-6)	In vitro: MDA-MB-231 cells, in vivo: MDA-MB-231 tumor-bearing female nude BALB/c mice model (DOX/CS-6 dose of 5/0.625 mg/kg)	Tumor inhibition rate: 84.4%, survival time ↑, inhibition rate of lung metastatic nodules: 80%, apoptosis ↑, anti-migration ↑, inhibit EMT activation, good in vivo biosafety, cleaved caspase 3 ↑, Bcl-2/Bax ↓, ROS ↑, COX-2 ↓, N-cadherin ↓, Vimentin ↓, VEGF ↓, MMP-9 ↓	[128]
Bufalin-loaded VES-CSO/TPGS-RGD mixed micelles (BU@VeC/T-RGD MM)	Bufalin → oxaliplatin + DOX	In vitro: HCT116/L-OHP and LoVo/ADR cells, in vivo: LoVo/ADR xenograft model (2.0 mg/kg bufalin)	Tumor inhibition rate: 65%, no obvious toxicity, apoptosis ↑, Bax ↑, Bcl-2 ↓, cleaved caspase 3/9 ↑, P-gp ↓	[129]
BF-ND-BUP-sMPs	Bufalin + nintedanib	In vitro: HepG2 cells, in vivo: H22 cells xenograft model (0.8 mg/kg bufalin and 1.05 mg/kg nintedanib)	Tumor inhibition rate: 84.2%, apoptosis ↑, CD31 ↓, α-SMA ↓, collagen fibers in the tumor microenvironment ↓, in vivo safety ↑	[130]
BF-ND-BUP-sMPs	Bufalin + nintedanib	In vitro: LLC cells	In vitro cytotoxicity ↑	[131]
LP-R/C@AC NPs	Apatinib + cinobufagin	In vitro: HGC-27 cells, in vivo: HGC-27 tumor-bearing nude BALB/c mice model (7.5 mg/kg apatinib and 0.2 mg/kg cinobufagin)	Tumor inhibition rate: 86.78%, ROS ↑, apoptosis ↑, cell cycle arrest at the S phase, angiogenesis ↓, immunity ↑, pyroptosis, activate autophagy, anti-migration ↑, VEGFR2 ↓, p-VEGFR2 ↓, p-STAT3 ↓, NF-κB ↓, p-NF-κB ↓, VEGF ↓, HIF-1α ↓, PD-L1 ↓, MCl-1 ↓, Bax ↑, Bcl-2 ↓, cleaved caspase 3 ↑, DFNA5 ↑, LC3-II/LC3-1 ↑, Cyclin D1 ↓, p21 ↑, MMP-9 ↓, Vimentin ↓	[132]
Le/Bu@mSiO$_2$-FA	Lenvatinib + bufalin	In vitro: 9810 cells, in vivo: 9810 cells xenograft model (10 mg/kg)	Tumor inhibition rate ↑, apoptosis ↑, migration and invasion ↓	[133]
Anti-CD40-BFL	Bufalin + anti-CD40 antibody	In vitro: B16 melanoma cells, in vivo: B16 tumor-bearing female C57/BL6 mice model (1 mg/kg bufalin and 8 mg/kg anti-CD40)	Tumor inhibition rate ↑, apoptosis ↑, Caspase 3/9 ↑, Cytochrome c ↑, active systemic immunity effect [Dendritic cells ($CD11c^+$, $CD45^+$), $CD4^+$ T cells ($CD3^+$, $CD4^+$), and $CD8^+$ T cells ($CD3^+$, $CD8a^+$)], reduced toxicity (ALT ↓, TNF-α ↓, IL-6 ↓, IL-1β ↓, IFN-γ ↓)	[134]
TV-SLN/NR-TISG	Toad venom + realgar	In vitro: HeLa and SKOV-3 cells	Inhibit cell proliferation, cell cycle arrest at the G2/M + S phase (HeLa cells) ↑, cell cycle arrest at the G0/G1 phase (SKOV-3 cells) ↑	[135]
Bufalin, cinobufagin and resibufogenin coloaded solid dispersion	Bufalin + cinobufagin + resibufogenin	–	–	[136]
Cetuximab conjugated compound immunoliposome loaded with bufalin and melittin	Cetuximab + bufalin + melittin	In vitro: sorafenib resistant SMMC-7721 cells, in vivo: sorafenib resistant SMMC-7721 tumor-bearing nude BALB/c mice model	Tumor inhibition rate ↑, apoptosis ↑, PERK ↑, IRE1α ↑, Chop ↑, Bax/Bcl-2 ↑, PARP ↑, Caspase 3 ↑	[137]

Toad skin extracts (TSE) and *Brucea javanica* oil (BJO) coloaded nanoemulsions (TSE-BJO NEs)	TSE + BJO	In vitro: HepG2 cells, in vivo: HepG2-bearing female BALB/c mice xenograft model (low-dose: TSE 3 mg/kg, BJO 6 mg/kg; high-dose: TSE 9 mg/kg, BJO 18 mg/kg)	Tumor inhibition rate ↑, apoptosis ↑, cell cycle arrest at the G2/M phase ↑, Ki-67 ↓, good in vivo biosafety	[138]
mTHPCandBU@VES-CSO/TPGS-RGD nanoparticles (T-B@NP)	Bufalin + mTHPC-mediated PDT	In vitro: HCT116 and CT26 cell, HCT116$^{SRC-3\ OE}$ cells, in vivo: CT26 tumor-bearing BALB/c male mice model (2.5 mg/kg bufalin, laser irradiation: 1 W/cm^2, 5 min, 660 nm)	Tumor inhibition rate: 84.2%, prolong mice survival rate, no obvious organ damage, apoptosis ↑, relief tumor hypoxia, inhibit tumor angiogenesis, HIF-1α ↓, SRC-3 ↓, CD31 ↓, VEGF ↓, ROS ↑	[139]
CeO_2@PAA@CS-1/Ce6@M (CPCCM NPs)	Cinobufagin + Ce6-mediated PDT	In vitro: MDA-MB-231 cells, in vivo: MDA-MB-231/Luc female BALB/c nude mice model, 4T1/Luc female BALB/c mice model (1 mg/kg cinobufagin, 2.5 mg/kg Ce6, laser irradiation: 0.2 W/cm^2, 5 min, 660 nm)	Tumor inhibition rate: 85.5%, anti-metastasis ↑, ROS ↑, HIF-1α ↓, MMP-9 ↓, good in vivo biosafety	[140]
A hybrid membrane derived from erythrocyte and SW480 cells (M) camouflaged Prussian blue (PB) NPs loading with cinobufagin (PC NPs) (PC@M NPs)	Cinobufagin + PB-mediated PTT	In vitro: SW480 cells, in vivo: SW480 tumor-bearing BALB/c female nude mice model, subcutaneous SW480 cancer model with complete tumor resection (2 mg/kg cinobufagin, 5 mg/kg PB NPs, NIR: 808 nm, 1.2 W/cm^2, 5 min)	Tumor inhibition rate: 84.57%, the metastatic nodules in the lung tissues: ~1 number, tumor recurrence rate and inhibition rate on post-surgical mice: 60% and 72.2%, regulate gut microbiota, inhibit EMT, good in vivo biosafety, reduce the cardiotoxicity of free Cinobufagin, apoptosis ↑, anti-metastasis ↑, ROS ↑, Bax/Bcl-2 ↑, Vimentin ↓, HIF-1α ↓, MMP-9 ↓, Ki-67 ↓	[141]
HA@RBC@PB@CS-6 NPs (HRPC)	Gamabufotalin + PB-mediated PTT	In vitro: MDA-MB-231 cells, in vivo: MDA-MB-231 tumor-bearing BALB/c mice model (1.25 mg/kg gamabufotalin and 5 mg/kg PB, NIR: 808 nm, 1 W/cm^2, 5 min)	Tumor inhibition rate: 93.4%, no obvious systemic toxicity, ROS ↑, apoptosis ↑, IKK ↓, IκB ↑, NF-κB ↓, P-Casp3 ↓, C-Casp3 ↑, P-PARP ↓, C-PARP ↑, Bcl-2 ↓, HSP70 ↓	[142]
Cinobufagin-loaded and folic acid-modified polydopamine (PDA) nanoparticle	Cinobufagin + PDA-mediated PTT	In vitro: A549 and LLC cells, in vivo: LLC tumor-bearing BALB/c male nude mice model (1 mg/kg cinobufagin, NIR: 808 nm, 2 W/cm^2, 5 min)	Tumor inhibition rate: 67%, Cleaved caspase-3 ↑, low hepatorenal toxicity	[143]
$Cu_{2-x}Se$-CB@MEM (CCM)	Cinobufagin + $Cu_{2-x}Se$-mediated PTT	In vitro: Ln229 cells, in vivo: Ln229-luc glioblastoma orthotopic nude mice model (14.7 mg/kg cinobufagin, 10 mg/kg $Cu_{2-x}Se$, NIR: 1064 nm, 1 W/cm^2)	Tumor inhibition rate ↑, prolong mice survival time, apoptosis ↑, cell cycle arrest at the G2/M phase, IMPDH1 ↓, Bcl-2 ↓, Bax ↑, cleaved caspase 9 ↑, Cyclin D ↓, CDK6 ↓, CDK4 ↓, Ki-67 ↓	[144]
Black phosphorus nanosheets (BPNSs) and bufalin coloaded temperature-sensitive supramolecular polypeptide hydrogel (BP-bufalin@SH)	Bufalin + BPNSs-mediated PTT	In vitro: HepG2 and SW480 cells, in vivo: HepG2 tumor-bearing mice model (100 nmol/L bufalin, 2.5 mL/kg containing 400 μg/mL BPNSs, NIR: 808 nm, 1 W/cm^2, 10 min)	Tumor inhibition rate ↑, prolong mice survival time (39 days), good in vivo biosafety, destroy mitochondrial transmembrane potential	[145]
Hybrid membrane (erythrocyte membrane and gastric cancer cell membrane, HM) wrapped indocyanine green (ICG) and gamabufotalin coloaded GOQD nanoparticles (GOQD-ICG-CS-6@HM nanoparticles, GIC@HM NPs)	Gamabufotalin + ICG-mediated PTT	In vitro: BGC-823 cells, in vivo: BGC-823 tumor-bearing mice model (2 mg/kg gamabufotalin, 5 mg/kg ICG, NIR: 808 nm, 1 W/cm^2, 5 min)	Tumor inhibition rate ↑, good in vitro biocompatibility and in vivo biosafety, apoptosis ↑, ROS ↑, P-Caspase3 and P-PARP ↓, C-Caspase3 and C-PARP ↑, Bcl-2 ↓, Bax ↑, Cyclin D1 ↓	[146]
HSA modified trimethyl chitosan (TMC) nanoparticles loading IR780 or bufalin (TIH or TBH)	Bufalin + IR780-mediated PTT	In vitro: 4T1 cells, in vivo: 4T1 tumor-bearing mice model (1 mg/kg bufalin, 1 mg/kg IR780, NIR: 808 nm, 3 W/cm^2, 3 min)	Tumor inhibition rate: 93%, inhibition of lung metastasis: 98.46%, apoptosis ↑	[147]
Photomagnetic dual-sensitive liposome coloaded with Fe_3O_4 nanoparticles and bufalin (BF-Fe_3O_4-L)	Fe_3O_4 nanoparticles + bufalin	In vitro: 4T1 cells, in vivo: 4T1 tumor-bearing lymphoid metastasis mice model (1 mg/kg bufalin, NIR: 808 nm, 2 W/cm^2, 5 min)	Tumor inhibition rate of sentinel lymph nodes: 91%, inhibition of lung metastasis: 81.5%, foot tumor inhibition rate: 81%	[148]

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and inhibiting EMT activation. Moreover, in vivo experiments demonstrated that the accumulation of GTDC@M-R NPs at tumor locations was almost twice as high as that of GTDC NPs, along with a significant tumor inhibition rate (84.4%) and a high inhibition rate for lung metastatic nodules (80%) through the inhibition of chemotherapy-activated cyclooxygenase-2 (COX-2), MMP-9 and VEGF (Figure 4). Furthermore, the GTDC@M-R NPs significantly reversed the

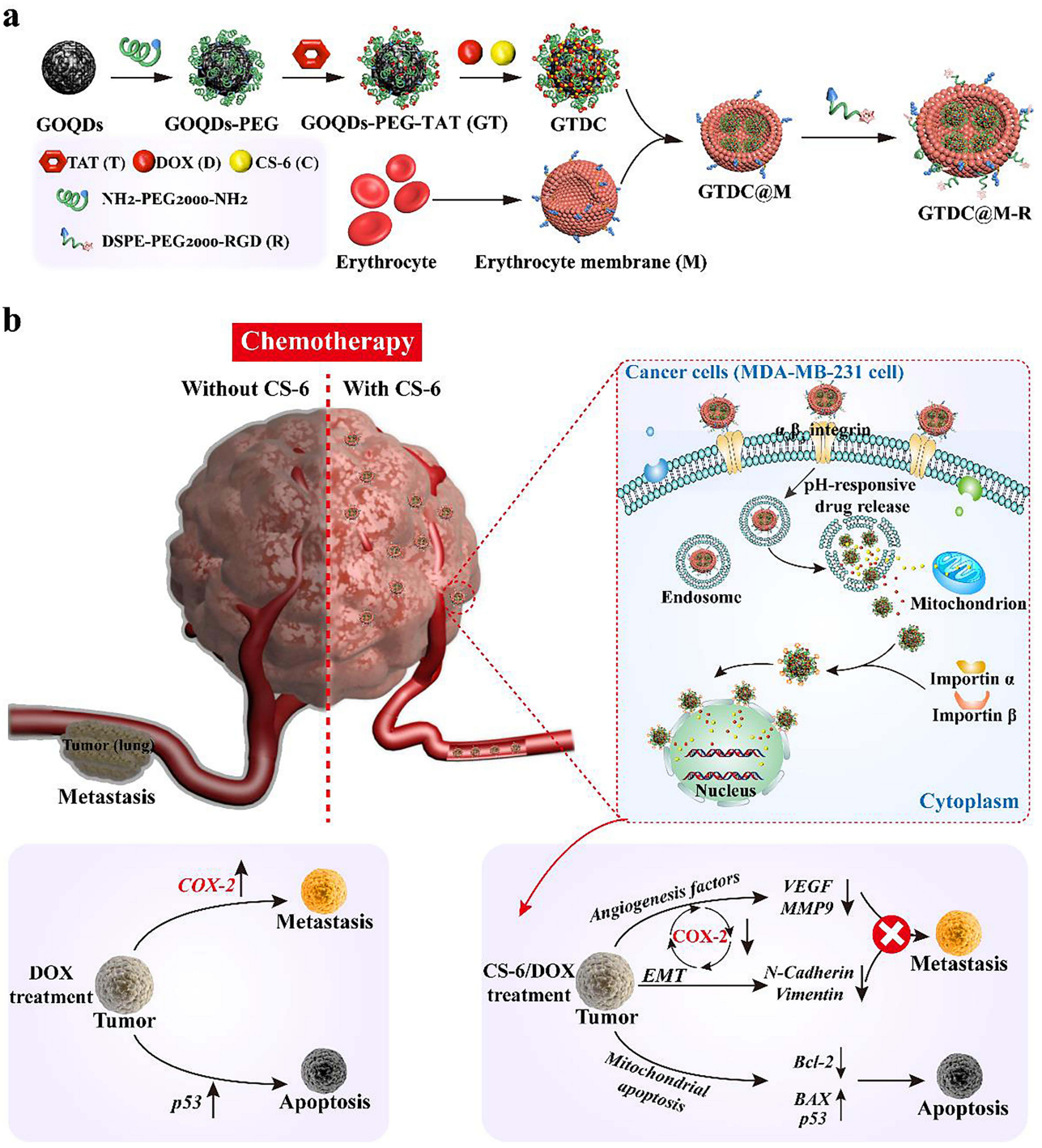

Figure 4 (**a**) Preparation scheme of RGD-modified erythrocyte membrane camouflaged GOQDs-PEG-TAT@DOX@CS-6 nanosystem (GTDC@M-R NPs). (**b**) Proposed mechanism of GTDC@M-R NPs mediated tumor ablation and metastasis inhibition, and GTDC@M-R NPs-mediated transfection of DOX and CS-6 in MDA-MB-231 cells. Reprinted from Acta Biomaterialia, Volume 113, Fan J, Liu B, Long Y, et al, Sequentially-targeted biomimetic nano drug system for triple-negative breast cancer ablation and lung metastasis inhibition, Pages 554–569, Copyright 2020, with permission from Elsevier.[128]

Abbreviations: GOQDs, Graphene oxide quantum dots; CS-6, Gamabufotalin; DOX, Doxorubicin; COX-2, Cyclooxygenase-2; VEGF, Vascular endothelial growth factor; MMP9, Matrix metalloproteinase 9; EMT, Epithelial-mesenchymal transition; Bcl-2, B-cell lymphoma; BAX, Bcl-2-like protein 4; NPs, Nanoparticles.

inflammatory reaction caused by the free drugs by decreasing white blood cell counts and increasing platelet counts, significantly reversed the negative effects of DOX on the liver (ALT and AST) and kidney (creatinine and blood urea nitrogen), and caused nearly no pathological changes in the main organs (heart, liver, spleen, and kidney).[128]

In addition, a vitamin-E–succinate-grafted chitosan oligosaccharide (VES-CSO) and RGD-modified bufalin-loaded TPGS multifunctional mixed micelles (VeC/T-RGD MMs) were constructed via the emulsion solvent evaporation method to enhance L-OHP/DOX-resistant colon cancer treatment. Compared with free bufalin, VeC/T-RGD MMs reduced P-gp expression and increased apoptosis in LoVo/ADR cells. Moreover, these micelles notably suppressed tumor growth (65%) in the LoVo/ADR tumor-bearing model compared with free bufalin (22%). Moreover, these micelles effectively reversed pathological damage, including cardiomyocyte necrosis, connective tissue proliferation, neutrophil infiltration, liver cell necrosis, renal tubular necrosis, and apoptosis, caused by free bufalin. This improvement can be attributed to the enhanced permeability and retention (EPR) effect and RGD-mediated active targeting.[129]

Codelivery of Active Ingredients of Toad Skin/Toad Venom and Small-Molecule Targeted Drugs

Nintedanib is a potent inhibitor of several tyrosine kinases. It specifically binds to ATP-binding sites located inside the kinase domains of VEGFR 1–3, fibroblast growth factor receptor (FGFR) 1–4, platelet-derived growth factor receptor (PDGFR) α/β and c-Src. It can also regulate the tumor microenvironment to inhibit cancer cells. Coloaded bufalin and nintedanib multifunctional albumin submicrospheres (BF-ND-BUP-sMPs) were developed via coaxial-electrospray technology to synergistically enhance the effect of HCC therapy. The tumor targeting ability was effectively enhanced by modification with biguanide and ursodeoxycholic acid. An in vivo study revealed that the BF-ND-BUP-sMPs had the greatest tumor growth inhibition effect (84.2%) on the H22 cell xenograft model, which was significantly greater than that of the BF-BUP-sMPs (66.5%) and ND-BUP-sMPs (58.7%). The mechanism involved enhanced cell apoptosis and changes in the tumor microenvironment structure through the inhibition of angiogenesis, tumor-associated fibroblasts and stroma by decreasing the levels of CD31, alpha-smooth muscle actin (α-SMA) and collagen fibers. Moreover, these BF-ND-BUP-sMPs could significantly attenuate cardiac tissue lesions and inhibit inflammation in the myocardial interstitium caused by bufalin-induced cardiotoxicity, which can be attributed to the dual modification of biguanide and ursodeoxycholic acid.[130] Moreover, the BF-ND-BUP-sMPs strengthened the tumor growth inhibitory effect on LLC cells. In vivo pharmacokinetic assays revealed that, compared with those of free bufalin and nintedanib, the $t_{1/2}$ values of bufalin and nintedanib in BF-ND-BUP-sMPs were 5.6 and 2.4 times higher, the area under the curve $(AUC)_{0\text{-}t}$ was 4.5- and 2.1-fold higher, and the mean residence time (MRT) was 3.4 and 2.1 times higher, respectively, indicating that the albumin shell improved sMP stability in the circulatory system.[131]

By binding to the ATP site of the catalytic domain of VEGFR2, apatinib, a tyrosine kinase inhibitor, can prevent tumor cells from proliferating, metastasizing, invading, and promoting angiogenesis. Biomimetic coloaded apatinib and cinobufagin liposomes with pH-responsive properties (LP-R/C@AC NPs), which have the benefits of evading the immune system and specifically homologously targeting tumor cells, were developed to effectively treat gastric cancer. The hybrid membrane derived from red blood cells and HGC-27 gastric cancer cells extended the $t_{1/2}$ and enhanced drug accumulation in solid and metastatic tumor tissues. The drug release assay revealed that approximately 82% of apatinib and 89% of cinobufagin were released from LP-R/C@AC NPs after 72 h of incubation at pH 5.2, whereas only approximately 44% and 52% of AP and CS-1 were released at pH 7.4, which could effectively decrease the toxicity and adverse reactions of free apatinib and cinobufagin. These liposomes can effectively fight cancer cells by blocking VEGFR2/signal transducer and activator of transcription 3 (STAT3) signaling through the induction of apoptosis, pyroptosis and autophagy; suppression of proliferation, angiogenesis and migration; and improvements in immunity and the hypoxic microenvironment. In addition, these liposomes efficiently suppressed tumor growth (86.78%), invasion and metastasis and improved tumor immunosuppression by inhibiting programmed death ligand-1 (PD-L1) and MMP-9 in an HGC-27 tumor-bearing nude BALB/c mouse model. Furthermore, the LP-R/C@AC NPs reversed the damage to the circulatory system caused by the free drugs and restored ALT levels to within the normal range. Moreover, other liver

and kidney indicators and H&E-stained pathological sections also revealed the biosafety of the LP-R/C@AC NPs in vivo, which is necessary and critical for clinical application.[132]

Lenvatinib is also a potent tyrosine kinase inhibitor that effectively suppresses the activity of several kinases, such as VEGFR 1–3, FGFR 1–4, KIT, PDGFR-α/β and RET. PEG- and folic acid (FA)-modified monodisperse mesoporous silica ($mSiO_2$) nanoparticles coloaded lenvatinib and bufalin (Le/Bu@$mSiO_2$-FA) were designed to synergistically enhance the therapeutic effect on cholangiocarcinoma (CCA). Compared with $mSiO_2$, $mSiO_2$-FA exhibited a superior intracellular drug delivery capacity in CCA 9810 cells. Additionally, Le/Bu@$mSiO_2$-FA exhibited both slow release and pH-responsive drug release properties, which effectively prevented side effects related to the drug concentration and enhanced the therapeutic effect (72 h, pH = 7.4/5.5, the rate of bufalin release = 33.47/74.27%). Le/Bu@$mSiO_2$-FA effectively inhibited the viability, migration, and invasion of 9810 cells. Furthermore, in the CCA tumor-bearing model, these nanoparticles dramatically decreased the tumor burden compared with lenvatinib or bufalin alone (Figure 5). Furthermore, no obvious changes in the ALT and AST contents and no obvious pathological damage to the heart, liver, spleen, lung, or kidney were observed, indicating that Le/Bu@$mSiO_2$-FA has good biological safety, which may be due to FA-mediated active targeting and pH-responsive drug release.[133]

Codelivery of Active Ingredients of Toad Skin/Toad Venom and Monoclonal Antibodies

T-cell–antigen-presenting cell interactions with anti-CD40 are crucial for the immune response. Anti-CD40 antibody-conjugated bufalin liposomes (BFLs) (anti-CD40-BFLs) were developed to enhance the effects of melanoma therapy and reduce systemic toxicity. Compared with free bufalin and anti-CD40, the anti-CD40-BFLs had the characteristics of sustained release. Compared with bufalin, the anti-CD40-BFLs had the strongest antitumor effect on B16 melanoma cells and tumor models. The enhanced therapeutic efficacy was partly due to bufalin-mediated apoptosis through caspase-dependent signaling; moreover, the interaction between anti-CD40 and CD40 adhesion molecules on antigen-presenting cells, such as dendritic cells, promoted the secretion of cytokines, leading to the generation of an ample number of

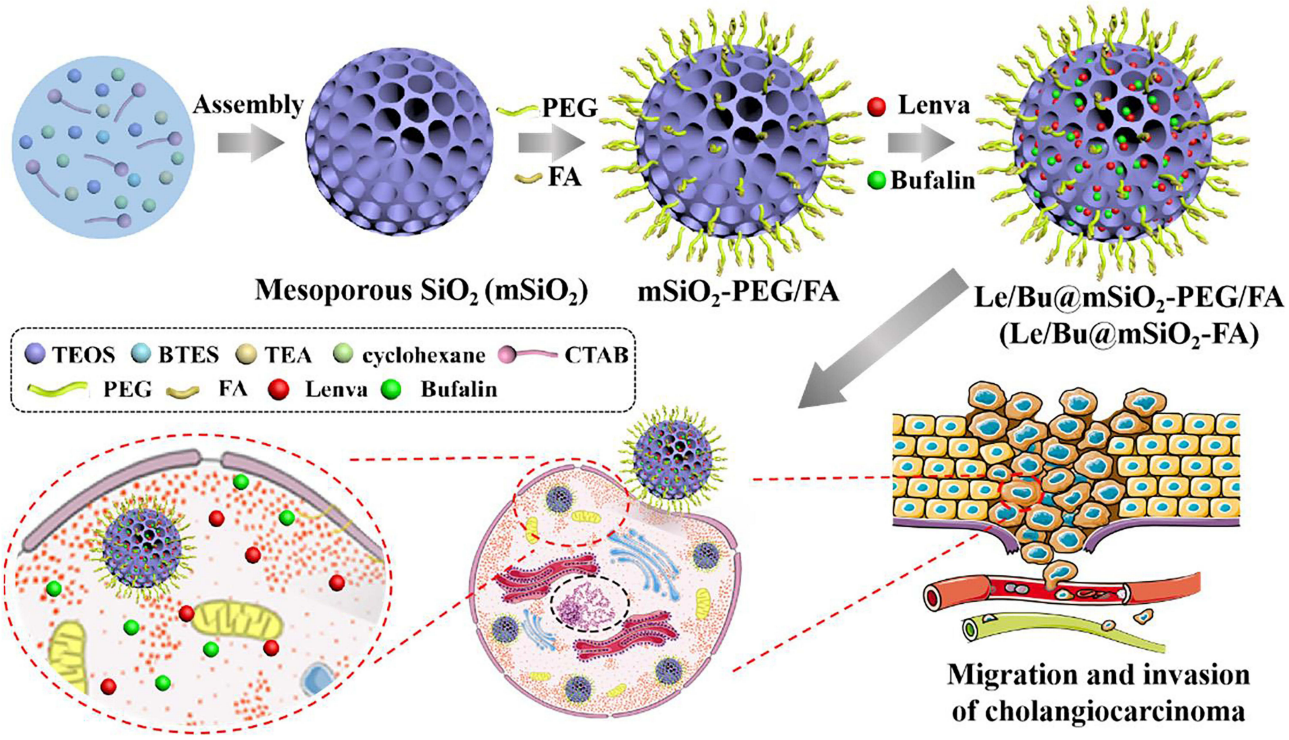

Figure 5 The synthesis process of Le/Bu@$mSiO_2$-FA. Reproduced with permission from Ning Z, Zhao Y, Yan X, Hua Y, Meng Z. Flower-like composite material delivery of co-packaged lenvatinib and bufalin prevents the migration and invasion of cholangiocarcinoma. Nanomaterials-Basel. 2022;12(12):2048. Copyright 2022, MDPI AG. (https://creativecommons.org/licenses/by/4.0/).[133]

Abbreviations: TEOS, Tetraethyl orthosilicate; FA, Folic acid; TEA, Triethanolamine; BTES, bis[3-(triethoxysilyl)propyl] tetrasulfide; CTAB, Hexadecyltrimethylammonium bromide; PEG, Polyethylene glycol.

cytotoxic T lymphocytes and a widespread immune response. Moreover, the administration of anti-CD40-BFLs resulted in no noticeable changes in body weight and notable decreases in the serum concentrations of TNF-α, IL-1β, IL-6, interferon-gamma (INF-γ), and alanine aminotransferase (ALT), indicating fewer adverse reactions.[134]

Codelivery of the Active Ingredients of Toad Skin/Toad Venom and the Active Ingredients of TCM

In China, local administration of realgar (tetraarsenic tetrasulfide) combined with toad venom is a frequent therapeutic approach for treating gynecological cancers, including ovarian and cervical cancer. Based on this information, a temperature-sensitive in situ gel coloaded with nanorealgar (NR) and toad venom-loaded solid lipid nanoparticles (TV-SLNs) (TV-SLNs/NR-TISG) was prepared for the treatment of cervical cancer via vaginal delivery. The coadministration of TV-SLNs and NR at a dosage ratio of 2:3 (w/w) resulted in the greatest inhibition of tumor cell proliferation. Following TV-SLN/NR treatment, HeLa cells exhibited more pronounced arrest in S and G2/M phases, whereas SKOV-3 cells exhibited greater arrest in G0/G1 phase than did the traditional powder group. The optimum formulation of TISG consisted of F127 (27.5%) and F68 (5%), resulting in gelation at a temperature of 33 ± 0.91 °C. Furthermore, this gel displayed good biocompatibility without inflammatory reactions in pathological sections when administered continuously for seven days, allowing the prolonged release of drugs by attachment to the vaginal mucosa.[135] In addition, a coloaded bufalin, cinobufagin and resibufogenin solid dispersion was prepared via spray congealing. Three drugs were shown to be molecularly dispersed inside the matrix, and the solid dispersion significantly increased the dissolution rate of the drugs, which was four times faster than the release rate of the physical mixture. Moreover, the simultaneous release of three medicines from the matrix was accomplished as a result of the outstanding molecular dispersibility and solubilization capability of F127. Furthermore, under controlled conditions, the solid dispersion remained physically stable for a minimum of one month.[136] The antitumor effect of solid dispersions needs to be further studied in the future.

Moreover, a cetuximab-conjugated immunoliposome loaded with bufalin and melittin was prepared to inhibit sorafenib resistance in HCC. The compound immune liposomes can effectively induce the complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity of cetuximab. Compared with free drugs, the liposomes can inhibit cell proliferation and trigger apoptosis more effectively via the activation of ER signaling. An in vivo study also revealed that the liposomes had a stronger antitumor effect than free melitoxin/bufarin and could more effectively prolong the survival time of tumor-bearing nude mice. In addition, the H&E staining results for the liposomes revealed no significant hepatic or renal toxicity compared with that of melittin or melittin/bufalin, which may be attributed to the fact that cetuximab can actively target HER1 to reduce the accumulation of the drug in other organs.[137] Based on the "unification of drugs and excipients" theory, toad skin extract (TSE) and *Brucea javanica* oil (BJO) coloaded nanoemulsions (TSE-BJO NEs) were also prepared. BJO not only is the main oil phase for the formation of nanoemulsions but also exerts a synergistic anti-liver cancer effect when combined with TSE. This nanoemulsion increased drug accumulation at the tumor site, extended the drug retention time, and resolved the issue of the fast clearance of hydrophobic active ingredients of toad skin in vivo. Moreover, the combined TSE-BJO NEs exerted synergistic antitumor effects by suppressing proliferation, arresting the cell cycle and triggering apoptosis in HepG2 cells and a HepG2 xenograft tumor model. In addition, the results of routine blood, liver and kidney indices and H&E staining of pathological sections all indicated that TSE-BJO NEs exhibited good biosafety.[138]

Codelivery of Active Ingredients of Toad Skin/Toad Venom and Photodynamic Therapy Drugs

PDT kills cancer cells through the generation of ROS; however, the hypoxic tumor microenvironment significantly limits its effectiveness. Since bufalin can significantly increase the effectiveness of PDT mediated by the photosensitizer meta-tetrahydroxyphenylchlorin (mTHPC) in treating colorectal cancer, VES-CSO/RGD-modified TPGS (TPGS-RGD) multifunctional nanoparticles were constructed to codeliver bufalin and mTHPC [mTHPCandBU@VES-CSO/TPGS-RGD nanoparticles (T-B@NPs)] and achieve synergistic colorectal cancer therapy. In vitro assays indicated that T-B@NPs increased the sensitivity of colorectal cancer cells to PDT via antiangiogenic effects and reduced hypoxia by targeting

SRC-3/HIF-1α/VEGF signaling. In addition, T-B@NPs have a sustained drug release behavior that favors the passive accumulation of nanoparticles in tumor tissues. In vivo studies using a CT26 tumor-bearing BALB/c mouse model demonstrated that T-B@NPs + laser had the strongest antitumor effect (84.2%), which was greater than that of T@NPs + laser (77.6%), B@NPs (65.2%), free mTHPC + laser (40.0%) and bufalin alone (45.8%), by relieving the oxygen deficiency and inducing antiangiogenic effects. Moreover, T-B@NPs + laser greatly prolonged the survival rate of the mice, and no obvious organ damage was observed during treatment, which was attributed to RGD-mediated active targeting and the antitumor ability in combination with PDT.[139]

Moreover, erythrocyte-cancer hybrid membrane-coated cinobufagin and Chlorin e6 (Ce6)-coloaded polyacrylic acid-modified cerium nanoparticles [CeO_2@PAA@CS-1/Ce6@M (CPCCM NPs)] were developed for synergistic chemo/PDT against TNBC. The hybrid erythrocyte/MDA-MB-231 cell membrane endows the nanomedicine with homologous targeting ability and long circulation times in the blood. The PAA modification could control the release of drugs. Approximately 22.31% of the cinobufagin was released from the CPCCM NPs after 5 h of incubation at pH 7.4, while the release rate increased to 39.78% at pH 6.8, and laser irradiation further accelerated drug release (72.84% within 90 min), which effectively prevented the side effects of free cinobufagin/Ce6 and enhanced the therapeutic effect. Interestingly, CPCCM NPs can also autonomously generate O_2 by decomposing excess H_2O_2 within tumor tissue and reprogramming the hypoxic tumor microenvironment, which accordingly enhances the effectiveness of cinobufagin and PDT. In vivo studies also revealed that cinobufagin + PDT had a high tumor growth inhibition rate (85.5%) and markedly prevented lung and liver metastases by inhibiting HIF-1α and MMP-9 expression. In addition, CPCCM NPs + laser could reduce the inflammatory response (white blood cells) or reverse the side effects (ALT and AST) caused by free cinobufagin or PDT, and H&E-stained pathological sections also revealed no obvious damage to the main organs, suggesting that the CPCCM NPs have obvious biosafety in vivo (Figure 6).[140]

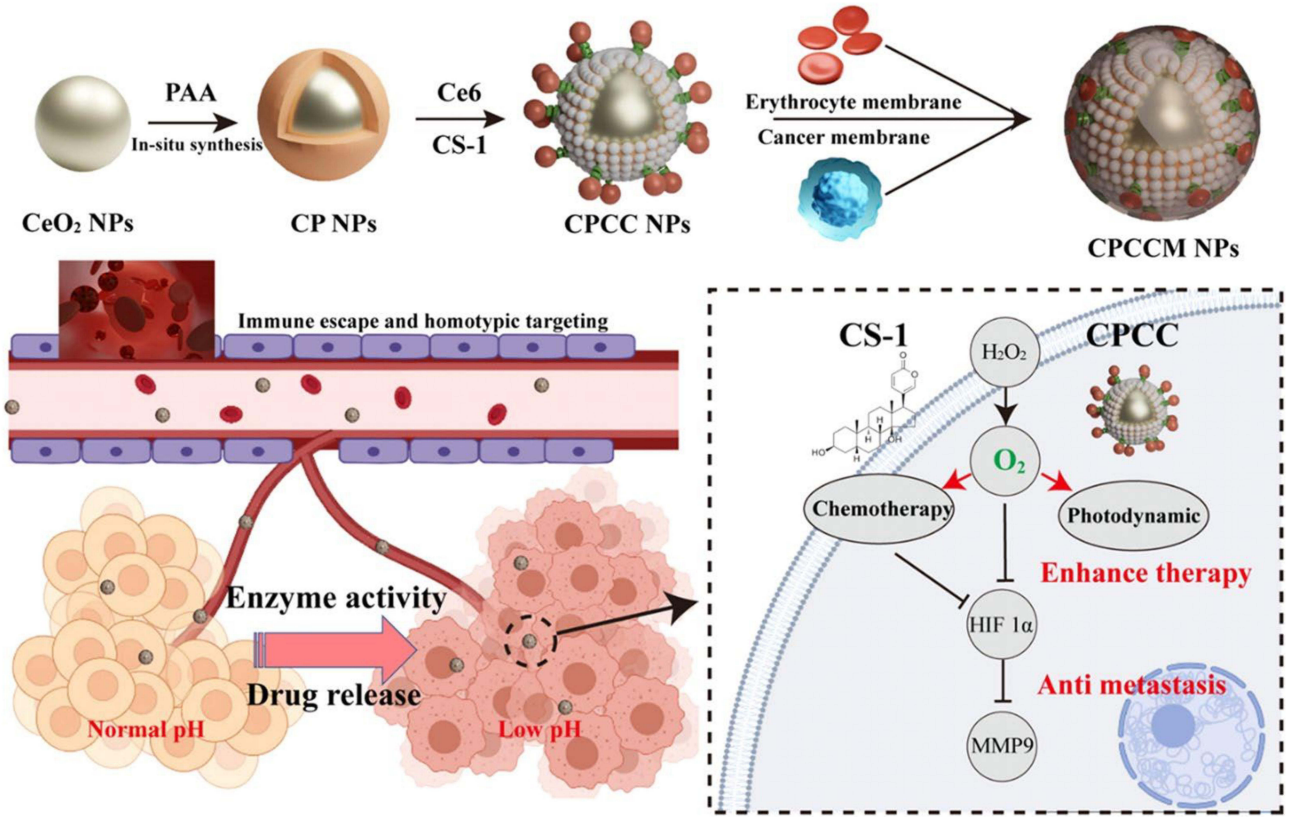

Figure 6 Schematic diagram of the designed strategy for the combined therapy of CPCCM NPs. Reproduced with permission from Zeng Z, Wang Z, Chen S, et al. Bio-nanocomplexes with autonomous O(2) generation efficiently inhibit triple negative breast cancer through enhanced chemo-PDT. J Nanobiotechnol. 2022;20(1):500. Copyright 2022, BioMed Central.[140]

Abbreviations: CS-1, Cinobufagin; Ce6, Chlorin e6; PAA, Polyacrylic acid; MMP9, Matrix metalloproteinase 9; HIF 1α, Hypoxia-inducible factor-1alpha; CPCC, Ce6 and CS-1 loaded cerium nanoparticles.

Codelivery of Active Ingredients of Toad Skin/Toad Venom and Photothermal Therapy Drugs

PTT is an optical treatment strategy that can convert light energy into heat energy for tumor treatment and has the advantages of minimal invasiveness, few adverse reactions, high specificity, and repeatable treatment. Some studies have shown that bufalin, cinobufagin and gamabufotalin combined with PTT can achieve synergistic chemo/photothermal therapeutic effects.

Hybrid erythrocyte/SW480 cell membranes (M) camouflaged with cinobufagin-loaded Prussian blue nanoparticles (PC@M NPs) were prepared for combined chemo/PTT against colorectal cancer. The camouflaged hybrid membrane exhibited enhanced cellular internalization and a favorable ability to escape the endolysosomal system, along with a strong homologous targeting capacity to tumor cells. In vitro release studies have shown that low pH (24 h, pH = 5.2/7.4, 59.2% vs 42.4%) and near-infrared (NIR) light stimulation (24 h, pH = 5.2, + laser irradiation reached 74.8%) can increase the release of drugs. Moreover, by increasing ROS levels and the Bax/Bcl-2 ratio and decreasing vimentin, MMP-9, and HIF-1a expression, PC@M NPs have the potential to both efficiently trigger apoptosis and suppress metastasis in colorectal cancer cells. In vivo studies also revealed that PC@M NPs + laser markedly inhibited tumor growth (84.57%) by activating mitochondria-driven apoptosis signaling and markedly prevented lung metastasis and recurrence by reducing the MMP-9 level and blocking the EMT process. In addition, PC@M NPs + laser effectively reduced the cardiotoxicity caused by cinobufagin alone, reduced the number of white blood cells due to its strong anti-inflammatory effect, and caused no obvious damage to the liver and kidney indices or major organs, which may be attributed to the excellent homologous targeting ability of the cell membrane, pH-responsive behavior and PTT ability. Notably, PC@M NPs + laser reversed the proliferation of detrimental bacteria and the reduction in the number of good bacteria in the intestinal tract of tumor-bearing mice (Figure 7).[141]

Red blood cell (RBC) membrane-camouflaged hyaluronic acid (HA)-modified PB NPs loaded with gamabutolin, referred to as HA@RBC@PB@CS-6 NPs (HRPCs), were developed for synergistic chemo/PTT against breast cancer. The erythrocyte membrane and HA on the nanoparticles increased the blood circulation time by 10 h, increased immune

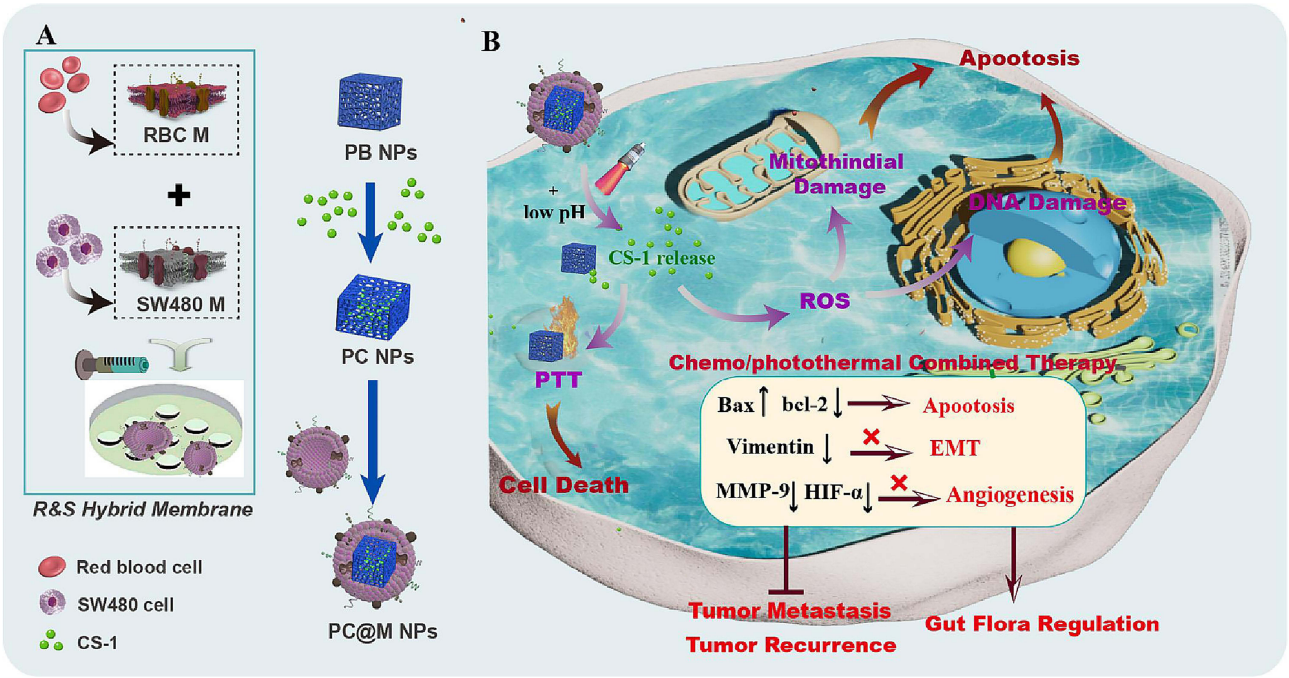

Figure 7 Schematic depiction of preparation (**A**) and in vivo application (**B**) of PC@M NPs. Reproduced with permission from Luo M, Tan C, Cao R, et al. Hybrid membrane camouflaged prussian blue nanoparticles with cinobufagin loading for chemo/photothermal therapy of colorectal cancer. Mater Design. 2023;232:112088. Copyright 2023, Elsevier Ltd.[141]

Abbreviations: CS-1, Cinobufagin; PTT, Photothermal therapy; ROS, Reactive oxygen species; Bax, Bcl-2-like protein 4; bcl-2, B-cell lymphoma; EMT, Epithelial-mesenchymal transition; MMP-9, Matrix metalloproteinase 9.

evasion by more than 60%, and increased drug accumulation at the tumor site by binding specifically to the CD44 receptor. Moreover, the release behavior of HRPC is pH- and temperature dependent. The cumulative rate of gamabutolin release at pH 5.0 was 33.40% after 4 cycles of laser on/off treatment, whereas it was only 19.34% at pH 7.4, which is favorable for increasing the release of drugs in the tumor region and reducing the adverse effects on other normal organs. Notably, the excellent antitumor activity of the gamabutolin-containing nanomedicine is partly due to its ability to suppress heat shock protein 70 (HSP70), which accordingly enhances the effectiveness of PTT. Moreover, in vivo studies also revealed that HRPCs + laser had the strongest antitumor effect (93.4%), which was superior to that of HRPCs (23.1%), RPCs + laser (45.4%) and free gamabutolin (58.8%) in an MDA-MB-231 tumor-bearing model. In addition, HRPCs + laser significantly reversed gamabutolin-induced hepatorenal toxicity (AST, ALT and creatinine levels returned to normal), and no damage to major organs (heart, liver, spleen or kidney) was detected.[142]

FA-modified cinobufagin-loaded polydopamine (PDA) nanoparticles were constructed for synergistic chemo/PTT against lung cancer. PDA nanoparticles can achieve active targeting of tumor cells via specific binding of FA and FA receptors. The PDA nanomedicine subsequently achieved pH-responsive and NIR irradiation-triggered drug release (12 h, pH = 7.4/5.0 released 24% vs 53%, pH = 7.4/5.0 + laser released 33%/74%). In vivo experiments on an LLC tumor-bearing model revealed that the PDA nanomedicine + NIR laser had excellent multimodal therapeutic effects (tumor inhibition rate: 67%). In addition, compared with free cinobufagin, the PDA nanodrug could decrease hepatorenal toxicity (ALT, AST, and creatinine), which may be attributed to FA-mediated active targeting and pH- and light-triggered drug release.[143]

An Ln229 glioma cell membrane camouflaged with cinobufotalin-loaded $Cu_{2-x}Se$ nanoparticles ($Cu_{2-x}Se$-CB@MEM, CCM) was designed to enhance orthotopic glioblastoma therapy through synergistic chemo/photothermal therapeutic effects. The coated Ln229 cell membrane allowed the nanoparticles to penetrate blood–brain barrier, and the nanoparticles exhibited a homologous tumor-targeting capacity. In vitro release studies revealed that CCM could achieve pH-responsive drug release (120 min, pH = 7.4/5.0, 39.6% vs 54.1% release, respectively). Cinobufotalin + $Cu_{2-x}Se$-mediated PTT exhibited remarkable glioma cell inhibitory efficacy through the induction of G2/M phase arrest and apoptosis. Moreover, CCM + laser significantly suppressed tumor growth and extended the survival time in an Ln229-luc glioblastoma orthotopic nude mouse model. In addition, CCM + laser reversed the negative effects of cinobufotalin on the liver (ALT and AST) and caused no obvious damage to the main organs (heart, liver, spleen, lung, and kidney).[144]

Moreover, black phosphorus nanosheets (BPNSs) and bufalin coloaded with a temperature-sensitive supramolecular polypeptide hydrogel (BP-bufalin@SH) were successfully developed for synergistic chemo/PTT. Upon NIR irradiation (808 nm, 1 W/cm^2), the temperature of the BP-bufalin@SH mixture rapidly increased and promoted the smart, photocontrolled release of bufalin, which significantly reduced the adverse effects of bufalin. This hydrogel was able to increase cell apoptosis by disrupting the $\Delta\Psi m$, achieve a remarkable tumor growth inhibition effect and prolong the survival time in a HepG2 tumor-bearing mouse model. In addition, an injection of BP-bufalin@SH did not cause infection or inflammation in mice, and no significant hepatorenal toxicity or organ damage was observed, indicating its excellent biosafety in vivo.[145]

Erythrocyte–gastric cancer hybrid cell membrane (HM)-wrapped indocyanine green (ICG) and gamabufotalin coloaded GOQD nanoparticles (GIC@HM NPs) were also successfully designed and established for combined photothermal–chemotherapy against gastric cancer. The camouflaged hybrid membrane not only improved the nanoparticles' biocompatibility and in vivo circulation time but also increased drug accumulation at the tumor site. Moreover, the release of gamabufotalin from the GIC@HM NPs could be accelerated by the acidic microenvironment and laser (72 h, pH = 7.4/5.4, 40% vs 60% released, pH = 5.4 + laser 80% release), which effectively reduced the adverse reactions of free drugs and improved the therapeutic effect. In vitro and in vivo experiments on BGC-823 cells and a BGC-823 tumor-bearing mouse model proved that GIC@HM NPs + laser had excellent synergistic antitumor effects. In addition, GIC@HM NPs + laser reversed the negative effects of the free drugs (AST, ALT, uric acid, creatinine and blood urea nitrogen levels returned to normal), and all the indices of the blood samples were within the normal range. No damage to major organs (heart, liver, spleen or kidney) was detected, indicating the excellent biosafety of GIC@HM NPs + laser in vivo.[146]

Furthermore, human serum albumin (HSA)-modified IR780 or bufalin-loaded trimethyl chitosan (TMC) nanoparticles (TIH or TBH) were prepared for synergistic chemo/PTT against metastatic breast cancer. Notably, TIH-mediated PTT was able to disturb the tumor microenvironment, allowing deeper drug penetration and accordingly leading to increased

accumulation of a second TBH dose in the tumor tissue. Moreover, TBH could responsively release bufalin in the acidic tumor environment, which could further enhance the therapeutic effect. Upon NIR irradiation (808 nm, 3 W/cm^2, 3 min), the TIH + laser and second TBH injection (TIH+L/TBH) resulted in a high tumor growth inhibition rate of 93% and a remarkable lung metastasis inhibition rate of 98.46%. In addition, good compatibility was revealed by the histological examination and weight changes, which may be related to the ability of HSA modification to improve drug targeting.[147]

Using superparamagnetic Fe_3O_4 nanoparticles as photothermal agents and magnetic targeting agents, a photomagnetic dual-sensitive liposome coloaded with Fe_3O_4 nanoparticles and bufalin (BF-Fe_3O_4-L) was prepared. Under the action of a magnetic field, BF-Fe_3O_4-L targeted sentinel lymph nodes located near the tumor and increased the drug uptake rate by 1.6 times. When irradiated by an 808 nm NIR laser (2 W/cm^2, 5 min), Fe_3O_4 nanoparticles can effectively convert light energy into thermal energy (46 °C) and bufalin can be released from liposomes to achieve combined photothermal therapy and chemotherapy for sentinel lymph node metastatic breast cancer. Compared with the PBS group, the BF-Fe_3O_4-L group presented a 91.0% inhibition of sentinel lymph node metastasis and an 81.5% reduction in distal lung metastasis. In addition, treatment with BF-Fe_3O_4-L did not result in sudden weight loss in mice, indicating good biosafety in vivo.[148]

Perspectives and Conclusions

As valuable traditional Chinese medicines, toad skin and toad venom have been shown to exert significant antitumor effects in clinical practice for more than one thousand years. This article reviews three aspects of combined antitumor therapeutic strategies based on toad skin- and toad venom-related preparations and active monomer components: clinical efficacy and safety, antitumor effects and mechanisms in vitro and in vivo, and nanodelivery systems. Clinical studies have shown that chemotherapy drugs, small-molecule targeted drugs or TACE combined with toad skin- and toad venom-related preparations can effectively improve the objective response rate of cancer patients, prolong the survival time of patients, improve the QOL of patients, improve the immune function of patients and reduce the incidence of adverse reactions. However, the current research has focused mainly on combinations with chemotherapy drugs, and no reports on combinations with tumor immunotherapy are available. In recent years, tumor immunotherapy involving PD-1/PD-L1 antibodies has greatly influenced the pattern of tumor therapy and has shown great application potential in tumor therapy. TCM preparations such as toad skin and toad venom also have unique advantages in improving the immune function of the body. Therefore, clinical trials of the combination of toad skin- and toad venom-related preparations with tumor immunotherapy are necessary in the future to better exploit the advantages of TCM in "strengthening the body's resistance to eliminate pathogenic factors". In addition, studies of the combined antitumor effect of toad skin- and toad venom-related preparations with additional targeted drugs, such as regorafenib, bevacizumab, and cetuximab, as well as the combined antitumor effect with new anticancer therapies, such as PDT, antibody–drug conjugates, and oncolytic virus therapy, are necessary to provide cancer patients with more options for personalized treatment plans. In addition, evidence-based medical studies have shown that Huachansu, a water-soluble extract of toad skin, has another unique role in the clinical treatment of cancer pain. Huachansu combined with the three-step analgesic method is superior to the three-step analgesic method alone in the treatment of cancer pain, improving QOL and reducing the occurrence of side effects, and has the advantages of shortening the onset time and prolonging the duration of pain relief. Moreover, it can reduce the average daily dosage of opioids and greatly improve the quality of life of patients with middle-stage and advanced cancer. However, some problems exist in the clinical studies reported thus far, such as a small number of case samples, insufficient rigorous study design, and inconsistent clinical efficacy evaluation indicators. In the future, randomized controlled trials with a reasonable design, rigorous implementation, standardized reporting, large sample sizes, multiple centers, and sufficient follow-up time should be conducted to verify the effectiveness and safety of toad skin- and toad venom-related preparations in the treatment of cancer and cancer pain.

Mechanistically, active ingredients in toad skin and toad venom (gamabufotalin, arenobufagin, bufalin, cinobufagin, and cinobufotalin) can enhance the efficacy of chemotherapy, targeted therapy, radiotherapy, hyperthermia, signaling pathway inhibitors, traditional Chinese medicine therapy, PDT, mRNA therapy, siRNA therapy and other therapeutic methods by inducing the apoptosis of tumor cells; arresting the cell cycle; inhibiting proliferation, migration and invasion; and inhibiting tumor angiogenesis in in vitro cell and in vivo animal models. In addition, the active ingredients

in toad skin and toad venom, especially bufalin, can effectively reverse resistance to chemotherapeutic drugs and targeted drugs by inducing the apoptosis of tumor cells, regulating cancer cell stemness, reducing M2 macrophage polarization, decreasing drug efflux pump activity, inhibiting the epithelial–mesenchymal transition, and other processes. In general, the antitumor mechanisms of toad skin and toad venom have not been studied in sufficient depth. How these preparations regulate the tumor microenvironment to exert antitumor immune effects needs to be elucidated. In addition, whether they can enhance antitumor effects through ferroptosis, pyroptosis, cell senescence, glycolysis and other pathways also needs to be further studied. In addition, TCMs, such as toad skin and toad venom, have multiple components and multiple targets, and the study of a single pathway or single active ingredient cannot easily interpret the therapeutic idea of the "holistic concept" of TCM. Network pharmacology combined with multiomics technology can explain the molecular mechanism of TCM in the treatment of diseases from multiple angles and aspects, which is consistent with the research idea of TCM. Therefore, in the future, multiomics technologies such as transcriptomics, proteomics, metabolomics and network pharmacology research methods can be applied to study toad skin and toad venom and reveal their antitumor mechanisms more comprehensively. In addition, Huachansu can significantly relieve a variety of types of cancer pain in the clinic and may be related to inhibiting inflammation, regulating opioid receptor pathways, affecting the function of Ca^{2+} and K^{+} channels, alleviating nerve compression by tumor tissue, and regulating the function of the 5-hydroxytryptamine system.[151] Unfortunately, the understanding of the analgesic mechanism of Huachansu combined with the three-step analgesic method is insufficient at present. How to systematically study its mechanism of inhibiting cancer pain at multiple levels and in multiple disciplines needs to be further explored by researchers.

Although toad venom and toad skin exhibit excellent antitumor activity, they also possess certain toxicity. Toad venom is a toxic TCM according to the 2020 edition of the Chinese Pharmacopoeia.[12] At present, with the widespread clinical use of toad venom- and toad skin-related preparations such as Huachansu injection and Toad venom injection, the frequent occurrence of adverse reactions has garnered widespread attention. Adverse reactions involve multiple organ systems, including the nervous, circulatory, and digestive systems, among which adverse reactions of the cardiovascular system should receive increased attention because of their serious consequences.[152,153] Clinical reports have shown that adverse reactions such as arrhythmia and shortness of breath may occur within 30 minutes after the administration of Huachansu injection.[154] Additionally, adverse reactions such as atrioventricular block, myocardial damage, and sinus arrhythmia can also occur after the administration of preparations containing toad venom.[155–157] Furthermore, abnormal changes in cardiac troponin I (cTn-I) levels were observed after intravenous injection of Huachansu injection (5 g/kg and 10 g/kg) in rats, confirming its cardiotoxicity.[158] In beagles, intravenous injection of 3 g/kg Huachansu injection immediately caused shortness of breath, with arrhythmia detected at 10 minutes posttreatment. Additionally, 3 g/kg Huachansu injection resulted in significant increases in cTn-I, CK-MB, and AST levels at 1.5 h and 3 h postinjection.[159] Moreover, the administration of a large dose of a 75% ethanol extract of toad venom resulted in gradually reduced activity, slow and deep respiration, a weakened or absent righting reflex before death, as well as slow and weak breathing in mice, with an LD_{50} of 0.6006 g/kg.[160] In addition, a study reported that IL-6 can exacerbate the inhibitory effect of Toad venom injection on the Ca^{2+} current, significantly increasing the risk of atrioventricular block.[161] Another study has shown that toad venom can impair normal myocardial function by affecting ion homeostasis, actin construction and cell apoptosis.[162] Numerous studies have confirmed that the main active ingredients of toad venom and toad skin are bufadienolides, which have similar structures to those of cardiac glycosides, and thus they can produce side effects such as cardiotonic effects in cancer treatment,[163] causing long-term Na^{+}-K^{+}-ATP blockage and resulting in cardiac arrest.[164] Currently, the cardiotoxicity of bufadienolides has been widely reported. For example, symptoms of cardiac glycoside toxicity, such as palpitations, tachypnea and convulsions, were observed in mice intraperitoneally injected with 1 mg/kg, 2 mg/kg and 10 mg/kg bufalin. Mice intraperitoneally injected with 1 mg/kg bufalin showed inverted T waves on the electrocardiogram, and mice injected with 2 mg/kg and 10 mg/kg bufalin showed 80% and 100% lethality, respectively,[165] with instant death observed after the injection of 30 mg/kg.[166] Moreover, studies have shown that inhibition of the $Na_v1.5$ channel, enhancement of the late sodium channel current and enhancement of the sodium–calcium exchange current are among the main mechanisms of the cardiotoxicity induced by toad venom.[167] In addition, an intraperitoneal injection of 10 mg/kg/day bufalin or cinobufagin induced 100% death at 1 and 2 days postadministration, respectively, with death occurring within 10 minutes after the intravenous injection of 1 mg/kg cinobufagin.[168] The LD_{50} of arenobufagin administered via intraperitoneal injection in mice was 16.2 mg/kg.[169] Furthermore, some studies have reported that the LD_{50} values of various

components of toad venom in mice are as follows: 41.0 mg/kg (intravenous), 96.6 mg/kg (subcutaneous), and 36.24 mg/kg (intraperitoneal) for toad venom; 2.2 mg/kg (intraperitoneal) for bufalin; 4.38 mg/kg (intraperitoneal) for cinobufagin; and 4.25 mg/kg (intravenous), 15 mg/kg (slow intravenous), 14 mg/kg (intraperitoneal), 124.5 mg/kg (subcutaneous), and 64 mg/kg (oral) for resibufogenin. In dogs, reported LD_{50} values for bufotalin are approximately 0.36 mg/kg (intravenous) and 0.98 mg/kg (oral minimal lethal dose).[170] In summary, the toxicity of toad venom and toad skin should not be underestimated, and ensuring their safe use is a key issue in their clinical application. At present, toad venom- and toad skin-related preparations are mainly used as clinical antitumor adjuvant drugs to enhance the effects of chemotherapy, radiotherapy, targeted therapy, and other treatments. However, most of the current studies on the cardiotoxicity of toad venom and toad skin are limited to single drugs, and an exploration of the toxicity and mechanism of clinical combination drugs is lacking. Since toxic reactions are often closely related to the dose, mode and course of administration and more factors need to be considered in combination administration than in single administration, the toxicity and mechanism of combined drug administration based on toad skin and toad venom should be further studied to better guide rational drug use in the clinic. In addition, some studies have shown that taurine,[171] phenytoin sodium, lidocaine and propranolol[172] can alleviate bufadienolide-induced cardiotoxicity or arrhythmia, suggesting that we can use these drugs in combination to alleviate toad venom- and toad skin-induced cardiotoxicity.

Compared with the conventional multidrug combination strategy, the multidrug combination strategy based on a nanodelivery system can achieve the accurate loading of combined drugs and carry the drug to overcome physiological and pathological barriers, complete the efficient enrichment in the tumor tissue, and achieve targeted delivery to tumor cells and controlled drug release, resulting in enhanced antitumor efficacy and reduced toxic effects. Although preclinical studies have shown that the codelivery strategy of the active ingredients of toad skin and toad venom (bufalin, gamabufotalin, and cinobufagin) combined with chemotherapy drugs, small molecule-targeted drugs, monoclonal antibodies, active ingredients of TCM, drugs for PDT and PTT can reduce the side effects of drugs on normal tissues, especially cardiac toxicity, can reverse the MDR of tumor cells to a certain extent, and achieve the synergistic therapeutic effect of "1+1>2", many problems remain to be solved to achieve the clinical transformation of nanomedicines. The optimal ratio of drugs is the key to realizing the synergistic effect of drugs, and how to screen the optimal compatibility ratio of combined drugs needs to be studied. How to select drug delivery carriers to achieve high drug loading rates, high encapsulation rates, and stable production technology and storage; how to achieve the optimal ratio of combined drugs unchanged and stable delivery into tumor tissues during the preparation of coloaded drug nanodelivery systems; and how to control the simultaneous release or sequential release of combined drugs need to be further studied. In addition, the toxicity of nanomaterials and the safety of the long-term application of nanomedicines also need to be studied. Liposomes are spherical vesicles with special bilayers composed of lipid components such as phospholipids and cholesterol. Due to their high biocompatibility, biodegradability, low toxicity, and wide range of drug delivery, liposomes have become some of the most widely studied nanocarriers in the current nanodrug delivery system. At present, a variety of drugs, such as doxorubicin hydrochloride, paclitaxel, irinotecan hydrochloride, daunorubicin, mifamurtide, vincristine and cytarabine, have been successfully encapsulated in liposomes for clinical use.[173] Notably, in August 2017, the US FDA officially approved the market of the world's first compound liposome (Vyxeos). Vyxeos is a liposomal compound preparation composed of cytarabine and daunorubicin at a molar ratio of 5:1 for the treatment of therapy-related acute myeloid leukemia (t-AML) and acute myeloid leukemia with myelodysplastic-related changes (AML-MRC). In addition, the irinotecan hydrochloride/floxuridine compound liposome (LY01616) is also undergoing clinical trials.[23] Therefore, liposomes can be used as the starting point for the development of nanomedicines for the active ingredients of toad skin and toad venom.

In conclusion, combined antitumor strategies based on related preparations and active ingredients of toad skin and toad venom, along with drug delivery systems, can improve cancer therapy through multiple pathways and targets, reduce adverse events, and provide better treatment options for patients with tumors. However, many areas for improvement in current clinical combination treatment strategies still remain. The mechanistic basis is the rational foundation of antineoplastic drug combination therapy. In the future, researchers should continue to conduct in-depth mechanistic studies of combination therapy. Concurrently, adequate single-agent clinical pharmacological and safety data of the proposed combination drugs should be obtained, including human pharmacokinetic parameters, safe dose ranges, and dose–exposure–effect relationships, to fully evaluate the possible drug interactions and the superimposed risk of toxicity in vital organs. These findings provide a basis for the scientific and reasonable selection of combined drug

dosages, formulation of dosage sequences, and design of safety and risk control measures. This approach could also provide a foundation for developing low-toxicity, high-efficiency, stable, and quality-controlled codelivery systems. Additionally, continuously enriching and improving the clinical trial technical guidelines for antitumor drug combination therapy is necessary to promote the scientific and orderly development of the combination of toad skin and toad venom, thus providing patients with combination therapy solutions with real clinical value. With further research, the combination of toad skin and toad venom is anticipated to have wider, safer, and more rational clinical applications, leading to more effective therapeutic outcomes.

Abbreviations

PDT, photodynamic therapy; TCM, traditional Chinese medicine; MDR, multidrug resistance; TACE, transarterial chemoembolization; QOL, quality of life; PFS, progression-free survival; OS, overall survival; HCC, hepatocellular carcinoma; DCR, disease control rate; ORR, objective response rate; NSCLC, non-small-cell lung cancer; PSA, prostate specific antigen; f-PSA, free PSA; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DOX, doxorubicin; ASK1, apoptosis signal-regulating kinase 1; JNK, c-Jun N-terminal kinase; FAK, focal adhesion kinase; Bax, Bcl-2-like protein 4; PDCD4, programmed cell death 4; GSK-3β, Glycogen synthase kinase 3beta; Bcl-xl, B-cell lymphoma-extra large; p-Akt, phosphorylated protein kinase B; mTOR, mechanistic target of rapamycin; MTX, Methotrexate; DHFR, dihydrofolate reductase; 5-FU, 5-Fluorouracil; MRP1, multidrug resistance-associated protein 1; VCR, Vincristine; CDC25, cell division cycle 25; CDK1, cyclin-dependent kinase 1; Bcl-2, B-cell lymphoma; ΔΨm, mitochondrial membrane potential; ROS, reactive oxygen species; L-OHP, Oxaliplatin; ADR, Adriamycin; P-gp, P-glycoprotein; DCT, Docetaxel; ABCB1, ATP-binding cassette subfamily B member 1; Nrf2, nuclear factor erythroid 2-related factor 2; HO-1, heme oxygenase-1; IRE1a, inositol-requiring enzyme 1a; TRAF2, tumor necrosis factor receptor associated factor 2; ER, endoplasmic reticulum; NF-κB, nuclear factor-kappaB; MDR1, multidrug resistance gene 1; OCT4, octamer-binding transcription factor 4; SOX2, sex-determining region Y-box 2; ABCG2, ATP-binding cassette transporter G2; MIF, macrophage migration inhibitory factor; SRC-3, steroid receptor coactivator 3, PI3K, phosphoinositide 3-kinase; MYH9, myosin heavy chain 9; USP7, ubiquitin-specific protease 7; EMT, epithelial-mesenchymal transition; S6K, S6 kinase; 4EBP1, eIF4E-binding protein 1; FOXO1, forkhead box O1; CIPNP, chemotherapy-induced peripheral neuropathic pain; PIPNP, Paclitaxel-induced peripheral neuropathic pain; TNF-α, tumor necrosis factor-alpha; IL-1β, interleukin-1beta; TRPV1, transient receptor potential vanilloid 1; ERK, extracellular signal-regulated kinase; PCNA, Proliferating cell nuclear antigen; CDK2, cyclin-dependent kinase 2; HUVECs, human umbilical vein endothelial cells; PDGF-BB, platelet-derived growth factor-BB; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; EGFR, epidermal growth factor receptor; HGF, hepatocyte growth factor; APAF-1, apoptotic protease activating factor-1; SOD, superoxide dismutase; Bad, BCL2-associated agonist of cell death; MAPK, mitogen-activated protein kinase; SOS-1, son of sevenless homolog 1; MMP-2, matrix metalloproteinase-2; RhoA, Ras homolog gene family, member A; uPA, urokinase-type plasminogen activator; TIMP1, tissue inhibitor of matrix metalloprotease 1; MCL-1, myeloid cell leukemia-1; HR, homologous recombination; γ-H2AX, phosphorylated histone H2AX; MM, multiple myeloma; BMSC, bone marrow stromal cells; IL-6, Interleukin-6; LC3B, light chain 3B; Atg, autophagy-related gene; Ptch1, patched homolog 1; Gli1, GLI family zinc finger 1; Gli3, GLI family zinc finger 3; GSH, glutathione; LC-MS, liquid chromatography-mass spectrometry; MALDI-MSI, matrix-assisted laser desorption ionization-mass spectrometry imaging; PC, phosphatidylcholines; PG, phosphatidylglycerol; PS, phosphatidylserine; PE, phosphatidylethanolamine; HIFU, high-intensity focused ultrasound; HIF-1a, hypoxia-inducible factor-1alpha; SDF-1, stromal cell-derived factor 1; CXCR4, C-X-C chemokine receptor type 4; MiR-497, MicroRNA-497; TRAIL, TNF-related apoptosis-inducing ligand; DR5, death receptor 5; cFLIP, cellular Fas-associated death domain-like interleukin-1β-converting enzyme inhibitory protein; XIAP, X-linked inhibitor of apoptosis protein; PTT, photothermal therapy; CA, Cholic acid; TPGS, Tocopherol polyethylene glycol 1000 succinate; NTCP, Na^+-taurocholate co-transporting polypeptide; GOQDs, graphene oxide quantum dots; MMP-9, matrix metalloproteinase 9; COX-2, chemotherapy-activated cyclooxygenase-2; VES-CSO, vitamin-E-succinate grafted chitosan oligosaccharide; EPR, enhanced permeability and retention; FGFR, fibroblast growth factor receptor; PDGFR, platelet-derived growth factor receptor; α-SMA, alpha-smooth muscle actin; AUC, area under the curve; MRT, mean residence time; STAT3, signal transducer and

activator of transcription 3; PD-L1, programmed death ligand-1; FA, folic acid; $mSiO_2$, monodisperse mesoporous silica nanoparticle; CCA, cholangiocarcinoma; INF-γ, interferon-gamma; NR, nano-realgar; TSE, Toad Skin extracts; BJO, *Brucea javanica* oil; mTHPC, meta-tetrahydroxyphenylchlorin; Ce6, Chlorin e6; RBC, red blood cell; HA, hyaluronic acid; HSP70, heat shock protein 70; PDA, polydopamine; NIR, near-infrared; BPNSs, black phosphorus nanosheets; HM, hybrid cell membrane; ICG, indocyanine green; HSA, human serum albumin; TMC, trimethyl chitosan; cTn-I, cardiac troponin I; t-AML, therapy-related acute myeloid leukaemia; AML-MRC, acute myeloid leukaemia with myelodysplastic-related changes.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

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