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Current status and future prospects of nanomedicine for arsenic trioxide delivery to solid tumors

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Abstract

Despite having a rich history as a poison, arsenic and its compounds have also gained a great reputation as promising anticancer drugs. As a pioneer, arsenic trioxide has been approved for the treatment of acute promyelocytic leukemia. Many in vitro studies suggested that arsenic trioxide could also be used in the treatment of solid tumors. However, the transition from *bench to bedside* turned out to be challenging, especially in terms of the drug bioavailability and concentration reaching tumor tissues. To address these issues, nanomedicine tools have been proposed. As nanocarriers of arsenic trioxide, various materials have been examined including liposomes, polymer, and inorganic nanoparticles, and many other materials. This review gives an overview of the existing strategies of delivery of arsenic trioxide in cancer treatment with a focus on the drug encapsulation approaches and medicinal impact in the treatment of solid tumors. It focuses on the progress in the last years and gives an outlook and suggestions for further improvements including theragnostic approaches and targeted delivery.

KEYWORDS

arsenic trioxide, cancer therapy, drug delivery, nanoparticles, theragnostics

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1 | INTRODUCTION

"The King of Poisons"[1] or a "magic bullet"[2]—the attributes researchers have given arsenic trioxide (ATO) in the course of its use in medical treatment reflect its paradoxical properties. Indeed, ATO has been described as healing and killing, as cancerous and anticarcinogenic, as an environmental toxin, and a novel chemotherapeutic. In the following, we want to shed some light on the double-edged sword ATO represents and on the most recent scientific approaches to use nanoparticles as an attempt to make the remedy outweigh the poison.

1.1 | From panacea to first line therapy of APL

In traditional Chinese medicine, ATO has been used for hundreds of years as a remedy for various diseases such as fever, infectious, or rheumatic disease.[2,3] In western medicine, Fowler solution (arsenite potassium) was used between the 18th and mid-20th century to treat a broad range of diseases such as asthma, cholera, syphilis, and hematological diseases like anemia, leukemia, and Hodgkin's disease.[1–3] As early as 1878, Cutler and Bradford described the effect of Fowler solution on white blood cells. In their case series, two healthy individuals and one patient with leukemia responded to arsenic treatment with a decrease in leukocytes, which was dramatic in the latter.[4] The emergence of roentgen therapy for leukemia at the beginning of the 20th century eviscerated the use of arsenite potassium until the case series of Forkner and Stephens in the 1930s discussed it as effective palliative therapy for chronic myeloid leukemia (CML).[5,6] The observed toxicity was described as mild and reversible.[6] Even though other authors did not dispute the efficacy of arsenic potassium in the treatment of CML, more cautious voices arose given the observation of sequelae like ceratosis, liver cirrhosis, or polyneuritis after long-term treatment.[7] Eventually, conventional chemotherapy displaced arsenic in the treatment of leukemia.[2] It was not until the late 20th century that ATO regained interest as a therapeutic agent for leukemia when Chinese researchers evaluated it for therapy of acute promyelocytic leukemia (APL). The studies conducted showed exceedingly high remission rates ranging from 72.7% to 90%.[8–10] The American Food and Drug Administration (FDA) acknowledged the efficacy of ATO for APL treatment with approval for relapsed and refractory APL in 2000,[11] followed by the European admission 2 years later.[12] Direct binding of the promyelocytic leukemia protein—retinoic acid receptor alpha (PML-RARα) oncogene leading to degradation was identified as a mean mechanism of action of ATO in PML.[13] Two key multicenter, randomized, controlled Phase III studies showed that the combination therapy of ATO and all-*trans*-retinoic acid (ATRA) was superior to conventional chemotherapy in terms of improved event-free survival and relapse rate, with adverse effects that were controllable and reversible with the suspension of treatment.[14,15] Subsequently, the admission of ATO got extended to newly diagnosed APL[12,16] and today, the combination therapy of ATO-ATRA is state of the art for APL treatment.

1.2 | ATO for treatment of solid tumors and drawbacks of ATO in clinical use

Even though the binding and degradation of PML-RARα is considered the main mechanism of action of ATO in APL, studies have elucidated that ATO sensitivity in APL is not merely mediated by PML-RARα expression.[17] Other mechanisms for ATO in APL such as generation of reactive oxidative species (ROS),[17] activation of C-Jun N-terminal kinase,[18] and induction of apoptosis associated with the downregulation of bcl-2 protein[9] have also been described. These observations, combined with the clinical success of ATO in APL treatment, have led to an advancing interest to evaluate the effects of ATO in, and to make it utilizable for, other malignancies as well. Indeed, the preclinical studies of ATO in other hematologic and solid tumor entities are promising as ATO turned out to induce apoptosis and inhibit proliferation in several malignant hematologic and solid tumor entities. Interestingly, a recent study by Chen et al.[19] identified ATO as capable of rescuing structural p53 mutants in cell lines derived

from hematological as well as solid malignancies, highlighting the broader potential of ATO as an anti-tumorigenic drug. Other mechanisms identified include, but are not limited to, induction of caspase-3,[20] generation of ROS,[21] alteration of the cell cycle,[22,23] reduction of stem cell markers,[24] and inhibition of glioma-associated oncogene family zinc finger (GLI) transcription factors.[25–27]

The first clinical Phase II studies evaluating the efficacy of ATO in solid tumors were initiated at the end of the last millennium.[28] Over the past 20 years, patients with different tumor entities such as hepatocellular carcinoma, glioblastoma, or lung cancer were treated with different therapy regimes of ATO alone or in combination therapy. A number of ongoing clinical trials evaluating ATO in solid tumors are registered at https://www.clinicaltrials.gov/ (i.e., in neuroblastoma—ClinicalTrials.gov number NCT03503864 and malignant glioma—ClinicalTrials.gov number NCT00275067). The results of those studies are still pending.

While some patients in the Phase II studies conducted showed stable disease[29,30] or partial remission,[29] no general clinical response could be observed upon ATO treatment[31,32] (see Subbarayan & Ardalan[33] for a review). On the contrary, severe adverse events (≥Grade 3) were described in the majority of clinical trials.[29–32,34–36] Although the adverse events were deemed as moderate and well-tolerated in a number of studies,[29,32,35,36] in other studies, side effects attributable to ATO lead to Grade 5 events[31] and treatment[29] respectively study discontinuation[37] in patients with solid tumors.

Well-known adverse effects of ATO, not only in patients with solid tumors but in APL patients as well, are QTc prolongation,[15,38] dermatological conditions like rashes or hyperkeratosis,[7,29] neurotoxicity,[15] and transaminase elevation.[14,15] What is more, the carcinogenic potential of arsenic compounds has been pointed out (see Martinez et al.[39] for a review) and carcinogenicity of ATO is considered an "important potential risk" by the European Medicines Agency (EMA).[12]

The poor clinical outcome in solid tumors stands in contrast to the antiproliferative, proapoptotic effect of ATO in many solid cancers in preclinical in vitro and in vivo models. For this circumstance, different explanations are conceivable. First, as APL is a hematologic malignancy, intravenously administered ATO is located where it needs to act: in the blood. It does not need to accumulate at a specific tumor site nor pass the blood–brain barrier (BBB), as it does when acting on a solid (brain) tumor. Therefore, insufficient concentrations of ATO reaching the tumor site are considered the main obstacle in the treatment of solid tumors.[40] Second, a priori or acquired resistance towards ATO has been described in APL patients[41–43] and seen in solid tumor cell lines[44,45] and is likewise imaginable in solid tumors in the clinic.

1.3 | Nanoparticles as an approach to overcome the shortcomings of ATO in solid tumors

In recent years, the advent of nanoparticles as novel drug delivery systems (DDSs) has offered new possibilities for improved delivery of chemotherapeutic drugs, for example, by increasing their bioavailability, decreasing their effects on healthy tissue, or enhancing their uptake by tumor cells (see Sun et al.[46] for a review). Utilizing DDSs for ATO delivery has been proposed as an efficient tool to eliminate some of the drawback of ATO use in therapy, such as (i) rapid clearance of ATO and its products from the blood,[47] and (ii) low specificity. Due to rapid clearance, a therapeutic dose of ATO is not reaching the tumor sites and a simple dosage increase of ATO is not feasible due to its systemic toxicity. However, utilizing DDSs offers an attractive approach to foster the antitumor effects of ATO and to possibly overcome the limitation of the insufficient enrichment at the tumor side while reducing its adverse effects. Moreover, nanotechnology offers the possibility of tailoring the DDDs to target different types of solid cancers specifically, for instance, by attaching specific targeting ligands to the carrier surface.

Different strategies of ATO delivery to solid tumor entities have been examined over the past several years. They differ regarding the encapsulation strategies, the kind of carrier material used and the type of tumor the

ATO-formulation aim to target. In this review, we focus on the newest development over the last few years and highlight some of the older studies, which were reviewed previously.[48]

2 | STRATEGIES OF ATO ENCAPSULATION

ATO (As_2O_3) is an amphoteric oxide (i.e., a compound able to react both as a base and as an acid) and its aqueous solutions are weakly acidic (H_3AsO_3). ATO dissolves readily in alkaline solutions and forms arsenites with the following pKa values: $H_2AsO_3^-$ (pK_{A1} = 9.22), $HAsO_3^{2-}$ (pK_{A2} = 12.10), and AsO_3^{3-} (pK_{A3} = 13.40).[49] To encapsulate ATO into a drug vehicle effectively, different strategies have been reported. These include: (i) reaction of ATO with transitions metals [M; such as Ni(II), Co(II), Zn(II), Mn(II), and Pt(II)] to form insoluble $MAsO_x$ complexes, (ii) binding ATO to thiol or amino functional groups, and (iii) anionic exchange. The different strategies utilized for different DDSs are summarized in Tables 1 and 2 and discussed in detail in the following chapter.

3 | MATERIALS USED AS DDSs OF ATO

DDSs can be classified based on the type of material which forms the nanocarrier as organic, inorganic and hybrid. Each of these groups has its advantages and disadvantages. For instance, liposomes often feature a low drug loading capacity and instability during storage. Meanwhile, mesoporous silica nanoparticles (MSNs) can load the drug efficiently due to the porous structure and extremely high specific surface area, but often exhibit a high burst drug release during systemic circulation. The pros and cons of each material class are shown and discussed in the text below.

3.1 | Organic

DDSs of ATO based on organic materials are summarized in Table 1 and include liposomes, proteins, dendrimers, and polymer nanoparticles.

3.1.1 | Liposomes

One of the first DDSs investigated for ATO delivery were liposomes.[79–81] Liposomes are spherical vesicles, which compose an internal aqueous cavity surrounded by a bilayer usually made of phospholipids. The hydrophobic lipid membrane can encapsulate strongly lipophilic drugs, whereas the hydrophilic interior can entrap water-soluble molecules such as ATO. However, there are two main drawbacks when using liposomes for ATO delivery—low carrier stability and diffusivity of ATO through the membrane, both resulting in an immature drug release. To address the issues, two main strategies have been proposed—utilizing liposomes based on arsenic contacting lipids (=arsonolipids)[80,81] and ATO loading in presence of transition metal ions.[82,83]

When ATO is loaded into liposomes, the neutral $As(OH)_3$ molecules (which are predominant at pH< 9.0) diffuse readily across the phospholipid membrane making the drug entrapment difficult. To overcome this issue, an encapsulation employing transmembrane gradients of transition metal ions (e.g., Ni(II), Co(II), Zn(II), and Pt(II)) to produce insoluble complexes with As(III) was proposed in 2006[82] and extensively studied in the later years. As described by Chen et al.,[82] to form the DDS, a liposome is first loaded with a salt of the transition metal (e.g., acetate), then ATO is added. During the cycle of loading ATO into a liposome, the neutral $As(OH)_3$ diffuses across the lipid membrane and reacts with the metal(II) ions to form insoluble metal(II) arsenite complexes inside the

TABLE 1 Overview of DDSs for ATO based on organic materials reported since 2014 and discussed in this review

Delivery vehicle	Method of ATO encapsulation	NP-size (analytical method)[a]	ATO loading (analytical method)[b]	Other characteristics and features	Type of solid cancer	In vivo studies	Reference
Liposome	$NiAsO_x$ precipitation	121.0 ± 2.4 nm (DLS)	0.78 ± 0.23 mM of As(ICP-OES)	Four weeks stability tests	Cervical cancer	–	Wang et al.[50]
Liposome	$NiAsO_x$ precipitation	100 - 400 nm (DLS)	24.2% (ICP-OES)	Systematic study of liposomes of different sizes and surface charge	Cervical cancer	–	Akhtar et al.[51]
Liposome	$MnAsO_x$ precipitation	92.4 ± 2.6 nm (DLS)	As: lipid = 0.73 ± 0.03 (AAS)	MRI detection of Mn(II); phosphatidylserine antibody as a targeting ligand	Glioma	–	Zhang et al.[52]
Polylactic acid (PLA)	Co-encapsulation with Fe_3O_4 NPs	213.6 nm (DLS)	13.9 ± 1.33% (AFS)	Combined with magnetic NPs	Hepatocellular carcinoma	–	Song et al.[53]
Poly(lactide-co-glycolide) (PLGA)	ATO & PLGA	233.6 nm (TEM)	2.87% (ICP-OES)	Cloaked by red blood cell membrane	–	–	Su et al.[54]
Poly(lactide-co-glycolide) (PLGA)	ATO & PLGA	127.5 ± 0.9 nm (DLS)	5.8% (ICP)	–	Hepatocellular carcinoma	✓	Hu et al.[55]
Poly(lactide-co-glycolide) (PLGA)	ATO & PLGA	249.1 ± 9.1 nm (DLS)	7.2 ± 0.2% (AFS)	Coated with polyethylene glycol and/or lactobionic acid	Hepatocellular carcinoma	✓	Song et al.[56]
Poly(lactide-co-glycolide) (PLGA)	ATO & PLGA	187.2 ± 10.6 nm (DLS)	7.2 ± 1.24% (AFS)	Polyethylene glycol and lactobionic acid modified chitosan	Hepatocellular carcinoma	✓	Song et al.[57]
Sodium alginate	ATO & sodium alginate (ion crosslinking)	163.2 ± 4.4 nm (DLS)	4.98% (ICP-OES)	Cloaked by red blood cell membrane	Hepatocellular carcinoma	✓	Lian et al.[58]
Polyamidoamine (PAMAM)	ATO & PAMAM	21.60 ± 6.81 nm (DLS)	2.82% (ICP)	pH-responsive; Arg-Gly-Asp as a targeting ligand	Glioma	✓	Lu et al.[59]

Abbreviations: ATO, arsenic trioxide; DDSs, drug delivery systems.

[a]Dynamic light scattering (DLS) and transmission electron microscopy (TEM).

[b]Inductively coupled plasma (ICP)–mass spectrometry/optical emission spectrometry (MS/OES); atomic absorption spectrometry (AAS); and atomic fluorescence spectrometry (AFS).

TABLE 2 Overview of DDSs for ATO based on inorganic and hybrid materials reported since 2014 and discussed in this review

Delivery vehicle	Method of ATO encapsulation	NP-size (analytical method)[a]	ATO loading (analytical method)[b]	Other characteristics and features	Type of solid cancer	In vivo studies	Reference
$GdAsO_x$	Gd&As co-precipitation in presence of dextran	400 ± 50 nm (TEM)	Gd/As = 1.77 (ICP-MS)	Phosphate-triggered ATO release	Hepatocellular carcinoma	✓	Chen et al.[60]
$GdAsO_x$	Gd&As co-precipitation	Rods: 27 ± 5 nm x 126 ± 36 nm (TEM)	Gd:As = 1:1.05 (ICP-OES)	Phosphate-triggered ATO release	Hepatocellular carcinoma	✓	Fu et al.[61]
$GdAsO_x$	Gd&As co-precipitation in presence of dextran	0.9–1.0 μm (DLS)	12.35 ± 0.84% (ICP-MS)	Phosphate-triggered ATO release	Hepatocellular carcinoma	✓	Zhao et al.[62]
Mesoporous silica	Thiol functional groups	100 ± 30 nm (TEM)	0.61 mmol/g (ICP-OES)	Cyclic peptide Arg-Gly-Asp-D-Phe-Lys as a targeting ligand	Breast cancer	✓	Wu et al.[63]
Mesoporous silica	Thiol functional groups	65 ± 5 nm (TEM)	20 mg/g	Carriers of radioisotopes of $[^*As]_2O_3$ (* = 72, 76, 74, 71) for PET image-guided drug delivery	–	✓	Ellison et al.[64]
Mesoporous silica	Amino functional groups	158.6 ± 1.3 nm (DLS)	11.42 ± 1.75% (ICP)	Coating with polyacrylic acid for pH-trigged ATO release	Hepatocellular carcinoma	✓	Xiao et al.[65]
Mesoporous silica	Amino functional groups	141.6 ± 3.6 nm (DLS)	8.19 ± 0.51% (ICP)	Coating with polyacrylic acid for pH-trigged ATO release; angiopep-2 as a targeting ligand	Glioma	✓	Tao et al.[66]

(Continues)

TABLE 2 (Continued)

Delivery vehicle	Method of ATO encapsulation	NP-size (analytical method)[a]	ATO loading (analytical method)[b]	Other characteristics and features	Type of solid cancer	In vivo studies	Reference
Mesoporous silica	$NiAsO_x$ precipitation	~ 120 nm (TEM)	As/SiO_2 = 1.05(EDX)	Folic acid as a targeting ligand; combined with magnetic nanoparticles for MRI detection	Hepatocellular carcinoma	✓	Chi et al.[67]
Mesoporous silica	$MnAsO_x$ precipitation	25 ± 2 nm (TEM); 30 ± 3 nm (DLS)	Mn:As = 1.5:1 (ICP-MS)	Mn(II) for MRI detection; pH-low insertion peptide (pHLIP) as a targeting ligand	Hepatocellular carcinoma	✓	Zhang et al.[68]
Mesoporous silica	$ZnAsO_x$ precipitation	35 ± 2 nm (TEM); 51 ± 3 nm (DLS)	–	pH-responsive	Hepatocellular carcinoma	✓	Huang et al.[69]
Mesoporous silica	$FeAsO_x$ precipitation	12.0 ± 1.3 nm (DLS)	–	Co-delivery with doxorubicin; pH-responsive	Hepatocellular carcinoma	–	Liu et al.[70]
Hollow silica	$NiAsO_x$ precipitation	30 ± 2 nm (TEM); 50.8 ± 3 nm (DLS)	Ni:As = 1.15:1 (ICP-MS); As/SiO_2 = 0.83 ± 0.06 (AFS)	pH-responsive; EGFR-Affibody as a targeting ligand	Hepatocellular carcinoma	✓	Zhao et al.[71]
Hollow silica	$MnAsO_x$ precipitation	52 nm (DLS)	80 mg/g (ICP-MS)	–	Hepatocellular carcinoma	✓	Chi et al.[72]
Hollow silica	$MnAsO_x$ precipitation	40 ± 2 nm (TEM); 55 ± 3 nm (DLS)	As/SiO_2 = 1.07 ± 0.06 (ICP-MS)	Mn(II) for MRI detection; pH triggered release	Hepatocellular carcinoma	✓	Zhao et al.[73]
Hollow silica-liposome	Amino functional groups	146 nm (DLS)	6.76% (TGA)	Arg-Gly-Asp as a targeting ligand	Hepatocellular carcinoma	✓	Fei et al.[74]

TABLE 2 (Continued)

Delivery vehicle	Method of ATO encapsulation	NP-size (analytical method)[a]	ATO loading (analytical method)[b]	Other characteristics and features	Type of solid cancer	In vivo studies	Reference
MOF (MFU-4*l*)	Anionic ligand exchange ($H_2AsO_3^-$)	109 nm (DLS)	237 mg/g (ICP-OES)	pH-responsive	Atypical teratoid/ rhabdoid tumors	–	Ettlinger et al.[75]
MOF (ZIF-8)	Anionic ligand exchange ($H_2AsO_3^-$)	68 ± 15 nm (TEM)	98 mg/g (ICP-OES)	pH-responsive	Atypical teratoid/ rhabdoid tumors	–	Ettlinger et al.[76]
MOF (ZIF-Fe_3O_4)	Anionic ligand exchange ($H_2AsO_3^-$)	97 ± 8 nm (TEM)	70 mg/g (ICP-OES)	pH-responsive, Fe_3O_4 for MRI detection	Atypical teratoid/ rhabdoid tumors	–	Ettlinger et al.[77]
MOF (Zn-MOF-74)	Ligand exchange (H_3AsO_3)	~ 100 nm (TEM)	153 mg/g (ICP-OES)	pH-responsive	–	–	Schnabel and Ettlinger[78]

Abbreviations: ATO, arsenic trioxide; DDSs, drug delivery systems; MOF, metal-organic frameworks; MRI, magnetic resonance imaging.

[a]Dynamic light scattering (DLS) and transmission electron microscopy (TEM).

[b]Inductively coupled plasma (ICP)–mass spectrometry/optical emission spectrometry (MS/OES); energy dispersive x-ray analysis (EDX); thermogravimetric analysis (TGA).

liposome. During the reaction, protons are released, which react with acetate ions to form acetic acid. Consequently, the weak acid diffuses out of the liposome in exchange for ATO. Both the formation of insoluble metal(II) arsenite complexes and the efflux of acetic acid facilitate the ATO uptake and entrapment in a liposome (Figure 1). For such systems of drug encapsulation in liposomes, the term "nanobin" (NB) was proposed.[82] For instance, it was shown that nanobin encapsulation of ATO (NB(Ni, As)) significantly improved pharmacokinetic properties of the drug and led to greater therapeutic efficacy compared with free ATO in an orthotopic model of triple-negative breast cancer.[84] In a follow-up work, the nanobins (NB(Ni, As)) were coated with a pH-sensitive polymer to enable pH-triggered drug release.[85] Nanobins were also used for co-encapsulation of arsenic and platinum drugs.[83]

Liposomes can be also functionalized with various targeting ligands to enable ATO delivery to specific cells. For instance, ATO-loaded liposomes were functionalized with folate ligands and their cellular uptake and antitumor efficacy were evaluated in folate receptor (FR)–positive human nasopharyngeal epidermal carcinoma (KB) and human cervical carcinoma (HeLa) cells, as well as FR-negative human breast carcinoma (MCF-7) cells.[86] The uptake of folate functionalized ATO-loaded liposomes by KB cells was three to six times higher than that of free ATO or liposomes without the targeting ligands. Zhang et al.[87] reported on nanobins (NB(Ni, As)) functionalized with urokinase plasminogen activator antibodies to promote targeted delivery to epithelial ovarian cancer cells (in which the urokinase system is overexpressed compared to normal cells). The targeted nanobins showed a fourfold higher uptake in ovarian cancer cells in comparison with nontargeted nanobins.

In the last years, delivering ATO using liposomes has also been studied to examine whether liposomal-encapsulated ATO could reduce the drug toxicity and improve the efficacy of ATO in treating human papillomavirus (HPV)-associated cancers. Wang et al.[50] showed that ATO encapsulated into liposomes in presence of Ni(II) ions induced apoptosis and reduced protein levels of HPV-E6 in HeLa cells more effectively than ATO alone. Akhtar et al.[51] altered the properties of liposomes such as size (from 100 to 400 nm) and surface charges and studied their influence on the efficiency of ATO delivery to cervical cancer cells. It was shown that neutral liposomes of 100 nm in size were the best-tested formulation, as they showed the least intrinsic cytotoxicity and the highest loading efficiency.

When Mn(II) ions are used as transitions metal to efficiently encapsulate ATO inside a liposome, drug nanocarrier suitable for magnetic resonance imaging (MRI), and thus theragnostic applications, can be prepared (Figure 1).[52] The formation of the Mn(II) arsenite precipitate in liposomes generates magnetic susceptibility effects, which can be detected as a dark contrast on T_2-weighted MRI. When accepted by cells, due to a low pH in endosome-lysosome, the Mn(II) arsenite complex decomposes, which results in a release of the As-drug and Mn(II) ions (i.e., a T_1 contrast agent that gives a bright signal in MRI). The convertible MRI signals (dark to bright) enable to follow not only the ATO delivery but also its release. Moreover, the liposomes were functionalized with phosphatidylserine (PS)-targeting antibodies to enable a specific binding of the nanodrug to PS-exposed glioma cells.

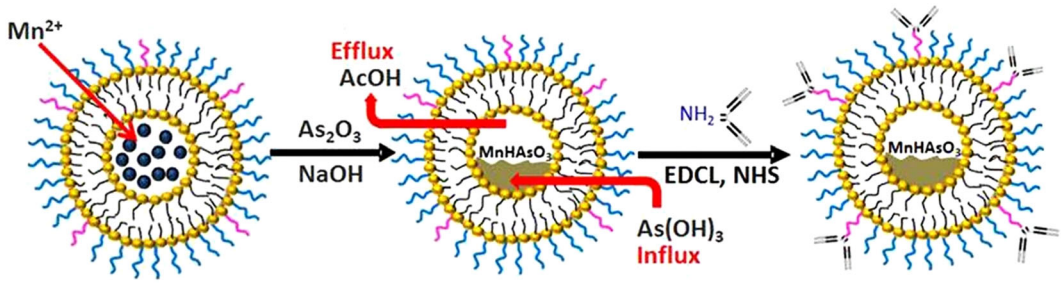

FIGURE 1 Schematic illustration of the preparation of liposomal nanocarrier of arsenite-manganese functionalized with targeting ligands. (Figure adapted from Zhang et al.[52]) [Color figure can be viewed at wileyonlinelibrary.com]

3.1.2 | Proteins

Zhou et al.[88,89] investigated albumin as a DDS for ATO. Albumin microspheres as a DDS for ATO were prepared using a chemical crosslink and solidification method and the synthesis was optimized with regard to the particle size and drug loadings.[88] In another work, ATO-loaded albumin microspheres were functionalized with a trans-activating transcriptional activator peptide (i.e., a cell-penetrating peptide) and the nano drug delivery into bladder cancer cells was evaluated.[89] The results indicated that the attached peptide enhanced intracellular permeation of the nano drug by translocating microspheres across the cell membrane.

3.1.3 | Polymers

Nanoparticles based on several different polymers have been proposed as DDSs for ATO. These polymers include polylactic acid (PLA),[53] poly(lactide-*co*-glycolide) (PLGA),[54–57,90] sodium alginate,[58] polyamidoamine (PAMAM) dendrimer,[59] pluronic F127 polymer,[91] or chitosan.[92]

Polymeric nanoparticles as DDSs of ATO are often prepared by a double emulsion (water-in-oil-in-water = w/o/w) solvent evaporation/extraction method. In the method, a polymer is dissolved in an organic solvent to form the organic phase. Then an aqueous phase containing ATO is emulsified into the organic phase by ultrasonication to form a primary emulsion, which is then added to an aqueous solution of a surfactant. The mixture is then stirred open to the air to evaporate the organic solvent and make the nanoparticle. This method was utilized, for instance, to prepare polylactic acid (Figure 2) or poly(lactide-co-glycolide) nanoparticles discussed in the following text.

Song et al.[53] used the double emulsion (w/o/w) solvent-evaporation method (Figure 2) to prepare ATO-loaded polylactic acid/magnetic nanoparticles. They studied the preparation conditions in detail to optimize the encapsulation efficiency and particle size distribution. It was shown that the ATO-nanoparticles (ATO-NPs) attached easily to hepatocellular carcinoma (HCC) cell line SMMC-7721 and had higher inhibition efficiency on SMMC-7721 cells than free ATO.

Another polymer examined as DDS for ATO was poly(lactide-*co*-glycolide) (PLGA).[90] Su et al.[54] reported on ATO-loaded PLGA nanoparticles cloaked by red blood cell membrane (RBCM) (Figure 3) to reduce their cytotoxicity as they demonstrated on the human embryonic kidney cell line 293T. Song et al.[56] coated PLGA nanoparticles with polyethylene glycol (PEG) and/or lactobionic acid to improve the carrier biocompatibility. In a follow-up study, they used PLGA nanoparticles coated with PEG and lactobionic acid modified chitosan for encapsulation and targeted release of ATO in liver cancer treatment.[57] The ATO-NPs showed only low cytotoxicity against normal human liver cells (LO2 cells) but effectively inhibited SMMC-7721 cells. Similar results were obtained also in in vivo studies, in which the NPs did not show toxic effects on kidney and liver, but could inhibit the growth of liver tumor. Hu et al.[55] also reported on ATO-loaded PLGA nanoparticles for HCC treatment. To evaluate the anticancer effects, HCC cell lines Huh7 and Bel-7402 were used. It was

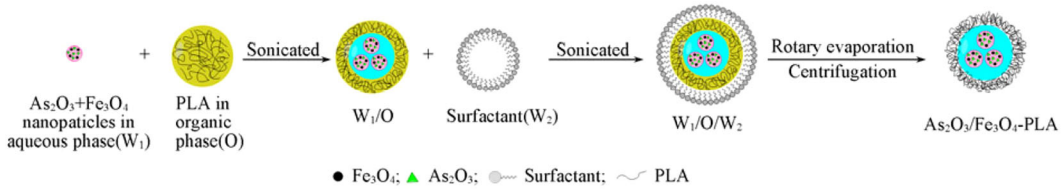

FIGURE 2 Synthesis route to arsenic trioxide (ATO)-loaded polylactic acid (PLA)/magnetic nanoparticles. (Figure reprinted with permission from Song et al.[53]) [Color figure can be viewed at wileyonlinelibrary.com]

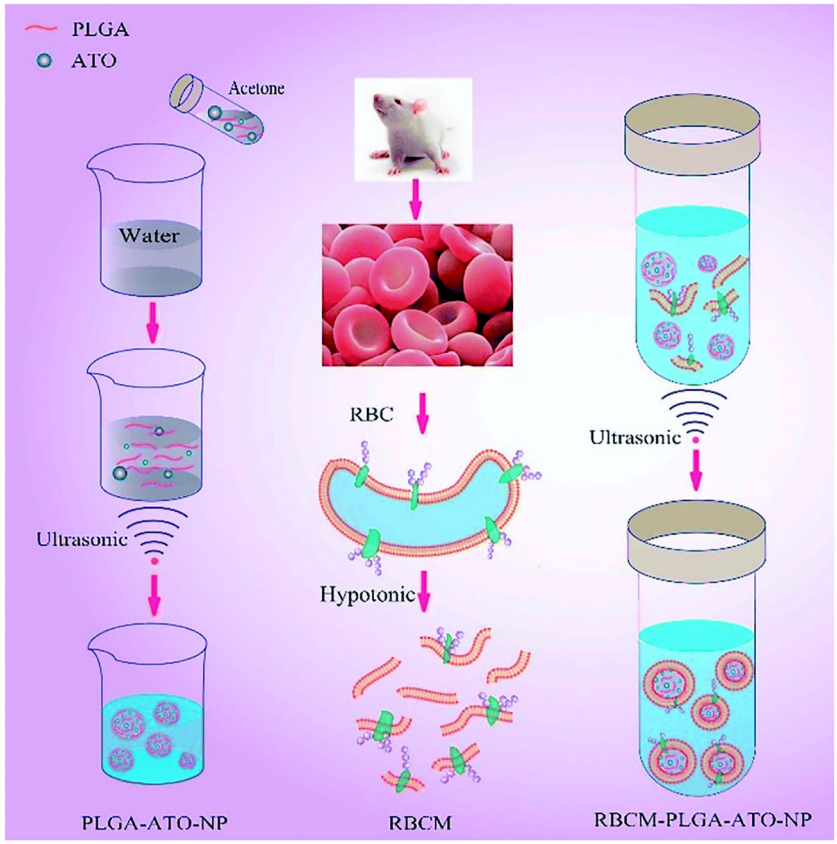

FIGURE 3 Synthesis route to arsenic trioxide (ATO)-loaded poly(lactide-co-glycolide) (PLGA) nanoparticles cloaked by red blood cell membrane (RBCM). (Figure adapted with permission from Su et al.[54]). NP, nanoparticle [Color figure can be viewed at wileyonlinelibrary.com]

reported that the nano drug had a better inhibition and promoted greater lactate dehydrogenase release in comparison to free ATO. In vivo the ATO-NPs induced a significant decrease in the expression of DNA methyltransferases, while the expression of N-terminal-cleaved gasdermin E was upregulated. As a consequence, the nanoparticles inhibited the tumor growth more than free ATO or a control.

Lian et al.[58] reported on sodium alginate nanoparticles (SANs) as a DDS for ATO. ATO-loaded SANs were prepared by the ion crosslinking method and were subsequently camouflaged with RBCM. The ATO-NPs had lower cytotoxicity than ATO on normal 293 (kidney) cells and exhibited antitumor effects on both NB4 (PML) cells and SMMC-7721 (HCC) cells. Moreover, it was shown also in in vivo studies that the ATO-NPs reduced the drug toxicity and improved the antitumor effects in comparison to ATO which caused mild lesions of main organs.

Lu at al.[59] reported on a pH-responsive dendrimer based on polyamidoamine (PAMAM) as a DDS of ATO. The surface of the nanoparticles was functionalized with an αvβ3 integrin targeting ligand to enable targeted delivery to glioma. In in vitro BBB model, the targeting ligand attachment heightened the cytotoxicity of the ATO-loaded nanoparticles, due to an increased uptake by C6 (glioma) cells. In vivo, the tumor volume of C6 glioma-bearing rats was reduced by 61.5 ± 12.3% after intravenous administration of the nano drug, and that was approximately fourfold higher than that of free ATO and twofold higher than that of the nano drug without the targeting ligands.

3.2 | Inorganic nanoparticles

As inorganic carriers, two types of materials were intensively studied—materials based on metal (or metal oxide) nanoparticles and silica nanoparticles. The overview of inorganic DDSs for ATO is given in Table 2.

3.2.1 | $GdAsO_x$ nanoparticles

As metal nanoparticles for ATO delivery, $GdAsO_x$ NPs were proposed. To synthetize such nanodrug, Chen et al.[60] co-precipitated As with Gd in the presence of dextran into $GdAsO_x$ NPs. It was proposed that the unloading of ATO from such nanoparticles could be triggered by endogenous phosphate ions present in the plasma and cytosol. In the release process, the arsenite ions would be exchanged by phosphate ions, and thus ATO release could be achieved. Indeed, the in vitro results showed that the nanoparticles gradually "dissolved" into fragments in a phosphate solution. In follow-up studies, the therapeutic effect of $GdAsO_x$ NPs on aggressive HCC was studied.[61,93] After administration of the ATO-NPs, arsenic accumulation within tumors was evaluated. It was found that the accumulation of the ATO-NPs was as much as 5%, which was ten times more than when only ATO was administrated.[61] Additionally, it was shown in a series of in vitro and in vivo experiments that after the administration of ATO-NPs, the phosphate concentration decreased, and as a consequence, the corresponding pH increased. Thus, exhausting the phosphate ions resulted in neutralizing the tumor acidity. Zhao et al. reported on dextran coated $GdAsO_x$ NPs for chemoembolization therapy of the rabbit VX2 liver tumor.[62] The nanoparticles were studied both in vitro and in vivo, and the results showed that the ATO-NPs caused severe necrosis via chemoembolization combinational therapy.

3.2.2 | Mesoporous silica nanoparticles

Nanoparticles formed by mesoporous silica have been extensively studied as DDSs not only for ATO.[94] MSNs are a class of inorganic porous material, which comprise open mesoporous channels with a diameter of 0.1–10 nm. Furthermore, their outer surface can be modified by attaching various molecules including targeting ligands for tumor specific drug delivery. The high material porosity enables high drug loading. However, due to nonspecific drug–material interactions, a burst drug release is often observed. To decrease the burst release and increase ATO loading, two main strategies were reported (Figure 4A,B). First, enhanced ATO binding via thiol[63,64] or amino functional groups[65,66] anchored on the surface of the mesoporous channels, and second—similar to the strategy for liposomes described above—an encapsulation of ATO in presence of transition metal ions to form insoluble $MAsO_x$ complexes.[67–70] To increase the ATO loading even more, the second approach was applied to hollow MSNs (Figure 4C).[71–73]

Thiol group and amino group functionalized MSNs

Silica nanoparticles functionalized with thiol groups were used to bind ATO to develop nano drug for treating MDA-MB-231 triple-negative breast cancer (TNBC).[63] The inner and outer surfaces of MSNs were functionalized with thiol groups not only for the ATO binding, but also to conjugate targeting agents to the outer surface. As a targeting ligand, cyclic peptide Arg-Gly-Asp-D-Phe-Lys (cRGDfK) was used. Human TNBC cells showed a higher uptake of ATO-MSNs which were functionalized with the targeting ligands compared to nontargeted ATO-MSNs. Moreover, it was shown in vivo in a mouse model of human TNCB, that the ATO-MSNs with targeting ligands effectively inhibited a tumor growth at a low ATO dosage of 0.75 mg/kg. Ellison et al.[64] reported on MSNs decorated with thiol functional groups as carriers of radioisotopes of [*As]arsenic trihydroxide (* = 72, 76, 74, 71). The idea was to combine the chemotherapeutic effects of ATO with positron emission tomography (PET) of [*As] for image-guided drug delivery.

Xiao et al.[65] reported on amino group functionalized MSNs capped with polyacrylic acid (PAA) for pH-trigged ATO release in acidic microenvironment of tumor. The antitumor efficacy of the ATO-NPs was investigated both in

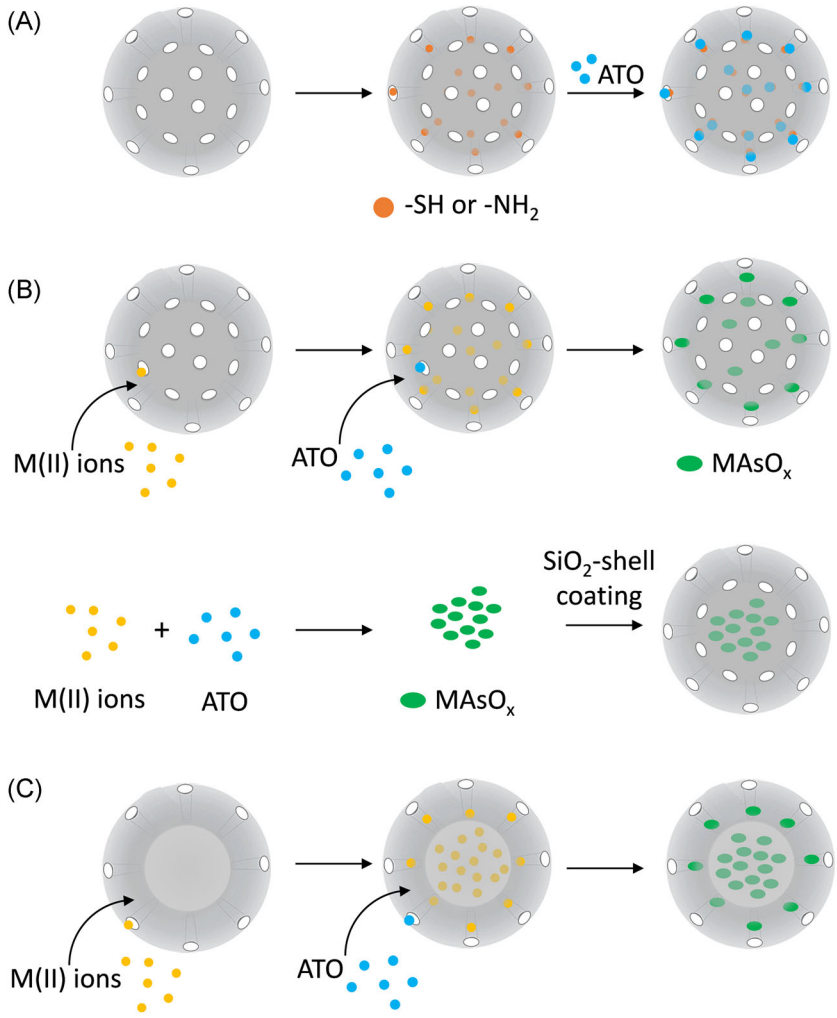

FIGURE 4 Schematic illustration of different synthesis approaches to prepare silica-based nanoparticles for arsenic trioxide (ATO) delivery [Color figure can be viewed at wileyonlinelibrary.com]

vitro (SMMC-7721 cell line) and in vivo (H22 xenografts). Similarly, also Tao at al.[66] proposed MSNs decorated with amino functional groups for pH-triggered ATO delivery. They reported on angiopep-2-conjugated core–shell silica-liposome hybrid nanovesicles for targeted and pH-triggered delivery of ATO to glioma. By performing a blood–glioma microdialysis, the targeting efficiency of the ATO-NPs was evaluated. Moreover, the improved glioma-specific distribution of ATO and its antitumor effects in comparison with free ATO could be shown both in vitro and in vivo.

MSNs with $MAsO_x$ complexes

To prepare silica nanoparticles with $MAsO_x$ complexes, two approaches were reported (Figure 4B)—(i) loading pre-synthesized MSNs with a transition metal salt and subsequently with ATO,[67] and (ii) pre-synthesizing $MAsO_x$ nanoparticles and coating them subsequently with a shell of mesoporous silica.[68–70] The advantage of the first approach is that the MSNs can be combined with other nanoparticles before ATO is loaded. For instance, MSNs were combined with magnetic iron oxide nanoparticles to enable not only ATO delivery but also real-time

monitoring via MRI, and thus a theragnostic function.[67] As a drug, ATO prodrug ($NiAsO_x$) was loaded into the mesopores. The surface of the nanoparticles was functionalized with folic acid as a targeting ligand to enhance the drug efficacy in treatment of HCC. The superior antitumor activity of the magnetic ATO-NPs in comparison to free ATO was confirmed by in vitro experiments with SMMC-7721 cells as well as by in vivo experiments with mice bearing H22 tumors. Moreover, the imaging ability of the magnetic nanodrug for real-time tumor monitoring by MRI was shown.

Another theragnostic agent combining ATO delivery and MRI was reported by Zhang et al.,[68] who developed $MnAsO_x$@SiO_2 core-shell nanoparticles. In the synthesis, first manganese arsenite complexes were prepared by a co-precipitation of manganese acetate and aqueous ATO. Then tetraethyl orthosilicate was added to coat the $MnAsO_x$ nanocomplexes with a silica shell. In a subsequent step, the nanoparticles were decorated with a pH-low insertion peptide (pHLIP), which was added to target an acidic tumor microenvironment. The targeting ability was confirmed in in vivo experiments with BALB/c mice, which further revealed that pHLIP could also considerably prolong the circulation time of the nano drug. Moreover, the released Mn(II) ions brighten the T_1 signal in MRI, which could be used for real-time monitoring of the chemotherapy treatment.

Huang et al.[69] reported on $ZnAsO_x$@SiO_2 NPs. In the synthesis, firstly $ZnAsO_x$ complexes were prepared, which were subsequently encapsulated in a SiO_2 matrix. The antitumor activity of the ATO-NPs was investigated in vitro with HCC cell lines (MHCC97L and Hep3b). In comparison to free ATO, the ATO-NPs promoted apoptosis and significantly inhibited proliferation, migration, and invasion of both tested cell lines. In in vivo experiments, the ATO-loaded NPs inhibited tumor growth by 2.2-fold and metastasis by 3.5-fold more than free ATO. Liu et al.[70] reported on a dual-drug loading, including ATO and doxorubicin (DOX), into MSNs and demonstrated a drug synergy and pH-triggered drug release for effective treatment of DOX resistant HCC cells. First, $FeAsO_x$ nanoparticles were prepared, followed by in situ coating with an amine-functionalized silica shell. Subsequently, DOX molecules were anchored on the nanoparticles by pH-sensitive imine bonds. It was shown that such NPs could enhance drug accumulation and cytotoxicity in DOX-resistant HuH-7/ADM cells.

Hollow silica nanoparticles

To increase the ATO loading capacity of MSNs even more, hollow silica nanoparticles (HSNs) were investigated as DDSs.[71–74] Zhao et al.[71] prepared HSNs via selective etching of Fe_3O_4@SiO_2-NH_2 with HCl. To introduce the drug, first the HSNs were treated with an aqueous nickel acetate solution, and then an aqueous ATO solution was used to prepare water-insoluble nickel arsenite complexes (=ATO prodrug) encapsulated inside the nanocarrier. To enable targeted delivery, epidermal growth factor receptor (EGFR)-Affibody molecules were attached to the carrier surface. The anticancer activity of the ATO-NPs was studied on four different cell lines (HeLa, RAW 264.7, HepG2, and SMMC-7721). Moreover, in vivo therapeutic study in mice bearing mouse hepatoma H22 tumors were carried out. Notably, the ATO-NPs were more effective in inhibiting tumor growth in comparison to free ATO. The authors discussed that this observation might be attributed to the possible ability of nanoparticle formulation to reduce renal clearance of ATO and an improved drug accumulation at the tumor site by the enhanced permeability and retention (EPR) effect.[95]

In another work, HSNs loaded with ATO in the presence of Mn(II) ions were examined in the treatment of HCC.[72] It was shown that the ATO-NPs could decrease the invasion of HCC cells not only in vitro but also in vivo without adverse side effects (determined by pathology tests of the main organ tissues). Zhao et al. demonstrated that HSNs containing manganese arsenite complexes could be used as a pH-sensitive multifunctional DDS capable of real-time monitoring of ATO release by activatable T_1 imaging in MRI (Figure 5). It was shown that in acidic environment, the simultaneous release not only of ATO but also of manganese ions was triggered. Subsequently, the released manganese ions increased the T_1 signal (bright signal) in MRI and thus, real-time visualization and monitoring of ATO release and delivery could be achieved. To functionalize the nanocarrier surface, glutathione (GSH) was used. The anticancer activity of the nano drug was studied both in vitro (HeLa, HepG2, SMMC-7721, and H22 cells) and in vivo (BALB/c mice and nude mice bearing human HCC tumors).

Fei et al.[74] prepared hybrid core-shell nanoparticles by coating HSNs (functionalized with amino groups) with a liposomal shell for controlled ATO release. The surface of the nanoparticles was functionalized with Arg-Gly-Asp (RGD)-ligands to enable targeted delivery. In vitro, the ATO-NPs showed good biocompatibility and low toxicity on HepG2, MCF-7, and LO2 cells. Moreover, due to the attached ligand, enhanced cellular uptake and a reduced half-maximal inhibitory concentration (IC_{50} value) of the nano drug could be detected. In addition, the targeting efficiency of the ligand functionalized ATO-NPs was also confirmed in an H22 tumor-xenograft mouse model.

3.3 | Hybrid

Hybrid materials consist of at least two constituents at the nanometer or molecular level. Commonly one of these components is inorganic and the other one organic in nature. Many of the materials discussed in the two previous chapters (organic and inorganic materials) and summarized in Tables 1 and 2, could be considered as hybrid or composite materials by the composition. For instance, inorganic nanoparticles coated with an organic polymer (to improve the nanocarrier's biocompatibility) or organic targeting ligands (to enable targeted delivery), or inorganic and organic particles combined with magnetic nanoparticles (to enable detection via MRI). However, these DDSs were already included in the previous chapters based on the type of the carrier material (either organic or inorganic) which was used for the ATO encapsulation. From this point of view, as a typical hybrid material, only metal-organic frameworks (MOFs) are considered in this section.

3.3.1 | Metal-organic frameworks

MOFs are porous crystalline coordination polymers. They comprise inorganic metal ions (or clusters) and organic ligands.[96] They exhibit outstanding properties including high internal surface area and chemical versatility. They have been suggested as promising materials for many different applications including gas storage, catalysis, and sensing,[97] but also drug delivery.[98] The most prevalent method described in published reports to capture drug molecules in MOFs is via noncovalent interactions.[98] In such cases, upon administration, the drug can easily diffuse from the material, and thus no control over the release is achieved. However, when administrating toxic drugs such as ATO, having a control of the drug release is crucial. Therefore, for ATO delivery, MOFs having possibilities to form a strong interaction with ATO, such as a chemical bond, were proposed and are summarized in Table 2.

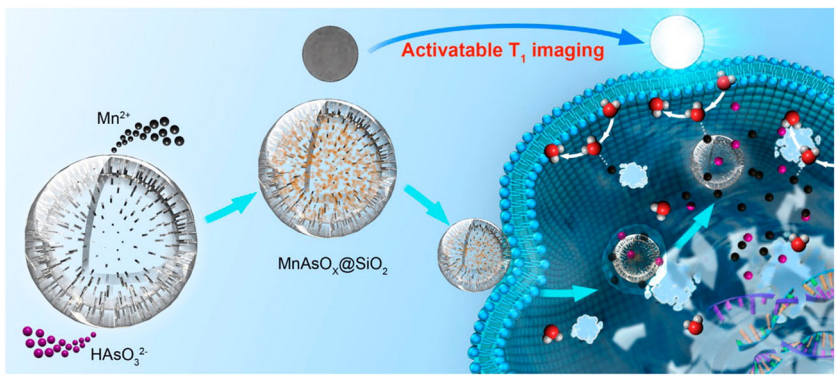

FIGURE 5 Schematic illustration of HSNs loaded with ATO and its drug release with activatable T1 imaging process inside cells enabled by released Mn(II)ions. (Figure reprinted with permission from Zhao et al.[73])
[Color figure can be viewed at wileyonlinelibrary.com]

The first MOF reported for ATO delivery was MFU-4*l* (MFU stands for Metal-Organic Framework Ulm University).[75] MFU-4*l* consists of Zn(II) ions and bis(1H-1,2,3-triazolo[4,5-b],[4′,5′-i])dibenzo[1,4]dioxin (H_2-BTDD) as a ligand. In the framework structure, $[Zn_5Cl_4(BTDD)_3]$, there are negatively charged chloride side ligands, which were shown to be postsynthetically exchangeable to arsenite ions. Cytotoxicity studies conducted on atypical teratoid/rhabdoid (ATRT) cell lines revealed that the ATO-NPs had similar effects on cancer cell lines as free ATO).[75] In another work, Ettlinger et al.[76] showed that a MOF called ZIF-8 (ZIF stands for zeolitic imidazole framework) could be an even more promising candidate for ATO delivery. Not only that it enables a high drug loading capacity (due to its high porosity), but additionally, due to pH-sensitive Zn–N coordinate bonds, a pH-triggered drug release can be achieved. Moreover, in in vitro cytotoxicity studies the ATO-ZIF-8 nanoparticles had a substantial cytotoxic effect on tested ATRT cell lines at low concentrations and the cytotoxic effect was similar to free ATO. In a related work reported by Ettlinger et al.,[77] ZIF-8 nanoparticles were combined with superparamagnetic iron oxide (Fe_3O_4) nanoparticles, which are common contrast agents used in MRI. A shell of ZIF-8 was grown around nanosized Fe_3O_4 clusters to prepare a core-shell structure, in which the core would be responsible for imaging via MRI, whilst the shell could function as DDS for treatment of ATRT. Both the imaging and therapeutic activity were demonstrated in vitro.

Another reported MOF for ATO delivery, which also displayed a prominent pH-triggered behavior, was Zn-MOF-74.[78] Zn-MOF-74 consists of Zn(II) ions and 2,5-dihydroxybenzene-1,4-dicarboxylate ligands and when desolvated, it contains a high density of vacant metal sites readily accessible for guest binding. It was shown that ATO could be successfully attached to these sites, and thus a high drug loading could be achieved. Moreover, it has been shown that the drug release, tested in a phosphate buffered saline, could be triggered by a pH change from 7.4 to 6.0. However, no additional biological studies have been reported.

4 | BENEFITS OF UTILIZING NANOPARTICLES FOR ATO DELIVERY

In addition to the chemical properties and encapsulation strategies of DDSs for ATO, the benefits which the ATO-formulations offer are of great interest too. The most recent studies dealing with ATO-NPs can be assigned to five categories regarding the benefit(s) which the nanoparticle formulation(s) is/are supposed to yield:

- Improvement of pharmacokinetics,
- Targeted delivery via surface modification,
- Theragnostic properties,
- Enhancement of Transarterial Chemoembolization (TACE), and
- Enhancement of BBB crossing.

4.1 | Improvement of pharmacokinetics

Since improvement of pharmacokinetics—such as controlled release or prolonged blood circulation half-life—is such a crucial point when it comes to nanomedicine, almost all studies evaluated dealt with this subject in one way or another.

4.1.1 | Controlled release of ATO

The most favored approach to achieve controlled release of ATO was to ensure pH-triggered release from the respective nanoparticle. Since acidic pH is a well-known characteristic of tumor tissue,[99] making ATO release

pH-dependently, with higher ATO release at lower pH value, ought to provide a kind of tumor-directed ATO delivery while sparing healthy tissue. pH-dependent release was achieved mainly through pH-labile bond respectively attachment between ATO and the nanoparticle.[52,59,69–73,75,76,78] Other researchers grafted pH-responsive material upon the surface of their nanoparticles to accomplish pH-dependent ATO release.[65,66,68] The degree of pH-selective release differed not only depending on the type of nanoparticle used, but on the exact composition of the respective nanoparticle.

Inorganic phosphate (Pi-)triggered ATO release was another way of obtaining controllable release. All four studies following this approach[60–62,93] used gadolinium-based nanoparticles, in which the arsenic could be exchanged by phosphate ions. Chen et al.[60] reported an outstanding ON/OFF specificity for their $GdAsO_x$ nanoparticles, with no arsenic release in the absence of Pi in vitro. Fu et al.[61] and Zhao et al.[62] attempted to introduce Pi-triggered ATO drug-eluting beats (DEBs) for the improvement of TACE therapy (see below) for HCC. As occlusion of the hepatic artery is a key characteristic of TACE, and intracellular Pi supply is limited upon occlusion, the Pi deprivation slowed down the drug release, avoiding high plasma peak levels of arsenic within the first hours of treatment compared to ATO alone.[62,93] Of note is that none of the studies testing for disturbance of Pi levels in plasma observed lasting changes of the very same.[60,93]

4.1.2 | Prolonged blood circulation and sustained release of ATO

In comparison to controlled release that is mediated by a defined trigger, sustained release of ATO eventually aims to prolong the circulation of ATO, allowing sufficient ATO concentrations to reach the tumor site before being metabolized and excreted. Controlled release can also lead to or be accompanied by sustained release. Zhao et al.[73] coated their pH-sensitive, ATO-containing HSNs with GSH and observed a higher retention time in blood, which they attributed to reduced interactions between the GSH-coated nanoparticles and serum proteins. Zhang et al.[68] observed that modifying their nanoparticles with pHLIP not only lead to pH-dependent release of ATO but also prolonged nanoparticle blood circulation in mice. Similar observations were made by Tao et al.[66] as well as by Xiao et al.,[65] that both grafted their nanoparticles with the pH-responsive PAA. The in vivo half-life of those PAA-coated nanoparticles was significantly prolonged compared with free ATO.[65,66]

Independent from pH-dependency, Lian et al.[58] achieved sustained release in vitro by camouflaging their ATO-loaded SANs with RBCM. As RBCM coating reduced the macrophage uptake in vitro and showed higher antitumor effect in vivo, the authors hypothesized that RBCM-SANs could escape the clearance by the immune system, enabling more ATO to reach the tumor site.[58] Two authors used RGD-conjugated nanoparticles as a targeted delivery system (see below) and observed sustained release, namely an enhanced half-time of ATO in vivo compared to uncoated nanoparticles and free ATO.[59,74]

Coating with PEG can reduce the uptake of nanoparticles by the reticuloendothelial system, nanoparticle accumulation in the liver and thereby increase the circulation lifetime.[100,101] Several authors verified sustained ATO release for PEGylated ATO-loaded nanoparticles in vitro as well as in vivo.[55,59,75,76]

4.1.3 | Enhanced ATO uptake by tumor cells

A manner of achieving favorable drug distribution in vivo is to obtain enhanced uptake of a drug by tumor cells. This could be achieved by modifying the surface of nanoparticles with specific targeting ligands[56,59,63,66,74] (see below). By contrast, Chen et al.[60] achieved enhanced arsenic accumulation in the tumor via a different mechanism. Their Pi-triggered nanoparticles showed a 10-fold accumulation of arsenic in the tumor tissue compared to free ATO, which they ascribed to the EPR effect of nanoparticles.[60] The EPR effect was also considered a reason for enhanced uptake of ATO-NPs in the tumor tissue observed by Tao et al.[66] and Huang et al.[69] Another nanoparticle system by

Chi et al.[72] lead to almost doubled arsenic uptake compared with free ATO into HCC cells, which the authors speculated might have been due to the rampant metabolism of tumor cells or easier internalization of nanoparticles via endocytosis.[72] Endocytosis was also identified as the most probable mechanism for enhanced uptake of arsenic from ATO-NPs compared with free ATO by Hu et al.[55]; likewise, they observed a doubling of arsenic concentration. A similar increase of arsenic accumulation could be observed by Fu et al.,[93] in whose study the arsenic level in rabbit VX2 tumors (a model for human HCC) was almost three times higher under treatment with ATO-NPs compared to free ATO. Long-term accumulation was described in a study by Zhao et al.,[62] who showed that with their nanoparticles used in the TACE procedure, intratumoral arsenic could be detected as long as seven days after the TACE procedure. Free ATO in turn was close to zero after the same time.[62] The enhanced uptake of arsenic into HCC cells in the study of Zhang et al.[68] showed pH-dependency, wherefore the authors ascribed the accumulation in tumor cells to the pH-triggered release properties of their nanoparticles.

4.2 | Targeted delivery via surface modification

A huge advantage of nanoparticles consists in their modifiable surface. In the past few years, several studies have shown, for instance, that chemical modification not only enabled nanoparticles to increase BBB penetration (see below), but also tuned the toxicity of nanoparticles as drug delivery vehicles.[59] Apart from general diversification of nanoparticle characteristics, surface modification of nanoparticles holds great potential in terms of targeted therapy. Attaching targeting ligands directed towards specific structures on tumor cells or the tumor microenvironment could possibly lead to an enhanced antitumor effect while sparing healthy tissue.

Recently, three studies evaluated nanoparticles modified with RGD for targeted delivery of ATO towards glioma, HCC, and TNBC cells.[59,63,74] RGD selectively binds $\alpha_v\beta_3$ integrin peptides, which are overexpressed by endothelial cells of the tumor vasculature and tumor cells.[102] Indeed, the authors showed that the tumor uptake of RGD-modified nanoparticles was higher compared to uncoated nanoparticles, which was accompanied by higher antitumor efficacy, namely lower tumor volume, larger area of tumor necrosis in vivo[59,63,74] and longer survival[59,74] compared with uncoated ATO-NPs and ATO alone. Beyond that, Fei et al.[74] confirmed that the transport of their RGD-modified nanoparticles was effectively dependent on $\alpha_v\beta_3$ integrins.

Another targeting ligand, lactobionic acid, was studied as a coating agent by Song et al. for HCC-directed ATO-NPs. Lactobionic acid is a disaccharide consisting of gluconic acid and galactose. Galactose-binding asialoglycoprotein receptor (ASGPR) is a receptor primarily expressed in the liver and not in other human tissues, therefore it constitutes an interesting target for HCC-directed drug delivery.[103] The authors showed for two different nanoparticle compositions that surface modification with lactobionic acid led to a decreased toxicity of ATO-NPs in normal hepatocytes in comparison to the toxic effect in HCC cells in vitro.[56,57] However, in vivo, only minimal reduction of tumor volume upon treatment with lactobionic acid-modified ATO-NPs could be detected compared with ATO alone. The authors predicated the advantage of lactobionic acid-modified nanoparticles in sparing the healthy tissue compared to free ATO, as confirmed by H&E staining of the liver and kidney.[57] It is of note that the preference for HCC cells is ought to be at least partly mediated by the EPR effect as ASGPR is not only expressed on HCC cells but on normal hepatocytes as well.[103]

Folic acid is yet another targeting ligand that aims at a receptor which is overexpressed on the surface of various cancers and has hence been identified as an attractive target for tumor-directed therapy: the folate receptor (see Assaraf et al.[104] for a review). Chi et al.[67] modified their HCC-directed nanoparticles with folic acid and observed enhanced in vitro toxicity, as well as enhanced apoptosis induction in HCC cells and improved in vivo tumor efficacy compared to unmodified nanoparticles and ATO alone.

Zhang et al.[52] used a different approach and targeted a structure, which is restricted to the inner membrane of viable, healthy cells but present on the outer membrane leaflet of numerous cancers: phosphatidylserine (PS). They bound F(ab')2 fragments of PGN635, a novel human monoclonal PS-targeting antibody, onto the surface of their

liposomal ATO-NPs to target glioma cells. Their in vitro study revealed that nanoparticle binding to glioma cells was PS-dependent.[52] However, in vivo experiments of this approach are still pending.

Finally, Tao et al.[66] modified their nanoparticles with angiopep-2, a specific ligand of the lipoprotein receptor-related protein (LRP) receptor. As Glioma and normal brain endothelial cells express LRP receptor on their surface, the authors proposed that functionalization of the nanoparticle surface with angiopep-2 could lead to increased accumulation of ATO in glioma. As a matter of fact, they verified that angiopep-2-modification led to a higher cellular uptake of nanoparticles by glioma and brain endothelial cells. The study revealed that targeted therapy with angiopep-2 was effective in vivo as it was shown by significantly decreased tumor volume, longer survival time and higher accumulation of the nanoparticles in tumor tissue.

4.3 | Theragnostic properties

Theragnostics describes the combination of therapy and diagnostics in one system. Visualization of drug-containing nanoparticles by integrating imaging agents into the nanoparticles is an attractive feature as it allows for image-monitored drug delivery. When it comes to theragnostic properties of ATO-NPs, two imaging agents prevailed the research: manganese and (superpara)magnetic iron oxide nanoparticles. Both can be detected by MRI.

Zhang et al.[52] examined liposomes consisting of arsenite and manganese ions, which were visible as dark contrast on T_2-weighted MRI imagines. Upon pH-triggered release of arsenite and manganese, liberated manganese ions caused a bright signal in T_1-weighted MRI, visualizing not only the location of nanoparticles but the release of ATO from those nanoparticles.[52] A different working group also made use of the fact that the release of manganese ions from their nanoparticles was proportional to the amount of released arsenic ions. They likewise observed brightening of the T_1 signal, which they confirmed in vivo by conducting MRI before and at several time points after nanoparticle-administration in mice. This time-dependent enhancement of the T_1 signal enabled real-time monitoring of ATO release.[68,73] Even though the manganese containing nanoparticles showed good in vitro[52] respectively in vivo[68,73] biocompatibility in the studies conducted, the toxic effects of free manganese demand further attention regarding tissue accumulation and long term effects.

In contrast to the bright T_1-imaging contrast manganese, iron oxide displays negative enhancement in T_2-weighted MRI. Ettlinger et al.[77] as well as Chi et al.[67] confirmed that nanoparticles with (superpara)magnetic iron oxide cores could be visualized via MRI. While the biocompatibility of magnetic iron oxide nanoparticles seems to be given,[105] further studies evaluating the in vivo distribution of ATO-NPs with iron oxide cores upon intravenous administration are pending.

4.4 | Enhancement of TACE for HCC treatment

For patients with intermediate-stage HCC, TACE has become a core treatment method. The method combines intra-arterial injection of a chemotherapeutic substance with embolization of tumor feeding vessels.[106] In a randomized trial, it could be demonstrated that TACE using drug-eluting beads (DEB-TACE) leads to a better tumor response with reduced adverse side effects compared with normal TACE.[107] HCC has been the tumor entity prevailing the most recent studies on ATO nanoparticles for drug delivery (see Tables 1 and 2). Therefore, it is only logical that certain studies focused on assessing the value of ATO-nano DEBs (ATO-NDEBs) for TACE. The studies by Fu et al.[93] and Zhao et al.[62] both focused on ATO-NDEBs from which ATO could be released in a Pi-triggered manner (see above). While Fu et al.[93] emulsified their ATO-NPs in lipiodol, which is also used for conventional TACE, Zhao et al.[62] coated their ATO-NPs with dextran. Both authors administered their ATO-NDEBs intra-arterially into VX2-tumor-bearing rabbits. They observed high intratumoral arsenic accumulation (see above) and low plasma arsenic levels compared with conventional TACE, indicating that the NDEB formulation prevented the

rushing out effect of ATO into the peripheral circulation.[62,93] Moreover, it was demonstrated that the liver and renal toxicity of ATO-NDEB was close to the sham group and much lower than the toxicity of conventional TACE with ATO, confirmed by H&E staining and blood levels of liver and kidney markers.[93]

4.5 | Enhancement of BBB crossing

The second most prevalent tumor entity used in the evaluation of nanoparticle-based drug delivery of ATO are brain tumors, namely glioma and ATRT (see Tables 1 and 2). As mentioned before, ATO has been shown to be a potent GLI-inhibitor (see above). GLI has been firstly identified to be amplified in human malignant glioma.[108] What is more, a subgroup of ATRT is characterized by an overexpression of GLI.[109] The desire to improve the characteristics of this potentially effective drug by nanoparticle encapsulation is therefore very reasonable. When it comes to brain tumors, the BBB constitutes a limiting factor to successful treatment as most drugs cannot pass it (see Pardridge[110] for a review). This problem has been addressed by evaluating ATO-NPs for transport across the BBB. While both studies showed higher BBB penetration of their modified ATO-NPs in vitro as well as higher antitumor efficacy in vivo,[59,66] the strategies differed. Tao et al.[66] used angiopep-2 as a targeting ligand for LRP receptors, present on both glioma as well as human brain endothelial cells (see above). The competition essay showed that transport of the NPs across the in vitro BBB model veritably relied on the targeting angiopep-2. Lu et al.[59] in turn coated their ATO-NPs with RGDyC, which is known to interact with integrin receptors expressed on the surface of neutrophils and monocytes.[111] The underlying idea was to target leukocytes in peripheral blood, stimulating phagocytosis of NPs and eventually enabling uptake into the brain across the BBB upon leukocyte recruitment.[59,111] Indeed, their ATO-NPs showed higher efficacy in vivo, but also decreased the cell viability of glioma cells in an in vitro BBB model. The leukocyte targeting therefore cannot be the only explanation for enhanced BBB uptake, which might at least partly be also attributable to the additional PEGylation the authors used. However, the exact mechanisms of RGDyC-mediated BBB crossing remain to be elucidated.

5 | CONCLUSION AND OUTLOOK

Evidently, the interest in evaluating nanomedicine for ATO delivery to solid tumors has emerged in the last years, especially for HCC and brain tumors. There are many aspects to consider when designing nanocarriers for ATO delivery. It is not just about the loading capacity, but also suitable carrier size, surface properties including an attachment of targeting ligands, options of triggered drug release or combination with imaging agents to form theragnostics. Encouragingly, more and more researchers have taken their nanoparticles to the in vivo stage, supposedly providing a better approximation to the efficacy of NPs than cell culture experiments. However, more data about biodistribution, in vivo safety and stability of NPs have to be gathered before ATO-NPs can be taken to the clinical stage. Given the numerous advances and attempts that have been made in the past few years, we hope that this review can provide an impetus and inspiration for future research on ATO-NPs.

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REFERENCES

1. Dilda PJ, Hogg PJ. Arsenical-based cancer drugs. *Cancer Treat Rev*. 2007;33(6):542-564.
2. Chen SJ, Zhou GB, Zhang XW, Mao JH, de Thé H, Chen Z. From an old remedy to a magic bullet: molecular mechanisms underlying the therapeutic effects of arsenic in fighting leukemia. *Blood*. 2012;117(24):6425-6437.
3. Waxman S, Anderson KC. History of the development of arsenic derivatives in cancer therapy. *Oncologist*. 2001; 6(S2):3-10.
4. Cutler EG, Bradford EH. Action of iron, cod-liver oil, and arsenic on the globular richness of the blood. *Am J Med Sci*. 1878;75(149):74-84.
5. Forkner CE, Scott TFM. Arsenic as a therapeutic agent in chronic myelogenous leukemia: preliminary report. *J Am Med Assoc*. 1931;97(1):3-5.
6. Stephens DJ, Lawrence JS. The therapeutic effect of solution of potassium arsenite in chronic myelogenous leukemia. *Ann Intern Med*. 1936;9:1488-1502.
7. Kandel EV, Leroy GV. Chronic arsenical poisoning during the treatment of chronic myeloid leukemia. *Arch Intern Med*. 1937;60(5):846-866.
8. Niu C, Yan H, Yu T, et al. Studies on treatment of acute promyelocytic leukemia with arsenic trioxide: remission induction, follow-up, and molecular monitoring in 11 newly diagnosed and 47 relapsed acute promyelocytic leukemia patients. *Blood*. 1999;94(10):3315-3324.
9. Shen ZX, Chen GQ, Ni JH, et al. Use of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia (APL): II. clinical efficacy and pharmacokinetics in relapsed patients. *Blood*. 1997;89(9):3354-3360.
10. Soignet SL, Maslak P, Wang ZG, et al. Complete remission after treatment of acute promyelocytic leukemia with arsenic trioxide. *N Engl J Med*. 1998;339(19):1341-1348.
11. U. S. Food and Drug Administration (FDA). Trisenox 21-248 approval letter. 2000.
12. European Medicines Agency (EMA). Trisenox EMEA/H/C000388/II/0058 assessment report. 2016.
13. Zhang X-W, Yan X-J, Zhou Z-R, et al. Arsenic trioxide controls the fate of the PML-RARa oncoprotein by directly binding PML. *Science*. 2010;328(5975):240-243.
14. Burnett AK, Russell NH, Hills RK, et al. Arsenic trioxide and all-trans retinoic acid treatment for acute promyelocytic leukaemia in all risk groups (AML17): results of a randomised, controlled, phase 3 trial. *Lancet Oncol*. 2015;16(13): 1295-1305.
15. Platzbecker U, Avvisati G, Cicconi L, et al. Improved outcomes with retinoic acid and arsenic trioxide compared with retinoic acid and chemotherapy in non–high-risk acute promyelocytic leukemia: final results of the randomized Italian-German APL0406 trial. *J Clin Oncol*. 2017;35(6):605-612.
16. U.S. Food and Drug Administration (FDA). Trisenox 021248/S-015 Supplemental Approval. 2018.
17. Davison K, Côté S, Mader S, Miller WH. Glutathione depletion overcomes resistance to arsenic trioxide in arsenic-resistant cell lines. *Leukemia*. 2003;17(5):931-940.
18. Davison K, Mann KK, Waxman S, Miller Jr., WH. JNK activation is a mediator of arsenic trioxide-induced apoptosis in acute promyelocytic leukemia cells. *Blood*. 2004;103(9):3496-3502.
19. Chen S, Wu J-L, Liang Y, et al. Arsenic trioxide rescues structural p53 mutations through a cryptic allosteric site. *Cancer Cell*. 2021;39(2):225-239.
20. Kumar S, Yedjou CG, Tchounwou PB. Arsenic trioxide induces oxidative stress, DNA damage, and mitochondrial pathway of apoptosis in human leukemia (HL-60) cells. *J Exp Clin Cancer Res*. 2014;33(1):42.
21. Amigo-Jiménez I, Bailón E, Aguilera-Montilla N, García-Marco JA, García-Pardo A. Gene expression profile induced by arsenic trioxide in chronic lymphocytic leukemia cells reveals a central role for heme oxygenase-1 in apoptosis and regulation of matrix metalloproteinase-9. *Oncotarget*. 2016;7(50):83359-83377.
22. Eyvani H, Moghaddaskho F, Kabuli M, et al. Arsenic trioxide induces cell cycle arrest and alters DNA methylation patterns of cell cycle regulatory genes in colorectal cancer cells. *Life Sci*. 2016;167:67-77.
23. Moghaddaskho F, Eyvani H, Ghadami M, et al. Demethylation and alterations in the expression level of the cell cycle–related genes as possible mechanisms in arsenic trioxide–induced cell cycle arrest in human breast cancer cells. *Tumor Biol*. 2017;39(2):1010428317692255.
24. Ding D, Lim KS, Eberhart CG. Arsenic trioxide inhibits Hedgehog, Notch and stem cell properties in glioblastoma neurospheres. *Acta Neuropathol Commun*. 2014;2:31.
25. Beauchamp EM, Ringer L, Bulut G, et al. Arsenic trioxide inhibits human cancer cell growth and tumor development in mice by blocking Hedgehog/GLI pathway. *J Clin Invest*. 2011;121(1):148-160.
26. Kerl K, Moreno N, Holsten T, et al. Arsenic trioxide inhibits tumor cell growth in malignant rhabdoid tumors in vitro and in vivo by targeting overexpressed Gli1. *Int J Cancer*. 2014;135(4):989-995.
27. Nakamura S, Nagano S, Nagao H, et al. Arsenic trioxide prevents osteosarcoma growth by inhibition of GLI transcription via DNA damage accumulation. *PLoS One*. 2013;8(7):e69466.

28. Murgo AJ. Clinical trials of arsenic trioxide in hematologic and solid tumors: overview of the National Cancer Institute Cooperative Research and Development Studies. *Oncologist*. 2001;6(S2):22-28.
29. Lin CC, Hsu C, Hsu CH, Hsu WL, Cheng AL, Yang CH. Arsenic trioxide in patients with hepatocellular carcinoma: a phase II trial. *Invest New Drugs*. 2007;25(1):77-84.
30. Owonikoko TK, Zhang G, Kim HS, et al. Patient-derived xenografts faithfully replicated clinical outcome in a phase II co-clinical trial of arsenic trioxide in relapsed small cell lung cancer. *J Transl Med*. 2016;14(1):111.
31. Beer TM, Tangen CM, Nichols CR, et al. Southwest Oncology Group phase II study of arsenic trioxide in patients with refractory germ cell malignancies. *Cancer*. 2006;106(12):2624-2629.
32. Vuky J, Yu R, Schwartz L, Motzer RJ. Phase II trial of arsenic trioxide in patients with metastatic renal cell carcinoma. *Invest New Drugs*. 2002;20(3):327-330.
33. Subbarayan PR, Ardalan B. In the war against solid tumors arsenic trioxide need partners. *J Gastrointest Cancer*. 2014;45(3):363-371.
34. Kim KB, Bedikian AY, Camacho LH, Papadopoulos NE, McCullough C. A phase II trial of arsenic trioxide in patients with metastatic melanoma. *Cancer*. 2005;104(8):1687-1692.
35. Kindler HL, Aklilu M, Nattam S, Vokes EE. Arsenic trioxide in patients with adenocarcinoma of the pancreas refractory to gemcitabine: A phase II trial of the University of Chicago phase II consortium. *Am J Clin Oncol Cancer Clin Trials*. 2008;31(6):553-556.
36. Kumthekar P, Grimm S, Chandler J, et al. A phase II trial of arsenic trioxide and temozolomide in combination with radiation therapy for patients with malignant gliomas. *J Neurooncol*. 2017;133(3):589-594.
37. Subbarayan PR, Lima M, Ardalan B. Arsenic trioxide/ascorbic acid therapy in patients with refractory metastatic colorectal carcinoma: A clinical experience. *Acta Oncol*. 2007;46(4):557-561.
38. Barbey JT, Pezzullo JC, Soignet SL. Effect of arsenic trioxide on QT interval in patients with advanced malignancies. *J Clin Oncol*. 2003;21(19):3609-3615.
39. Martinez VD, Vucic EA, Becker-Santos DD, Gil L, Lam WL. Arsenic exposure and the induction of human cancers. *J Toxicol*. 2011;2011:431287.
40. Swindell EP, Hankins PL, Chen H. Miodragović CDSU, O'Halloran T V. Anticancer activity of small molecule and nanoparticulate arsenic(III) complexes. *Inorg Chem*. 2013;52(21):12292-12304.
41. Goto E, Tomita A, Hayakawa F, Atsumi A, Kiyoi H, Naoe T. Missense mutations in PML-RARA are critical for the lack of responsiveness to arsenic trioxide treatment. *Blood*. 2011;118(6):1600-1609.
42. Liu J, Zhu HH, Jiang H, Jiang Q, Huang XJ. Varying responses of PML-RARA with different genetic mutations to arsenic trioxide. *Blood*. 2016;127(2):243-250.
43. Sun Y, Kim SH, Zhou DC, et al. Acute promyelocytic leukemia cell line AP-1060 established as a cytokine-dependent culture from a patient clinically resistant to all-trans retinoic acid and arsenic trioxide. *Leukemia*. 2004;18(7):1258-1269.
44. Chen X, Zhang M, Liu LX. The overexpression of multidrug resistance-associated proteins and gankyrin contribute to arsenic trioxide resistance in liver and gastric cancer cells. *Oncol Rep*. 2009;22(1):73-80.
45. Zhang YK, Dai C, Yuan C, et al. Establishment and characterization of arsenic trioxide resistant KB/ATO cells. *Acta Pharm Sin B*. 2017;7(5):564-570.
46. Sun T, Zhang YS, Pang B, Hyun DC, Yang M, Xia Y. Engineered nanoparticles for drug delivery in cancer therapy. *Angew Chem Int Ed Engl*. 2014;53(46):12320-12364.
47. Robles-Osorio ML, Sabath-Silva E, Sabath E. Arsenic-mediated nephrotoxicity. *Ren Fail*. 2015;37(4):542-547.
48. Akhtar A, Wang SX, Ghali L, Bell C, Wen X. Recent advances in arsenic trioxide encapsulated nanoparticles as drug delivery agents to solid cancers. *J Biomed Res*. 2017;31(3):177-188.
49. Mohan D, Pittman CU, Jr. Arsenic removal from water/wastewater using adsorbents: a critical review. *J Hazard Mater*. 2007;142(1-2):1-53.
50. Wang X, Li D, Ghali L, et al. Therapeutic potential of delivering arsenic trioxide into HPV-infected cervical cancer cells using liposomal nanotechnology. *Nanoscale Res Lett*. 2016;11(1):94.
51. Akhtar A, Wang SX, Ghali L, Bell C, Wen X. Effective delivery of arsenic trioxide to HPV-positive cervical cancer cells using optimised liposomes: a size and charge study. *Int J Mol Sci*. 2018;19(4):1081.
52. Zhang L, Zhang Z, Mason RP, Sarkaria JN, Zhao D. Convertible MRI contrast: sensing the delivery and release of anti-glioma nano-drugs. *Sci Rep*. 2015;5:9874.
53. Song X, You J, Wang J, Zhu A, Ji L, Guo R. Preparation and investigation of arsenic trioxide-loaded polylactic acid/magnetic hybrid nanoparticles. *Chem Res Chin Univ*. 2014;30:326-332.
54. Su J, Liu G, Lian Y, et al. Preparation and characterization of erythrocyte membrane cloaked PLGA/arsenic trioxide nanoparticles and evaluation of their in vitro anti-tumor effect. *RSC Adv*. 2018;8:20068-20076.
55. Hu J, Dong Y, Ding L, et al. Local delivery of arsenic trioxide nanoparticles for hepatocellular carcinoma treatment. *Sig Transduct Target Ther*. 2019;4:28.

56. Song X, You J, Shao H, Yan C. Effects of surface modification of As_2O_3-loaded PLGA nanoparticles on its anti-liver cancer ability: an in vitro and in vivo study. *Colloids Surfaces B Biointerfaces*. 2018;169:289-297.
57. Song X, Wang J, Xu Y, Shao H, Gu J. Surface-modified PLGA nanoparticles with PEG/LA-chitosan for targeted delivery of arsenic trioxide for liver cancer treatment: inhibition effects enhanced and side effects reduced. *Colloids Surfaces B Biointerfaces*. 2019;180:110-117.
58. Lian Y, Wang X, Guo P, et al. Erythrocyte membrane-coated arsenic trioxide-loaded sodium alginate nanoparticles for tumor therapy. *Pharmaceutics*. 2020;12(1):21.
59. Lu Y, Han S, Zheng H, et al. A novel RGDyC/PEG co-modified PAMAM dendrimer-loaded arsenic trioxide of glioma targeting delivery system. *Int J Nanomedicine*. 2018;13:5937-5952.
60. Chen FY, Yi JW, Gu ZJ, et al. Inorganic phosphate-triggered release of anti-cancer arsenic trioxide from a self-delivery system: an in vitro and in vivo study. *Nanoscale*. 2016;8:6094-6100.
61. Fu X, Liang Q, Luo R, et al. An arsenic trioxide nanoparticle prodrug (ATONP) potentiates a therapeutic effect on an aggressive hepatocellular carcinoma model via enhancement of intratumoral arsenic accumulation and disturbance of the tumor microenvironment. *J Mater Chem B*. 2019;7(19):3088-3099.
62. Zhao J, Li YS, Liu ZX, et al. Nanosized drug-eluting bead for transcatheter arterial chemoembolization (ND-TACE). *J Mater Chem B*. 2020;8(37):8684-8694.
63. Wu X, Han Z, Schur RM, Lu Z-R. Targeted mesoporous silica nanoparticles delivering arsenic trioxide with environment sensitive drug release for effective treatment of triple negative breast cancer. *ACS Biomater Sci Eng*. 2016;2(4):501-507.
64. Ellison PA, Chen F, Goel S, et al. Intrinsic and stable conjugation of thiolated mesoporous silica nanoparticles with radioarsenic. *ACS Appl Mater Interfaces*. 2017;9(8):6772-6781.
65. Xiao X, Liu Y, Guo M, et al. pH-triggered sustained release of arsenic trioxide by polyacrylic acid capped mesoporous silica nanoparticles for solid tumor treatment in vitro and in vivo. *J Biomater Appl*. 2016;31(1):23-35.
66. Tao J, Fei W, Tang H, et al. Angiopep-2-conjugated "core-Shell" hybrid nanovehicles for targeted and pH-triggered delivery of arsenic trioxide into glioma. *Mol Pharm*. 2019;16(2):786-797.
67. Chi X, Zhang R, Zhao T, et al. Targeted arsenite-loaded magnetic multifunctional nanoparticles for treatment of hepatocellular carcinoma. *Nanotechnology*. 2019;30(17):175101.
68. Zhang K, Lin H, Mao J, et al. An extracellular pH-driven targeted multifunctional manganese arsenite delivery system for tumor imaging and therapy. *Biomater Sci*. 2019;7(6):2480-2490.
69. Huang Y, Zhou B, Luo H, et al. ZnAs@SiO_2 nanoparticles as a potential anti-tumor drug for targeting stemness and epithelial-mesenchymal transition in hepatocellular carcinoma via SHP-1/JAK2/STAT3 signaling. *Theranostics*. 2019;9(15):4391-4408.
70. Liu H, Zhang Z, Chi X, et al. Arsenite-loaded nanoparticles inhibit PARP-1 to overcome multidrug resistance in hepatocellular carcinoma cells. *Sci Rep*. 2016;6:31009.
71. Zhao Z, Zhang H, Chi X, et al. Silica nanovehicles endow arsenic trioxide with an ability to effectively treat cancer cells and solid tumors. *J Mater Chem B*. 2014;2(37):6313-6323.
72. Chi X, Yin Z, Jin J, et al. Arsenite-loaded nanoparticles inhibit the invasion and metastasis of a hepatocellular carcinoma: in vitro and in vivo study. *Nanotechnology*. 2017;28(44):445101.
73. Zhao Z, Wang X, Zhang Z, et al. Real-time monitoring of arsenic trioxide release and delivery by activatable T1 imaging. *ACS Nano*. 2015;9(3):2749-2759.
74. Fei W, Zhang Y, Han S, et al. RGD conjugated liposome-hollow silica hybrid nanovehicles for targeted and controlled delivery of arsenic trioxide against hepatic carcinoma. *Int J Pharm*. 2017;519:250-262.
75. Ettlinger R, Sönksen M, Graf M, et al. Metal-organic framework nanoparticles for arsenic trioxide drug delivery. *J Mater Chem B*. 2018;6(40):6481-6489.
76. Ettlinger R, Moreno N, Volkmer D, Kerl K, Bunzen H. Zeolitic imidazolate framework-8 as pH-sensitive nanocarrier for "Arsenic Trioxide" drug delivery. *Chem - A Eur J*. 2019;25(57):13189-13196.
77. Ettlinger R, Moreno N, Ziółkowska N, et al. In vitro studies of Fe_3O_4-ZIF-8 core–shell nanoparticles designed as potential theragnostics. *Part Part Syst Charact*. 2020;37(12):2000185.
78. Schnabel J, Ettlinger R, Bunzen H. Zn-MOF-74 as pH-responsive drug-delivery system of arsenic trioxide. *ChemNanoMat*. 2020;6(8):1229-1236.
79. Kallinteri P, Fatouros D, Klepetsanis P, Antimisiaris SG. Arsenic trioxide liposomes: encapsulation efficiency and in vitro stability. *J Liposome Res*. 2004;14(1-2):27-38.
80. Fatouros D, Gortzi O, Klepetsanis P, et al. Preparation and properties of arsonolipid containing liposomes. *Chem Phys Lipids*. 2001;109(1):75-89.
81. Gortzi O, Papadimitriou E, Kontoyannis CG, Antimisiaris SG, Ioannou PV. Arsonoliposomes, a novel class of arsenic-containing liposomes: effect of palmitoyl-arsonolipid-containing liposomes on the viability of cancer and normal cells in culture. *Pharm Res*. 2002;19(1):79-86.

82. Chen H, MacDonald RC, Li S, Krett NL, Rosen ST, O'Halloran TV. Lipid encapsulation of arsenic trioxide attenuates cytotoxicity and allows for controlled anticancer drug release. *J Am Chem Soc*. 2006;128(41):13348-13349.
83. Chen H, Pazicni S, Krett NL, et al. Coencapsulation of arsenic- and platinum-based drugs for targeted cancer treatment. *Angew Chem Int Ed Engl*. 2009;48(49):9295-9299.
84. Ahn RW, Chen F, Chen H, et al. A novel nanoparticulate formulation of arsenic trioxide with enhanced therapeutic efficacy in a murine model of breast cancer. *Clin Cancer Res*. 2010;16(14):3607-3617.
85. Lee SM, Lee OS, O'Halloran TV, Schatz GC, Nguyen ST. Triggered release of pharmacophores from [Ni($HAsO_3$)]-loaded polymer-caged nanobin enhances pro-apoptotic activity: a combined experimental and theoretical study. *ACS Nano*. 2011;5(5):3961-3969.
86. Chen H, Ahn R, Van den Bossche J, Thompson DH, O'Halloran TV. Folate-mediated intracellular drug delivery increases the anticancer efficacy of nanoparticulate formulation of arsenic trioxide. *Mol Cancer Ther*. 2009;8(7):1955-1963.
87. Zhang Y, Kenny HA, Swindell EP, et al. Urokinase plasminogen activator system-targeted delivery of nanobins as a novel ovarian cancer therapy. *Mol Cancer Ther*. 2013;12(12):2628-2639.
88. Zhou J, Zeng F, Xiang G, Xie S, Wei S. Preparation of arsenic trioxide albumin microspheres and its release characteristics in vitro. *J Huazhong Univ Sci Technolog Med Sci*. 2005;25(3):310-312.
89. Zhou J, Wang QH, Liu JH, Wan YB. Effects of Tat peptide on intracellular delivery of arsenic trioxide albumin microspheres. *Anticancer Drugs*. 2012;23(3):303-312.
90. Zhao SS, Lu Q, Zhang DS. Preparation of arsenic trioxide-loaded PLGA Nanoparticles And Investigation Of Its Inhibitory Effects On Proliferation Of Rabbit Vascular Smooth Muscle Cells. *In Vitro. AMR*. 2009;60-61:125-129.
91. Ma Y, Zhang C, Chen X, et al. The influence of modified pluronic F127 copolymers with higher phase transition temperature on arsenic trioxide-releasing properties and toxicity in a subcutaneous model of rats. *AAPS PharmSciTech*. 2012;13(2):441-447.
92. Da Sacco L, Masotti A. Chitin and chitosan as multipurpose natural polymers for groundwater arsenic removal and As_2O_3 delivery in tumor therapy. *Mar Drugs*. 2010;8(5):1518-1525.
93. Fu X, Luo R-G, Qiu W, et al. Sustained release of arsenic trioxide benefits interventional therapy on rabbit VX2 liver tumor. *Nanomedicine*. 2020;24:102118.
94. Manzano M, Vallet-Regi M. Mesoporous silica nanoparticles for drug delivery. *Adv Funct Mater*. 2020;30:1902634.
95. Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. *J Control Release*. 2000;65(1-2):271-284.
96. Kaskel S. The Chemistry of Metal-Organic Frameworks. *Synthesis, Characterization, and Applications*. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA; 2016.
97. Farrusseng D. Metal-Organic Frameworks. *Applications from Catalysis to Gas Storage*. Weinheim, Germany: Wiley-VCH; 2011:392.
98. Yang J, Yang Y-W. Metal–organic frameworks for biomedical applications. *Small*. 2020;16:1906846.
99. Kato Y, Ozawa S, Miyamoto C, et al. Acidic extracellular microenvironment and cancer. *Cancer Cell Int*. 2013;13(1):89.
100. Calvo P, Gouritin B, Chacun H, et al. Long-circulating pegylated polycyanoacrylate nanoparticles as new drug carrier for brain delivery. *Pharm Res*. 2001;18(8):1157-1166.
101. He X, Nie H, Wang K, Tan W, Wu X, Zhang P. In vivo study of biodistribution and urinary excretion of surface-modified silica nanoparticles. *Anal Chem*. 2008;80(24):9597-9603.
102. Ulbrich K, Holá K, Šubr V, Bakandritsos A, Tuček J, Zbořil R. Targeted drug delivery with polymers and magnetic nanoparticles: covalent and noncovalent approaches, release control, and clinical studies. *Chem Rev*. 2016;116(9):5338-5431.
103. Shi B, Abrams M, Sepp-Lorenzino L. Expression of asialoglycoprotein receptor 1 in human hepatocellular carcinoma. *J Histochem Cytochem*. 2013;61(12):901-909.
104. Assaraf YG, Leamon CP, Reddy JA. The folate receptor as a rational therapeutic target for personalized cancer treatment. *Drug Resist Updat*. 2014;17(4-6):89-95.
105. Nosrati H, Salehiabar M, Fridoni M, et al. New insight about biocompatibility and biodegradability of iron oxide magnetic nanoparticles: stereological and in vivo MRI monitor. *Sci Rep*. 2019;9:7173.
106. Villanueva A. Hepatocellular carcinoma. *N Engl J Med*. 2019;380(15):1450-1462.
107. Malagari K, Pomoni M, Kelekis A, et al. Prospective randomized comparison of chemoembolization with doxorubicin-eluting beads and bland embolization with BeadBlock for hepatocellular carcinoma. *Cardiovasc Intervent Radiol*. 2010;33(3):541-551.
108. Kinzler KW, Bigner SH, Bigner DD, et al. Identification of an amplified, highly expressed gene in a human glioma. *Science*. 1987;236(4797):70-73.

109. Ho B, Johann PD, Johann PD, et al. Molecular subgrouping of atypical teratoid/rhabdoid tumors: a reinvestigation and current consensus. *Neuro Oncol*. 2020;22(5):613-624.
110. Pardridge WM. Blood-brain barrier delivery. *Drug Discov Today*. 2007;12(1-2):54-61.
111. Qin J, Chen DW, Hu H, Cui Q, Qiao MX, Chen B. Surface modification of RGD-liposomes for selective drug delivery to monocytes/neutrophils in brain. *Chem Pharm Bull*. 2007;55(8):1192-1197.

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