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REVIEW

# New Progress in Improving the Delivery Methods of Bisphosphonates in the Treatment of Bone Tumors



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**Abstract:** Bone tumors are tumors that occur in the bone or its accessory tissues, including primary tumors and metastatic tumors. The main mechanism of bisphosphonate is to inhibit the resorption of destructive bone, inhibit the activity of osteoclasts and reduce the concentration of blood calcium. Therefore, bisphosphonates can be used for malignant hypercalcaemia, pain caused by osteolytic bone metastasis, prevention of osteolytic bone metastasis, multiple myeloma osteopathy, improving radiosensitivity and so on. However, the traditional administration of bisphosphonates can cause a series of adverse reactions. To overcome this disadvantage, it is necessary to develop novel methods to improve the delivery of bisphosphonates. In this paper, the latest research progress of new and improved bisphosphonate drug delivery methods in the treatment of bone tumors is reviewed. At present, the main design idea is to connect bisphosphonate nanoparticles, liposomes, microspheres, microcapsules, couplings, prodrugs and bone tissue engineering to targeted anti-tumors systems, and positive progress has been made in in vitro and animal experiments. However, its safety and effectiveness in human body still need to be verified by more studies.

**Keywords:** bone tumor, bisphosphonate, drug delivery system, tumor bone metastasis, bone defect repair

## Introduction

Bone tumors are tumors that occur in the bone or originate from the muscle system, including primary tumors and metastatic tumors. Primary bone tumors are malignant bone tumors formed directly by uncontrollable growth of tumor cells, including giant cell tumors of bone, osteosarcoma, multiple myeloma (MM), and Ewing's sarcoma. According to a report released by the American Cancer Society, MM accounted for 0.25% of all male cases and 0.17% of all female cases in 2020.[1] Metastatic bone tumors refer to malignant tumors of other tissues and organs outside the bone, including cancer, sarcoma and other malignant lesions metastasized to bone. According to statistics, metastatic bone tumors are more common than benign and malignant primary bone tumors. One of the most common metastatic sites of malignant tumors is bone, most of which occurs in patients with advanced breast or prostate cancer, as well as some lung, colon, gastric, bladder, uterine, rectal and thyroid or renal cancer patients.[2–4] According to a report released by the American Cancer Society, among global cancer cases in 2020, breast cancer accounted for 5.2% of all female cases, prostate cancer accounted for 3.86% of all male cases, and lung cancer accounted for 3.78% of males and 1.77% of females.[1]

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Tumor bone metastasis can cause a series of serious complications that acutely affect patients' survival time and quality of life, such as hypercalcaemia, spinal cord compression, pathological fracture, chronic persistent bone pain and so on, that is, bone-related events.[5]

There is a vicious cycle between tumor cell proliferation and bone formation. As the beginning of the cycle, tumor cells promote the excessive release of RANKL from osteoblasts to promote the differentiation and activation of osteoclasts and accelerate the process of osteolysis. There are a large number of growth factors that can promote the growth of tumor cells in bone, and the acceleration of osteolysis can promote the release and activation of these cytokines. This "vicious circle" not only accelerates the process of bone destruction but also promotes the progression of tumor cells.[2]

At present, the main clinical methods of conventional treatment of bone tumors are surgery, chemotherapy and radiotherapy. Among them, chemotherapeutic drugs are usually unable to be accurately enriched at the site of bone tumors because of the lack of bone targeting ability, and thus the therapeutic effect of chemotherapy is often not ideal and will cause certain toxicity and side effects to patients. The commonly used bone-targeted therapeutic drugs are denosumab and bisphosphonate (BPs). Denosumab is a human IgG2 monoclonal antibody that inhibits the formation, function and survival of osteoclasts by targeting binding to RANKL, but unlike bisphosphonates, it does not participate in bone remodelling. Bisphosphonate can preferentially bind to bone with active bone metabolism and release from bone matrix in the procedure of bone resorption, which can effectively inhibit the survival and activity of osteoclasts, thus reducing the occurrence of bone resorption caused by osteoclasts.[6] The common feature of bone tumor disease is the enhancement of osteoclastic bone resorption.[7] BPs are very effective in improving the quality of life of patients, and therefore they have been widely used in the clinic. In the treatment of tumor bone metastasis, its mechanism is to inhibit osteoclast absorption, indirectly inhibit tumor growth or directly exert antitumor effects, which can inhibit tumor metastasis and reduce the occurrence of bone-related events.[8–10] The mechanism of BPs in the treatment of multiple myeloma is that they not only have a direct inhibitory effect on myeloma cells but also indirectly affect tumor growth by preventing bone resorption and inhibiting the further release of tumor stimulating factors.[11] Therefore, BPs have become the standard treatment for solid tumor bone metastasis, multiple myeloma and other bone tumors. Bisphosphonates are also used in the treatment of primary bone tumors. The use of bisphosphonates reduces pain and bone damage in patients with bone tumors and improves quality of life. In addition, another function of BPs is to bring other pharmacological drugs into the bone to play a role in tumor treatment.[12] Figure 1 shows the schematic diagram of how BPs specifically inhibit the vicious cycle of bone destruction caused by tumors.

Bisphosphonates are divided into three generations according to different side chains of the molecular structure. The first generation is a simple P-C-P structure, represented by clodronate and etidronic acid. The second-generation BPs have side chains that contain amino groups, including pamidronic acid and alendronic acid (ALN). The third generation introduces a saturated hydrocarbon chain or nitrogen-containing imidazole ring on the nitrogen atom, represented by zoledronic acid (ZOL) and ibandronic acid. First- and second-generation BPs can relieve the pain of bone metastasis, prevent or delay SREs and improve the quality of life of patients. On this basis, third-generation BPs can also significantly reduce the hypercalcaemia of malignant tumor bone metastasis, increase bone mineral density and reduce the disorder of bone metabolism. Zoledronic acid or pamidronic acid can be applied to all patients with active multiple myeloma. At the same time, ZOL is also fit to the treatment of multiple myeloma-related hypercalcaemia, which is better than pamidronic acid.[13] At present, third-generation BP drugs

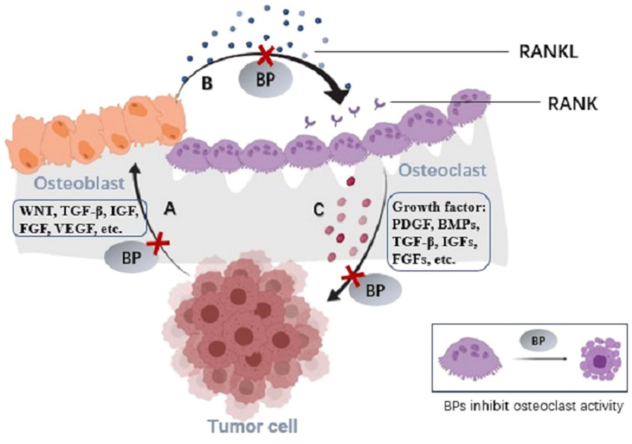
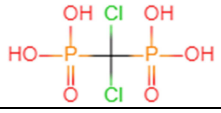
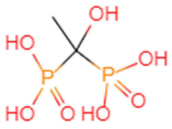
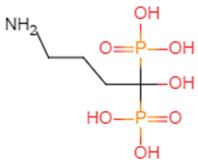
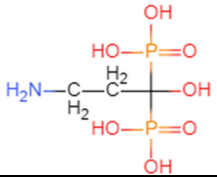
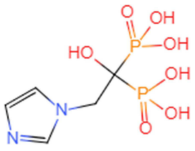
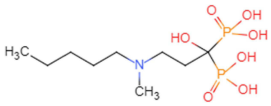
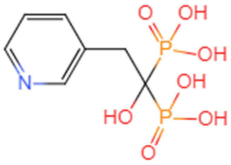
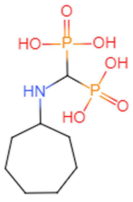



**Figure 1** Schematic of vicious circle of bone destruction caused by tumor and the mechanism of bisphosphonate inhibiting bone resorption. (**A**) Tumor cells promote osteoblast to secrete RANKL. (**B**) Osteoblasts release RANKL, promote the differentiation and activation of osteoclasts and accelerate the process of osteolysis. (**C**) Osteoclasts release and activate growth factors that promote tumor cell growth. Among them, bisphosphonates can curb every link in the above vicious circle, inhibit the absorption of osteoclasts, and thus inhibit tumor growth.

are widely applied in clinical practice. The common drugs of bisphosphonates and their clinical applications are shown in Table 1.

The common ways of administration of bisphosphonates are oral and parenteral routes. Oral clodronate, intravenous pamidronate and intravenous zoledronic acid have been used in the treatment of tumor bone metastasis and myeloma bone disease.[14] However, bisphosphonates are very polar molar molecules that are difficult to absorb after oral administration. If they are not first bound to the

**Table 1** Clinical Application of Common Bisphosphonates

| Generation | Name | Structure | Indication | Route of Administration |
|---|---|---|---|---|
| First Generation | Clodronate |  | Osteolytic bone metastasis caused by cancer and osteoporosis. Hypercalcemia | Intravenous drip, Oral |
| First Generation | Etidronic acid |  | Osteoporosis | Oral |
| Second Generation | Alendronic acid |  | Prevention and treatment of osteoporosis in postmenopausal women. Increase the bone mass of male patients with osteoporosis. Osteoporosis caused by glucocorticoid. Bone Paget's disease. | Oral |
| Second Generation | Pamidronic acid |  | Paget's disease. Hypercalcemia in malignant tumors. Osteolytic bone metastasis and osteolytic lesions of multiple myeloma. | Intravenous drip |
| Third Generation | Zoledronic acid |  | Paget's Disease of Bone | Intravenous drip |
| Third Generation | Ibandronic acid |  | Postmenopausal osteoporosis | Intravenous drip |
| Third Generation | Risedronic acid |  | Postmenopausal osteoporosis | Oral |
| Third Generation | Incadronic acid |  | Bone metastatic pain caused by malignant tumor | Intravenous drip |

**Note**: (The information about indication and route of administration in Table 1 comes from https://www.fda.com).

bone, they will be excreted quickly from the body.[15] Oral drug compliance is poor and can hardly slow down the development of skeletal complications. Intravenous injection of a certain dose of bisphosphonate can avoid potential gastrointestinal intolerance but may cause an acute reaction in the body, that is, low fever, accompanied by changes in serum lymphocytes and other haemograms and short-term discomfort.[12] More effective bisphosphonates are a major step forwards in relieving pain caused by bone disease and preventing bone-related events.[16,17]

Following the traditional method, it is difficult to transfer the drug to the lesion site, with low efficacy, large adverse reactions, and long-term, high-dose, and extensive bisphosphonates that cause a series of adverse reactions.[18] The problems in the use of bisphosphonates can be summarized as follows: (i) BPs have high water solubility; are metabolized too quickly, as the drug concentration reaches a peak in the stomach or blood for a short time, then decays rapidly and is excreted out of the body; the drug use efficiency is low; and the drug needs to be used frequently; (ii) irritation and side effects are caused by high water solubility, such as ALN leading to gastric erosion in rabbits; (iii) only bisphosphonate acts directly on osteoclasts and inhibits the rate-limiting step in cholesterol biosynthesis pathway, and thus it is difficult to provide more osteogenic elements; and (iv) bone loss and injury need to be filled immediately to avoid further fractures and injuries caused by gaps; therefore, in situ fixation and filling of bone repair and reconstruction materials are beneficial to bone reconstruction and recovery.

To overcome these shortcomings, it is necessary to prepare novel methods to improve the delivery of bisphosphonates.[19] Different carriers, such as liposomes, nanoparticles, microspheres, microcapsules and conjugates, have been developed, showing fruitful results in basic research and preclinical trials. Liposomes, nanoparticles, biological carriers and other drug carriers can be used to make a new drug delivery system so that drugs can selectively act on bone tissue to achieve the purpose of targeted therapy.[20–22] It can be coupled with imaging agents, antitumor drugs, radiopharmaceuticals or other biomedical materials and target bone tissue, which can significantly improve the inhibitory effect on tumor cells and help to reduce the dose.[23–25]

This article summarizes the research progress of novel methods to improve the delivery of bisphosphonates based on different types of preparations by consulting the literature, thereby offering a novel perspective for the in-depth study of bisphosphonate bone-targeting drugs for tumor treatment. At present, the main trend of research and development is to use bisphosphate as the target to deliver chemotherapeutic drugs through nanocarriers for the treatment of osteoma or bone metastases, that is, the combined application of nanotechnology and bone-targeted drug delivery systems. In addition, bone tissue engineering is a new method to repair bone defects based on the combination of seed cells, growth factors and scaffold materials to construct an artificial bone substitute material to repair the defects.

## Selection of Drugs Connected to Bisphosphonates

In the current literature and research, the drugs connected with bisphosphonates are mainly chemotherapeutic drugs, including paclitaxel, docetaxel, doxorubicin, methotrexate and so on. Paclitaxel and anthracycline are the main drugs for the treatment of breast cancer,[26] and methotrexate is the drug for the treatment of osteosarcoma. In future studies, chemotherapy drugs for lung cancer, such as gemcitabine, pemetrexed and vinorelbine, and tyrosine kinase inhibitors, such as gefitinib and axitinib, can be considered. Monoclonal antibodies can also be considered linked drugs, such as trastuzumab for breast cancer and bevacizumab for lung cancer. Endocrine drugs for the treatment of breast cancer can also be linked with bisphosphonates, such as letrozole, exemestane, and flurvist. Drugs related to the treatment of myeloma, such as the protease inhibitor bortezomib, lenalidomide and thalidomide, can also be regarded as combined drugs.

The following article will specifically introduce new methods to improve the delivery of bisphosphonates.

## Nanoparticles

Nanomedicines possess the features of small particles, large specific surface area, and high activity. They are mainly divided into two categories: nanomolecular drugs and nanocarrier drugs. The former is directly processed from raw materials into nanoparticles, and the latter is a pharmaceutical preparation made by combining nanoscale materials with drugs in a certain way. Nanocarrier drugs can prolong the circulation time of drugs, reduce the accumulation of drugs in nontarget tissues, and increase the positioning of drugs at target tumor sites through passive/active targeting.

Due to the lack of a blood circulatory system in bone tissue, it is difficult for nanocarriers to enrich in bone

tumors through enhanced permeability and retention effects. Researchers try to increase the enrichment of the material in the tumor in bone tissue by modifying bone targeting molecules to improve the effect of diagnosis and treatment. To further improve the targeting effect of nanoparticles on bone lesions, researchers often use bisphosphonates that have affinity with mineral areas in bone tissue as targeting ligands.[21,27] At present, a variety of nanocarrier drugs using bisphosphonates have been developed, including polymeric nanoparticles, metal-organic framework nanoparticles, metal/inorganic nanoparticles, polymer-drug conjugates, polymer nanocarriers, polymeric micelles and dendrimer nanoparticles.

## Polymeric Nanoparticles

The coupling of drugs and polymers is one of the most studied and promising methods for cancer treatment. At present, the widely studied polymer nanoparticles used in tumor immunotherapy include polylactic acid (PLA), poly (lactic-co-glycolic acid) (PLGA), polycaprolactone (PCL), poly(cyanoacrylic acid) (PCA), poly(g-glutamic acid) (g-PGA), polyethylene glycol (PEG) and polyethyleneimine (PEI)-polystyrene (PS). As an efficient delivery system, polymer nanoparticles can protect the contents from the environment and provide a sustained and adjustable release rate for the contents through a targeted strategy.

Otaka et al[28] synthesized an amphiphilic phospholipid polymer containing ALN, namely, poly(2-methacryloyloxyethyl phosphorylcholine-butyl methacrylate), and the polymer encapsulated docetaxel. Their results suggest that ALN plays a role in promoting bone accumulation in the polymer-docetaxel complex. The compound has certain anticancer activity against several breast cancer cells without hindering the pharmacological effect of docetaxel. Bone-targeted phospholipid polymers are a potential solubilizing excipient for the preparation of docetaxel and delivery of hydrophobic drugs to bone tissue through blood administration.

Li et al[29] developed a new ZOL nano-preparation (ZOL-NPs). The ZOL-calcium nanocomposites encapsulated by anionic lipids were encapsulated in PLGA copolymer nanoparticles and emulsified with acid-sensitive pyrolyzable emulsifier polyethylene glycol (2000). ZOL-NP led to a significantly lower distribution of ZOL in mice with orthotopic implantation of breast tumors. With the diffusion of compounds in the polymer and the promotion of hydrolysis and/or erosion of PLGA, the compounds in the nanoparticles are released. In PLGA nanoparticles, it is difficult for water molecules to spread to the polymeric nanoparticle layer and come into contact with layer-by-layer coated ZOL-Ca nanocomposites, which leads to the dissociation of ZOL-Ca complexes and the release of ZOL. Notably, ZOL-NPs can achieve the same amount of free ZOL and cannot inhibit tumor growth. These results suggest that this technique may be used to apply bisphosphonates more effectively in the treatment of extraosseous tumors.

Vanderburgh et al[30] developed a bone-targeting nanoparticle (BTNP) containing the amphiphilic diblock copolymer poly [(sodium propionate)-block-(ALN acrylamide-N-dimethylacrylamide)] [PPS-b-P (ALN-co-DMA)], which can encapsulate the small molecule Gli2 inhibitor GANT58 and preferentially deliver it to bone-related tumors. Among them, ALN provides bone targeting for GANT58-BTNPs, which increases the concentration of GANT58 in tumor-related bone relative to nontargeted NPs and provides benefits through the direct anti-absorption function of ALN. The aggregation of ALN on tumor-related bone can be optimized by changing the mole fraction of BTNPs in the hydrophilic polymer, the binding of active bone, or perhaps with the help of good permeability and retention. The results of their study in the intracardiac tumors cell injection model of bone metastasis of breast cancer showed that GANT58-BTNPs may effectively target the source of the mechanism of tumor bone metastasis, that is, to inhibit the activation of osteoclasts induced by bone-related tumors and the resulting bone destruction, which is expected to be used in the treatment of tumor bone metastasis.

PLGA is a copolymer synthesized by PLA and PGA that has been used to produce a variety of therapeutic devices and has been organized into several different formulations, such as nanoparticles, particles, hydrogels, stents and sponges.[31,32]

Swami et al[33] designed polymer nanoparticles that can deliver therapeutic drugs to bones in a spatiotemporal manner, which consist of PLGA, PEG and bisphosphonate, thereby increasing local drug concentrations and reducing nontargeted effects. The engineered NPs were prepared by mixing two different ratios of the synthetic polymer PLGA-b-PEG and the ALN-conjugated polymer PLGA-b-PEG-ALN. The new delivery system they developed tested the efficacy in a mouse model of multiple myeloma (MM). Their research shows that this bone-targeting specific anticancer therapy based on NP can be used as a clinically related method to improve drug delivery, thus inhibiting the tumor progression of MM.

Similarly, Raichur et al[34] prepared anchored bone nanoparticles with selective bone affinity and methotrexate release nanoparticles by coupling ZOL with PLGA nanoparticles. Comprehensive evaluation methods, including infrared spectroscopy and nuclear magnetic resonance studies, in vitro dissolution studies, bone localization studies, in vivo biodistribution of experimental animal model studies and a series of studies, show that ZOL is an effective bone-targeted drug delivery carrier. This multipronged strategy of coadministration of chemotherapeutic drugs and bone-targeted drugs may help supplement the therapeutic effect by reducing the dose of bioactive parts and drug capsule delivery carriers.

Chen et al[35] developed a bone- and tumor-targeted nanodrug delivery system containing a PLGA core and polyethylene glycol succinate (ALN-TPGS)/FA-TPGS coating to selectively release paclitaxel into bone tumor areas. Paclitaxel-loaded ALN-TPGS/FA-TPGS/PLGA NPs (AFTPNs), on the one hand, have a strong affinity for hydroxyapatite in bone, resulting in specific binding; on the other hand, AFTPNs significantly enhance the cytotoxicity of 4T1 cancer cells with high folate receptor expression by promoting cell uptake. What is exciting is that paclitaxel-carrying AFTPNs targeting both bone and 4T1 tumor cells accumulate well in bone metastases in vivo, which effectively inhibits tumor progression and improves the survival rate of treated mice. Mice treated with paclitaxel-AFTPNs significantly reduced bone destruction and bone loss while preventing adverse effects on their normal tissues. These results suggest that paclitaxel-AFTPN, a dual nanodrug delivery system, has certain potential to be used in the treatment of metastatic breast cancer in the future.

Miller et al[36] reported a conjugate of N-(2-hydroxypropyl) methacrylamide (HPMA) copolymer, ALN and paclitaxel, which can reduce microvessel density and induce apoptosis of circulating endothelial cells in mice, thus playing an antiangiogenic role. With the participation of the HPMA copolymer, the inhibitory effect of this conjugate on bone metastases of 4T1 breast cancer is better than that of paclitaxel alone or in combination with ALN. Moreover, in contrast with the clinically used paclitaxel formulated in cremophor/ethanol, treatment with bone-targeted polymer conjugates had greater efficacy, higher tolerability and intravenous administration.

Nguyen et al[37] designed targeted nanoparticles with PLGA as the core, ALN-modified lipids and doxorubicin, which can achieve the targeted treatment of bone tumors. As one of the three-layer structures of nanostructures, ALN plays a role in stabilizing and driving nanoparticles to reach the target. The closely packed phospholipid-conjugated ALN in the nanostructure can form a hydrated shell that prevents the nanoparticles from being decomposed. The bone targeting efficiency of the drug in vitro was studied in the bone model established by hydroxyapatite crystal, and it was found that it had obvious accumulation. The selectivity of the drug compared to internalization in non-targeted mouse melanoma was confirmed by internalization study. Therefore, the nanodrug can not only target bone but also provide a large number of antitumor drugs to improve the therapeutic effect.

Xu et al[38] prepared organic-inorganic hybrid nanoparticles composed of a hyaluronic acid/polyethylene glycol polymer shell and a nanohydroxyapatite core to carry ZOL. The results of a series of in vivo and in vitro studies show that the nanoparticles have a stable structure, high drug loading efficiency, sustained release effect and good biocompatibility. It has also been confirmed that the application of low-dose nanoparticles has low visceral toxicity and low cytotoxicity, which is beneficial to the treatment of osteosarcoma in the future.

Bisphosphonate nanoparticle systems can also be used to transport DNA to participate in gene therapy. Mekhail et al[39] synthesized an ALN-functionalized gelatine biopolymer that can target DNA to osteoblasts for gene therapy. It was proven that the transfection efficiency of the polymer combined with DNA and Gemini surfactant on embryonic kidney cells and osteoblast-like cells was higher than that of the control group. Finally, ALN gelatine can be used for bone-targeted drug delivery and can participate in the gene delivery of gene therapy.

Polymer nanoparticles can protect unstable loads and deliver them smoothly to the target site, so they have the potential to improve drug delivery. However, there are many biological obstacles to achieving effective drug delivery, including the ability to evade the immune system, target specific cells and tissues, and transport load to specific areas of the cell. As a result, examples of polymer delivery systems used in the clinic are still limited.

## Metal–Organic Frameworks Nanoparticles

In recent years, metal-organic frameworks (MOFs), as unexpectedly developing porous organic-inorganic hybrid materials, have been extensively used in the research of

integrated smart carriers for cancer diagnosis and treatment. They consist of metallic ions/ion clusters as nodes and organic ligands as pillars bridged by coordination bonds. MOF materials have steadily become possible smart carrier materials in the application and research of cancer diagnosis and treatment due to their outstanding properties, such as easy synthesis, various compositions, high porosity, large particular surface area, adjustable size, easy surface modification, and good biocompatibility. Therefore, MOF nanoparticles with the potential to carry a certain concentration of anticancer drugs are expected to become a new method of drug delivery.[40–42]

Au et al[43] reported an organic skeleton (nMOF) ZOL consisting of a CaZOL core and PEG surface. At the same time, to improve the tumor targeting of this nanosized metal-organic skeleton, they added folic acid (FOL)-targeting ligands to the nMOF. Their studies confirmed that FOL-targeted CaZOL nMOFs can effectively inhibit cell proliferation and induce apoptosis, thus significantly improving the antitumor activity of ZOL in vivo.

Pang et al[44] used ZOL to functionalize immunostimulated cytosine phosphate ZOL (CpG-) nanoparticles into bone-targeted immunostimulatory MOF nanoparticles. In vitro studies have confirmed that this type of MOF nanoparticle can not only promote the polarization of macrophages to the proinflammatory M1 phenotype but also reduce the formation of osteoclasts and inhibit the formation of bone resorption. What is more encouraging is that in vivo studies have proven that it can effectively reduce the osteolysis and destruction caused by bone metastasis in breast cancer model mice and slow the growth and progression of tumors. The functional MOF system developed by these researchers has injected new ideas into the treatment of tumor bone metastasis and laid a foundation for further exploration in the future.

Of course, while paying attention to the achievements that MOFs bring to the development of bone-targeted therapy for tumors, their shortcomings should also be considered. Research on drug delivery based on MOFs is still in the basic research stage, and its metabolic process, metal residue and safety should also be considered. Researchers should further study the metabolism and safety of MOFs in vivo. Due to the lack of clinical practice, drug delivery systems based on MOFs still lack strict standards. We should put forwards preliminary quality standards for the application of MOFs in the field of biomedicine to ensure the quality of basic research. We should increase the research investment in MOFs from production to clinical application, metabolism and safety in vivo, promote the communication of biology, chemistry, materials, medicine and other disciplines, and realize the transformation of clinical results based on MOF drug delivery systems.

## Metal/Inorganic Nanoparticles

Inorganic nanoparticles include metal nanoparticles, carbon nanotubes and silicon-based nanoparticles, among which metal nanoparticles are highly valued in cancer immunotherapy because of their accuracy and controllability of transmission. Compared with other nanoparticles, metal nanoparticles have a higher density, are more easily absorbed by bones, have unique optical characteristics and can be used in tumor ablation combined with immunotherapy regulated by metal nanoparticles. Inorganic nanoparticles have high drug loading, convenient functional modification and no immunogenicity, and thus they are good drug carriers and tumor therapeutic agents.

### Ag2S QD-Based Nanosystem

In the study of Li et al,[45] doxorubicin was encapsulated in the hydrophobic layer around the $Ag_2S$ quantum dots connected to the surface of ALN, thus achieving the dual effects of bone-targeted drug delivery and tumor chemotherapy. This ALN/doxorubicin@$Ag_2S$ nanosystem specifically arrives and remains in bone tissue, and at the same time, triggered by the tumor microenvironment with certain characteristics, doxorubicin is specifically released to the tumor site on demand, thus specifically killing cancer cells and minimizing toxicity to normal bone marrow tissue. At the same time, the nanosystem effectively inhibits osteoclast formation and promotes ALN-mediated osteogenic differentiation, thus significantly avoiding cancer-related osteolysis.

### Mesoporous Silica Nanoparticles

Silica nanoparticles show superb potential in the discipline of tumor diagnosis and therapy due to their special physical and chemical properties. A drug delivery system based on silica nanoparticles can passively or actively target tumor tissues and achieve controllable release of drugs at tumor sites through stimulus responses, efficiently enhancing the concentration of antineoplastic drugs at tumor sites and contributing to the high treatment efficiency.

Sun et al[46] prepared ZOL-anchored mesoporous silica nanoparticle (MSN)-targeting skeletons, which were characterized by temporally and spatially controllable delivery of doxorubicin. They used BPSs not only as a medicine for

treating bone diseases but also as a combination of these two characteristics of bone targeting ligands for the therapy for bone metastases. The antitumor results indicated that doxorubicin@MSN-ZOL exhibited excellent toxicity against human lung adenocarcinoma cells and markedly impaired cell migratory abilities in vitro. This drug delivery system is expected to be the treatment of cancer bone metastasis in the future.

### Iron Oxide Nanoparticles

Lalatonne et al[47] prepared a bone-targeting nanosystem of dimethylol ferric bromide nanoparticles with a superparamagnetic surface coupled with bisphosphonic acid molecules, which was combined with a magnetic contrast agent of magnetic resonance imaging and bisphosphonates. They confirmed that bisphosphonate nanoparticles have a strong adsorption effect on hydroxyapatite through adsorption studies and magnetic resonance imaging measurements. In addition, cell experiments show that the hybrid nanomaterial has no toxic effect on cells, suggesting that the system can be used for diagnosis and treatment.

### Carbon Nanotube Nanoparticles

Dlamini et al[48] attached the synthesized bisphosphonates (alendronate, pamidronate and nelidronate) to multiwalled carbon nanotubes (MWCNTs). MWCNTs are a novel type of nanotherapeutic entity with great potential. Because of their unique and excellent properties, MWCNTs have become an ideal drug delivery system. It not only allows different functional groups to functionalize different drug parts introduced by noncovalent and covalent drugs but also carries various kinds of parts designed for new treatments or diagnoses. After research, it was observed that the drug release curve was associated with the pH value and the drug delivery system. BP-conjugated MWCNTs tend to release faster at lower pH values. After the combination of ALN and MWCNTs, the cell survival rate decreased, indicating that the efficiency of ALN combined with multiwalled carbon nanotubes was improved. The ultimate conclusion is that bisphosphonates combined with water-soluble MWCNTs are anticipated to be more effective in treating cancer. Due to the enhancement of targeted release of active drugs in human breast cancer cells, the efficacy has been improved.

## Conjugated with Therapeutic Proteins

The targeting properties of BPs can be achieved by synthesizing dendritic molecules with protein modifications. To impart protein mineral affinity, BPs are chemically bound to therapeutic proteins. This conjugate, when implanted into the mineral matrix, controls the local delivery kinetics of the protein.[49]

Murphy et al[50] synthesized model peptide-based molecules containing poly (aspartic acid), poly (glutamic acid), or a bisphosphonate (pamidronate) to confer bone-binding properties to proteins and other biological agents that lack specific targeting capacity. Their results show that the adsorption of coupling peptides on hydroxyapatite is significant, indicating that bisphosphonates and oligopeptide conjugates have a broad prospect in improving new bioactive molecules for bone-specific applications.

Protein BP conjugates are being explored as new therapeutic agents, in which protein components are expected to provide pharmacologically active parts, while BP components will give protein mineral affinity.

## Polymeric Micelles

The polymer micelle has a hydrophobic interior core and a hydrophilic outer shell and has the characteristics of simple preparation, simultaneous loading of multiple drugs and easy surface modification. Hydrophobic drugs can be enveloped in the hydrophobic core of micelles, and hydrophilic drugs can be loaded by chemical coupling.

Xue Dong and associates[51] designed an innovative multifunctional redox responsive CD44 receptor targeting the polymer drug alendronate-hyaluronic acid-S-curcumin copolymer (ALN-OHA-S-S-CUR) based on CUR and ALN according to the tumor growth environment. Cell uptake experiments showed that the embedded ALN-OHA-S-S-CuR micelles could efficiently transport CuR into the cytoplasm. The results of cell uptake and cytotoxicity experiments show that ALN-OHA-SS-CUR micelles loaded with CuR have reduced responsiveness and CD44 receptor targeting. The three-dimensional permeation experiments show that the polymer micelles developed by them can enter the tumor tissue, indicating that the micelles have a certain potential to become a tumor therapeutic drug delivery system. This approach additionally provides a novel research idea for improving the stability and solubility of hydrophobic drugs.

Liu et al[52] functionalized an amphiphilic triblock copolymer (PEG-PGlu-PPhA) composed of polyglutamic acid, polyphenylalanine and polyethylene glycol with ALN to prepare bone homing polypeptide-based polymer micelles with chemotherapy docetaxel for the treatment modalities to cure bone metastases of breast cancer, which slowed

disease progression and improved the survival rate in a syngeneic murine model of breast cancer bone metastasis. Using an immunologically active mouse breast cancer model that spreads to the bones, the study confirmed that targeted micelle-based therapy significantly leads to a reduction in tumor burden and prolongs the survival rate of animals, demonstrating the effectiveness of this targeted strategy for delivering chemotherapy to the bone.

Polymer micelles self-assembled by amphiphilic block copolymers have many advantages, such as improving water solubility, improving biocompatibility, reducing side effects and prolonging cycle time. However, micelle distribution and drug release are difficult to track in cancer cells. Therefore, a drug delivery system with both diagnostic imaging and treatment capabilities is needed.

## Polymer-Drug Conjugates

In the polymer-drug conjugation system, drugs are conjugated with polymers and delivered as covalent conjugates with water-soluble and biodegradable polymers. The coupling of therapeutic drugs and polymer carriers has various benefits in pharmacy and clinical treatment, such as improving the solubility of drugs, prolonging circulation in the body, controlling release and enhancing safety. Today, different development types of polymer-drug conjugates (including polymer nanoparticles, polymer-small molecule drug conjugates, dendrimers, polymer-macromolecule conjugates) have accomplished the latest positive results progress.

### Polymer-Small Molecule Drug Conjugates

The combination of small molecular bioactive substances with polymer carriers can improve the water solubility of drugs, enhance stability, prolong the half-life of plasma, and deliver in living cells. There are many advantages, such as changing the biological distribution in vivo and the possibility of targeted delivery by adding targeted parts.

Schott et al[53] covalently coupled the amino-bisphosphonate ALN with the anti-metabolic 5-fluoro-2′-deoxyuridine (5-FdU) to produce N4- (butyl-(4-hydroxy-4-phosphono)phosphate) −5 -fluoro-2′-desoxyuridine (5-FdU-alendronat, 5-FdU-ALN), which was an effective and innovative duplex drug targeting bone. Finally, 5-FdU-ALN, prepared by coupling amino-bisphosphonates and antimetabolites through N-alkyl bonds, has preferential cytotoxicity to cancer cells in contrast to the control group and much less toxicity in vitro and in vivo. It is expected to be used in the clinical treatment of bone metastasis in the future.

Yuan et al[54] thought that multiple chemotherapeutic drugs with similar properties could have synergistic effects in the treatment of cancer; thus, ZOL was coupled with PLGA nanoparticles containing a variety of anticancer drugs, namely, gemcitabine and epirubicin. In an in vitro experiment at the cellular level, the new nanocarrier enhanced the uptake of nanoparticles by human osteosarcoma cancer cells and increased the regression of tumors in mice in vitro.

To achieve the purpose of targeted therapy of osteosarcoma, Zhao et al[55] prepared paclitaxel nanoparticles with dopamine as a surface modification and ALN as a ligand by dopamine polymerization. In vitro experiments showed that the inhibitory effect of targeted nanoparticles containing ALN on osteosarcoma cells was stronger than that of nontargeted nanoparticles. Similarly, in vivo distribution studies have shown that the targeted nanoparticle system has significantly more accumulation in tumors than nontargeted nanoparticle systems. In addition, the therapeutic effect of ALN-targeted nanoparticles is better than that of paclitaxel and can significantly reduce the side effects of paclitaxel. In summary, a series of experiments and data show that the ALN-mediated nanoparticle system may be an effective method for targeted therapy of osteosarcoma.

### Dendrimer Nanoparticles

Dendrimer polymers are mainly used in drug-polymer conjugation in the direction of drug delivery. Dendrimers are synthetic polymer materials with clear three-dimensional structures that consist of three basic units: core, core repeating unit and surface functional group. Its mass, size, shape and surface chemical properties can be controlled with high precision. Dendritic polymers such as PAMAM and PEI have traits such as clear chemical structure, monodispersity, easy realization of multiple functions and multivalent modification and have been broadly studied.

Shao-bo Bai and associates[56] used the solvent evaporation approach to prepare pH-sensitive nanoparticles containing ALN and polyamidoamine (PAMAM) with docetaxel-loaded ALN-PAMAM nanoparticles to treat bone metastases from lung cancer. In vitro results showed that docetaxel considerably enhanced the anticancer activity of docetaxel and suppressed osteoclast formation. Docetaxel@ALN-PAMAM delivers docetaxel and ALN to bone metastases at the same time, showing a synergistic impact that appreciably enhances the

anticancer activity of docetaxel and inhibits the formation of osteoclasts and inhibits bone resorption, pain response and the growth of bone metastases. Therefore, ALN-modified PAMAM enhances the therapeutic effect in the body.

Although a number of polymer nanoparticle conjugates have entered clinical development, they have not been approved to be put on the market thus far. Despite repeated setbacks in this field, polymer-drug coupling technology is becoming increasingly mature. As an increasing number of new drugs in this field enter the late clinical stage, polymer-drug coupling will enter the harvest period of the market in the next few years.

## Liposomes

Liposomes are hollow synthetic spherical particles, and the outer shell is bimolecular lipid. In current targeting guides and vector systems, liposomes have attracted remarkable interest due to their simple preparation, nontoxicity, non-immunogenicity, convenient carrying and release of a range of drugs, and modification to deliver the target drug to a specific site.[57,58]

According to the research of La-Beck et al,[59] ZOL liposomes and ALN formulations improve the anticancer efficacy of chemotherapy with cytotoxic agents and adoptive T cell immunotherapy in mouse models of cancer. To enhance the transport and delivery of antineoplastic drugs, they chose a long-cycle, leak-free polyethylene glycol liposome formulation. Formulations with "invisible" properties can enhance permeability and retention, thus increasing the accumulation of drug payloads in tumor tissues. They passively encapsulated ZOL or ALN (sodium or ammonium salt) in the aqueous segment of liposomes. They proposed that pegylated alendronate liposomes (PLAs) have a good therapeutic index, can be passively targeted and accumulate in tumors and that the biocompatibility of liposome carriers and preclinical anticancer efficacy have the greatest clinical translation potential.

Dos Santos Ferreira et al[60] prepared ALN-coated pH-sensitive liposomes containing adriamycin and compared them with nontargeted liposomes or free doxorubicin to evaluate the efficacy of the above drug preparations in a mouse breast cancer bone metastasis model. After a series of studies on antitumor activity, it was found that drug delivery modification, encapsulation of pH-sensitive liposomes and liposome surface modification of ALN bone-targeting carriers are the keys to obtaining stronger antitumor activity. The results demonstrate that the addition of the bone-targeting ligand ALN to the surface of encapsulated doxorubicin in pH-sensitive liposomes further increased the tumor specificity of this drug, allowing for an improved antitumor therapeutic effect. Compared with free doxorubicin, this higher specificity helps to reduce toxicity to the heart, thereby improving the survival rate of patients and improving the treatment index.

Similarly, Song et al[61] introduced Brij78 (polyoxyethylene stearyl ether) and pamidronate (Pa)-conjugated PA-Brij78 into DPPC/Chol liposomes to prepare PA surface functional liposomes. The in vitro binding of PB liposomes embedded with adriamycin to hyaluronic acid (HA), the temperature-dependent release of doxorubicin and the cytotoxicity were studied. The preparation could quickly release the active drug through gentle heating, and when combined with HA, the main component of bone, it was still temperature sensitive. Then the liposome complex was used to treat human lung cancer A549 cells to simulate the treatment of tumors bone metastasis. Their results indicate that this temperature-sensitive bone-targeted liposome preparation as a drug delivery system has considerable potential for the treatment of tumor bone metastasis or other bone diseases.

Because low molecular weight heparin (LMWH) can prolong the blood circulation time of liposomes and show an antimetastatic effect, Hao Wu and associate[62] modified liposomes with LMWH and ALN to deliver the antineoplastic drug doxorubicin. The mechanism may be that heparanase secreted by tumor cells can degrade LMWH and eliminate the shielding of lipids in vitro. In addition, the interaction between heparanase and LMWH also increased the retention time of liposomes. The results of pharmacodynamic evaluation in orthotopic osteosarcoma model and bone metastatic cancer model showed that the system could significantly inhibit tumors growth and metastasis.

Luisa Stella Dolci and associates[63] used spray coagulation technology to load a considerable quantity of ALN (up to 30% w/w) with solid lipid microparticles (MPs) into biomimetic calcium phosphate cement. Using a coculture model of osteoblasts and osteoclasts, in vitro experiments were carried out on bone cement with the best drug release properties, coagulation, hardening, and mechanical properties. The results show that the method of strengthening bone cement adopted by researchers can effectively improve the effect of ALN on inhibiting excessive bone

resorption and promoting bone formation. Moreover, the combination of MPs and ALN can inhibit the activity of osteoclasts and promote the activity and early differentiation of osteoblasts. It is an appropriate bisphosphonate sustained-release system.

Chang et al[64] developed paclitaxel-, alendronate- and transferrin-modified liposomes (ALN-/Tf-modified paclitaxel-L) based entirely on dioleoyl phosphatidic acid (DOPA), which mediates bone affinity and tumor targeting provided by transferrin by means of DOPA and the phosphate group in alendronate. The drug delivery system has a potential bone affinity because of the substantial divalent phosphate groups exposed to the surface of liposomes containing DOPA and alendronate and enhances the uptake of MM1S cells by modifying transferrin. In addition, they studied the advantages of this design by evaluating cell transport, cytotoxicity, apoptosis induction, and therapeutic effects in vivo. Finally, the study validated that ALN/TF-modified paclitaxel-L has excellent stability, an excessive HAP binding rate and can improve cell uptake, cytotoxicity and the potential to result in cell apoptosis. Moreover, the significance of this design in terms of bone targeting and antitumor effects in vivo has been verified.

Marra et al[65] developed an invisible liposome formulation containing zoledronic acid (LipoZOL) as a drug delivery system, which reduces the binding of ZOL to bone by enhancing the permeability retention effect and improves its extraosseous tumor site bioavailability. In addition, they used two different in vitro and in vivo models of human cancers to study whether the LipoZOL formula has stronger antitumor properties than standard (free) ZOL. The inhibitory effect of LipoZOL on the growth of different cancer cell lines in vitro was better than that of free ZOL. Similarly, the tumor growth inhibitory effect of LipoZOL on a multiple myeloma mouse model and human prostate cancer was remarkably stronger than that of ZOL, and the overall survival rate was improved. In addition, in prostate cancer transplanted tumors, after treatment with LipoZOL, angiogenesis events were strongly suppressed without evidence of necrosis. To further explore this hypothesis, they also confirmed that LipoZOL has good antitumor activity and tolerance in preclinical animal models of solid and haematopoietic malignant tumors.

Anada and his collaborators[66] synthesized a novel amphiphilic molecule containing a BP head group that can recognize and bind hydroxyapatite (HA). They confirmed that adriamycin-loaded liposomes containing BP groups have a high affinity for HA, and thus adsorption on the surface of HA can drastically inhibit the growth of tumor cells. This indicates that liposomes, particularly those targeting bone tissue, can act as extraordinary carriers for antitumor drugs. Bisphenol A liposomes can be selectively distributed on the bones, which is helpful for parts with a high turnover rate. In addition, BPA liposomes may play an essential role in improving the permeability and retention of targeted drug delivery systems for bone.

Although liposomes have more advantages as carriers than free drugs, liposomes still have limitations in tumor delivery efficiency and nontargeted accumulation. To improve the antitumor activity of nanodrugs, it is necessary to better understand the extravasation mechanism of nanodrugs in different tumor types and normal blood vessels.

Table 2 summarizes the application of the above antineoplastic drugs and new carriers in bisphosphonate drug delivery systems.

## Microspheres

The microsphere refers to the particle dispersion system formed by drug dispersion or adsorption in a macromolecule or polymer matrix. As microsphere preparation has a long-term and slow-release or targeting effect, it can greatly enhance the convenience and adherence of patients, which has highlighted its advantages in the clinic and is a potential dosage form.

Shi et al[67] used solid/oil/water (s/o/w) or water/oil/water (w/o/w) technology to prepare new polylactic acid-glycolic acid (PLGA)-cross hydroxyapatite (HA) microspheres, in which HA particles were used as the BP release medium. In this study, ALN was loaded on HA nanoparticles through strong BP-Ca chelation, and the HA particles loaded with ALN were further wrapped in PLGA. The prepared microspheres can be released stably for a long time, and in in vitro studies, the PLGA/HA-ALN controlled release system can inhibit the growth of osteoclasts and promote an increase in osteoblasts, thus promoting bone repair. From this study, it can be concluded that PLGA/HA hybrid microspheres have the conditions to become and perform well as carriers of ALNs and are likely to be used as injectable multifunctional carriers in the specific application of bone repair therapy in the future.

In the past, many researchers have used various microsphere drug delivery systems made of biodegradable synthetic polymers, such as PLGA and PCL.[68,69] Although synthetic polymers are relatively simple to use in the research and development of pharmaceutical preparations, there are some shortcomings in the application of

**Table 2** Chemotherapeutic Drugs and Their Carriers Used in Bisphosphonate Drug Delivery System

| Drug Carrier | Reference | Method Type | Chemotherapeutic Drugs |
|---|---|---|---|
| Bone-targeting phospholipid polymer (contains alendronic acid) | [28] | Polymeric nanoparticles | Docetaxel |
| Coupling of ZOL with PLGA nanoparticles | [34] | Polymeric nanoparticles | Methotrexate |
| Alendronic acid -TPGS/FA-TPGS/PLGA nanoparticles | [35] | Polymeric nanoparticles | Paclitaxel |
| Coupling of alendronic acid and N-(2-hydroxypropyl) methacrylamide Copolymer | [36] | Polymeric nanoparticles | Paclitaxel |
| Nanoparticles with PLGA as the core and alendronic acid modified lipids | [37] | Polymeric nanoparticles | Doxorubicin |
| The hydrophobic layer surrounding the Ag2S QDs (alendronic acid is connected to the surface) | [45] | $Ag_2S$ QD - based nanosystem | Doxorubicin |
| Mesoporous silica nanoparticles anchored by ZOL | [46] | Mesoporous silica nanoparticles | Doxorubicin |
| Bone homing polypeptide-based polymer micelles (contains alendronic acid) | [52] | Polymeric micelles | Docetaxel |
| Alendronic acid -PAMAM nanoparticles | [56] | Dendrimer nanoparticles | Docetaxel |
| PH sensitive liposomes encapsulated with alendronic acid | [60] | Liposome | Doxorubicin |
| Pamidronate-Brij78 liposomes | [61] | Liposome | Doxorubicin |
| Liposomes modified with alendronic acid and LMWH | [62] | Liposome | Doxorubicin |
| Alendronic acid and transferrin modified liposomes based on dioleyl phosphatidic acid | [64] | Liposome | Paclitaxel |
| Bisphenol A liposomes (contains bisphosphonates groups) | [66] | Liposome | Doxorubicin |

polymers; that is, they may contain residues or impurities of initiators and other compounds, which may not be able to achieve the most effective cell growth.

The repair of bone defects through tumor bone metastasis is a series of complex biological events controlled by a series of bone growth factors.[70] Based on the fact that chitosan (CH) has a series of distinct characteristics, such as biodegradability, low toxicity, good biocompatibility and promotion of absorption, Wu et al[71] constructed chitosan and nanohydroxyapatite (NHA)-ALN complex microspheres by emulsification and cross-linking strategies. Finally, the loading effectivity and sustained release of hydrophilic ALN have been notably improved so that it can be locally and constantly released in the bone nanoenvironment. In the study of osteogenesis in vitro, this system reflects its ability to enhance the osteogenic activity of animal adipose stem cells. As a result, CH/nHA-ALN composite microspheres have the potential to be used as local materials for the treatment of bone defects.

Likewise, Wu et al[72] prepared a novel microsphere-scaffold hybrid system (CM-ALNs) for bone tissue engineering applications and improved drug delivery by combining a poly(L-lactic acid)/nanohydroxyapatite (PLLA/nHA) matrix with chitosan/hydroxyapatite microspheres (CH/nHA-ALN) loaded with ALN (CH/nHA-ALN). Among them, the natural biomaterial chitosan is used as a drug controlled release adjuvant in the above system. They studied the characteristics and osteogenic differences of the scaffold system in vitro and studied the overall performance of bone regeneration in vivo in a rabbit model of severe segmental bone defects. The results show that CM-ALNS scaffolds are promising for use in bone tissue engineering and drug delivery in the future.

In addition, to effectively release bisphosphonate ALN locally and controllably, Chan Woo Kim and associates[73] innovatively adopted bioabsorbable calcium phosphate (CAP) microspheres and an in situ preparation strategy. The important advantage of this method is that microsphere carriers with excellent performance can be prepared, the load of the microsphere can be synchronized with the formation of the microsphere, and the load of ALN is allowed to be higher. The in vitro experimental results preliminarily confirmed that the bioabsorbable cap microspheres had an effect on inhibiting the formation and proliferation of osteoclasts.

In addition, Juan P Cattalini and associates[74] prepared ALN microspheres and effectively loaded them into a novel multifunctional nanocomposite scaffold, which was cross-linked by therapeutic ions (such as calcium ions and copper ions) with nanobioactive glass and alginate with high entrapment efficiency. Among them, ALN is introduced into the stent with high entrapment efficiency and effectively released from the stent in a controlled manner. In terms of viscoelasticity, the biological activity and mechanical properties of the scaffold are close to those of endogenous cancellous bone tissue. In addition, the extract of the biomaterial used in the scaffold promoted the formation of bone vessels in bone and endothelial cells in vitro, indicating that the matrix of the scaffold has the potential to promote bone repair and regeneration and angiogenesis.

Although the idea of combining drug-loaded microspheres with more drugs is mostly in the animal experimental stage, the experimental results show great advantages and reveal the new direction of bone targeted therapy of drug-loaded microspheres.

## Microcapsules

Microcapsule refers to the drug library microcapsule in which the solid or liquid drug (capsule core) is wrapped by the capsule material. The drugs contained in microcapsules can be released in specific parts and media, and have different release characteristics, such as slow release, controlled release or targeted release.

Bisphosphonates can also be used in the diagnosis of bone-related tumors, in which the specific method of fluorescence imaging is to combine bisphosphonates with fluorescent dyes or nanoparticles containing fluorescent molecules for fluorescence imaging of bone. Robert V. Bell and associates[75] proposed an effective method for preparing calcium phosphate (CAP) microcapsules using stable oil-in-water droplets to synthesize branched copolymers as templates. They successfully prepared the fluorescent capsule by connecting the BP conjugate containing fluorescein to the cap capsule. The wall of the microcapsule was decorated with a fluorescein-bisphosphonate conjugate to make it fluorescent. The fluorescence property of the fluorescein group comes from the adsorption mode of Fluo-BP and the fluorescein group, in which the adsorption mode of Fluo-BP on the cap shell is due to the coordination covalent interaction between the calcium atom in the cap shell and the phosphate group in BP. The BP section allows modifications in the chemical structure, imparting the possibility of offering particular functional groups as sections of the capsule wall. It is worth noting that acidified BP molecules can still be recovered by the dissolution of the capsule wall, suggesting that they may be used for diagnosis.

However, at present, microcapsules still lack a simple encapsulation method that is suitable for all core materials; the operation method is not continuous, which is not conducive to joint production, and it is not easy to recycle when the microcapsules are out of waste.

## Bisphosphonate Conjugate

Chemotherapeutic drugs can eliminate or reduce some bone tumors and reduce the occurrence of bone-related events, but at the same time, chemotherapeutic drugs can also have many adverse effects on nontarget organs, which may significantly limit the number of drugs that patients can use. In this case, if the therapeutic drug can be directed to the tumor in the bone, it will be an effective treatment. An instructive mechanism for overcoming this difficulty is the coupling of antineoplastic drugs to bisphosphonates, which allows drugs to be delivered to bones and released in affected areas.[76,77]

Holmberg et al[78] developed a new type of polybisphosphonate conjugate that has both antibone resorption and antitumor effects. The bone organs of newborn mice were cultured with zoledronic acid as a positive control. The ability of polydiphosphonate conjugates to inhibit bone resorption of osteoclasts was detected by 45Ca-labelled bone mineral material in a bone resorption experiment. The tumor cytotoxicity of prostate cancer and breast cancer cell cultures was studied by fluorescence cytotoxicity tests and apoptosis tests. In the above studies, polydiphosphonate conjugates had stronger osteoclast inhibitory effects and enhanced antitumor effects than zoledronic acid.

Varghese et al[79] proposed a new synthesis method of hyaluronic acid-bisphosphonate conjugates with free hydrazide functional groups, which can be used as antiosteoclast and antitumor agents in injectable hydrogel formulation drugs. The effectiveness of prodrugs lies in the fact that HA-BP, which is cleaved to a suitable size by Hase, is internalized by CD44-positive cells under receptor-mediated endocytosis. The hyaluronic acid-bisphosphonate complex in the form of hydrogel can be released controllably at the implant site while preventing systemic exposure to the drug. The hydrazide groups in hyaluronic acid-bisphosphonate complexes are usually used for cross-linking and integration into hydrogel matrices and can also be used to explore the acyl hydrazone connection of other drug molecules.

Agyin et al[80] creatively prepared a proteasome inhibitor-bisphosphonate conjugate, which can strongly attenuate the growth and proliferation of 5TGM1 and RPMI8226 myeloma cell lines in vitro. In addition, the selection of suitable binders for bisphosphonates is necessary for the effective synthesis of these bone-targeted affinity conjugates because connectors that are too stable cannot release drugs, and connectors that are too unstable may lead to early drug release. This study lays a foundation for providing effective bone-targeting drugs for curative therapy for multiple myeloma.

Wang et al[81] created a new bone-targeted bortezomib proteasome inhibitor by using bortezomib to connect BP residues lacking anti-osteoclast activity with new chemical ligands. Their study creatively confirmed that a bone-targeted bortezomib conjugate has a good antitumor effect on multiple myeloma in mice. There are two innovations in drug design. First, it uses BP, with no anti-absorption activity, which allows us to validate the effect of bortezomib separately. The other is to use a chemical connector that stably exists in the blood, but this connector is likely to be hydrolysed under the folding of osteoclasts at the absorption site in an acidic microenvironment, thus releasing the active drug from the bone surface. The results showed that the combination of BP-bortezomib and bone matrix could reduce the burden of multiple myeloma and that the effect of reducing bone loss was significantly better than that of bortezomib alone. BP-bortezomib may represent a new treatment method for patients with multiple myeloma, with better overall efficacy and fewer systemic adverse reactions.

## Prodrug

Through prodrug design, modifying the drug into a nontoxic prodrug and releasing the original drug after activation under specific conditions is a common strategy in medicinal chemistry. Antitumor prodrugs can not only improve the physical and chemical properties and pharmacokinetic behaviour of the drug but, more importantly, through reasonable drug design, can also improve the tumor targeting of chemotherapeutics and realize the selective and controllable release of chemotherapeutics, reduce the poisonous and adverse effects of the drug and improve its antitumor activity.

By covalently conjugating the antitumor compound doxorubicin to a bone-targeting hydroxy bisphosphonate carrier, Emmanuelle David and associates[82] converted the 12b80 compound into a prodrug through a customized linker designed to specifically trigger the release of doxorubicin in the acidic bone tumor microenvironment. In rodents, 12B80 rapidly and consistently targets bone tissue and tumor-associated ectopic bone and loads more effectively with adriamycin in the tumor bone environment than unresected adriamycin. Thus, 12B80 showed significantly lower toxicity than doxorubicin, promoting a strong antitumor effect against bone-related tumors in rodents, showing the quantitative strength of a certain effect in animals at dose and being more effective than doxorubicin/zoledronic acid combination. Their study end results may additionally be used for reference in targeted therapy of bone metastasis.

Webster et al[83] designed and synthesized a bisphosphoramide prodrug, which releases the corresponding bisphosphonates through intracellular activation and can unveil multiple negative charges with only two enzyme activation events. Their results indicated that after the nitroaryl delivery group was reductively activated, the bisphosphonamide prodrugs were rapidly activated to release the corresponding BP. Then, they revealed that compared with the parent bisphosphonate, the two bisphosphonate prodrugs notably improved bisphosphonate activation and anticancer activity in vitro.

Tanaka et al[84] synthesized bisphosphonate prodrugs, which are catechol bisphosphonates that enter the cell, where esterase converts them into active acids. Bisphosphonates are more effective than their corresponding acids in stimulating γδ T cells to secrete TNF-α in response to a range of tumor cells. It was found that they efficaciously confined the increase in tumor cells. Thus, the study provided some evidence that the combination of nitrogen-containing bisphosphonates and γδT cells performed better than when used alone. The use of bisphosphonate prodrugs to bind γδT cells can significantly enhance the application of γδT cells in

immunotherapy of various tumors. The final result is that the combination of tetrakis-pivaloyloxymethyl 2-(thiazole-2-ylamino) ethylidene-1,1-BP and γδT cells with other cancer immunotherapies can treat an extensive variety of adult and child solid tumors that currently cannot be successfully treated with chemotherapy or targeted therapy.

In the study of Zhu et al,[85] a qualitative boron-targeted bortezomib-loaded micelle was constructed for the therapy of breast cancer-induced bone metastases. This composite micelle uses ALN as a bone-targeting ligand and encapsulated bortezomib-catechol conjugate as cargo. Both in vitro and in vivo studies have demonstrated that ALN-NP precursor drug micelles exhibit many beneficial properties, including reduced systemic toxicity and improved therapeutic efficacy, compared with free drugs or controlled micelles. Therefore, this work provides a new combined strategy for tumor-related bone-targeted therapy.

Hochdörffer et al[86] prepared two kinds of novel water-soluble prodrugs by synthesis. Using doxorubicin as an anticancer agent and BPs as the bone-targeting portion, anthracene can be released by enzymes or anthracycline antibiotics through the protease B enzyme under acidic pH conditions. The addition of acid-sensitive or cathepsin B cleavable junctions to doxorubicin-BP prodrugs is a positive approach to particularly release doxorubicin. Their studies have demonstrated the sufficient stability of water-soluble prodrugs in human plasma, as well as the high stability of hydroxyapatite and natural bone.

Matsumoto et al[87] synthesized a heterocyclic BP prodrug with a phosphonate moiety derived from a pivoxil group and lacking a hydroxyl "bone hook" on the prototype carbon. Lipophilic BP prodrugs were transformed into an active form by intracellular esterases when entering tumor cells. This study shows that BP prodrugs may additionally enhance the effectiveness of BP in the treatment of haematopoietic and nonhaematopoietic solid tumors. Among them, the most active BP prodrug is 2-(thiazole-2-ylamino) ethylidene-1,1-bisphosphonate (7). In addition to its direct effect on tumor growth, the compound can enhance the toxicity of VG2VD2T cells in vitro and can bind to selectively metastatic VG2VD2T cells in vivo to enhance disease control in tumor mouse models.

Mizuta et al[88] synthesized a prodrug analogue of fluorine-containing zoledronate, in which the phosphonate was partially masked by pivaloyloxymethyl, and found that they significantly enhanced Vγ2Vδ2T cell-mediated cytotoxicity, promoted the expansion of peripheral blood Vγ2Vδ2T cells, and therefore stimulated Vγ2Vδ2T cells to kill tumor cells. In short, the development of BP prodrugs can more effectively block isoprene biosynthesis, thereby reducing the growth of tumor cells and triggering tumor immunity through Vγ2Vδ2T cells, thereby opening up a new approach for cancer treatment.

Prodrug strategies have become an indispensable part of the research and development of bisphosphonate drug delivery systems, which were only regarded as rescue measures for new drug defects in the past. Moreover, it has been transformed into the optimal design in the early stage of bisphosphonate drug delivery system research and development, which undoubtedly plays an important role in improving the success rate of research and development, as well as improving the pharmaceutical properties and clinical effects of bisphosphonates.

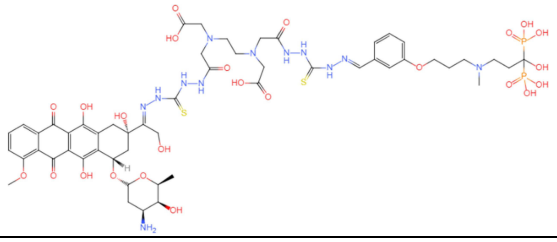
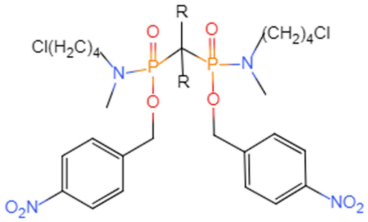
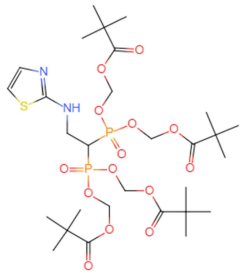
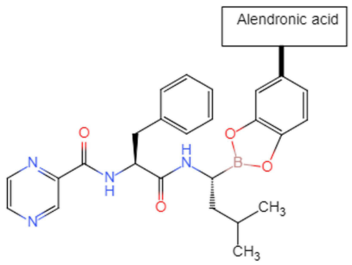
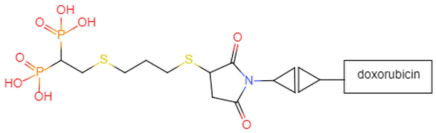
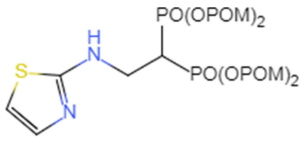
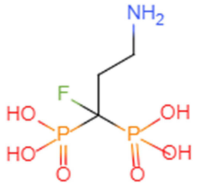
Table 3 summarizes the bisphosphonate prodrugs mentioned above for antitumor therapy.

## Bone Tissue Engineering

Bone tissue engineering aims to create biologically functional tissue that can be integrated and degraded in vivo to treat diseased or damaged tissue. It refers to the isolation of high concentrations of autologous osteoblasts, bone marrow stromal cells or chondrocytes. After culture and expansion in vitro, cells are implanted on a natural or artificial cell scaffold or extracellular matrix with good biocompatibility and can be gradually degraded and absorbed by the human body. The ultimate goal of bone tissue engineering is to repair bone tissue defects. The mechanism of bone tissue engineering combined with bisphosphonate in repairing bone is shown in Figure 2 In the previous overview of microcapsules, the application of bone tissue engineering has been mentioned, and the application of bisphosphonates in bone tissue engineering will be summarized below.

Because thermosensitive hydrogels are suitable for the treatment of bone injury, hydrogels formed in situ can promote the healing of injured bone by releasing drugs, growth factors and bioactive molecules. Morsi et al[89] prepared a chitosan-glycerophosphate thermosensitive hydrogel, which easily loaded risedronic acid, which inhibited bone resorption in situ. They arranged a series of studies to test and prove the in vitro characteristics and compatible osteogenic ability of the hydrogel carrier preparation. This hydrogel has good biological characteristics in vitro and can be used as an alternative to bone tissue engineering and related surgery. Thavornyutikarn et al[90] coated ALN

**Table 3** Prodrug of Bisphosphonate for Tumor Treatment

| Prodrug | Structure | Efficacy |
|---|---|---|
| 12b80 compound[82] |  | High affinity of bone support, specific release of doxorubicin, low cytotoxicity and cell uptake of prodrugs. |
| Bisphosphora–midate prodrug[83] |  | Significantly enhanced anti-cancer activity |
| Tetrakis-pivaloyloxy–methyl 2-(thiazole-2-ylamino) ethylidene-1, 1- bisphosphonate (7)[84] |  | Improve the effectiveness of related cancer immunotherapy |
| Prodrug micelles (alendronic acid - nanoparticle)[85] |   | Reduce systemic toxicity, improve therapeutic effect |
| Doxorubicin bisphosphonate prodrug[86] |   | Good stability and high affinity |
| 2-(thiazole-2-ylamino) ethylidene-1, 1-bisphosphonate[87] |   | Directly act on tumor growth, expand cytotoxic Vg2Vd2T cells in vitro, and enhance tumor control |
| Fluorine—containing zoledronate prodrug[88] |  | Sensitize tumor cells for killing, expand Vγ2Vδ2 T cells for adoptive cell therapy |

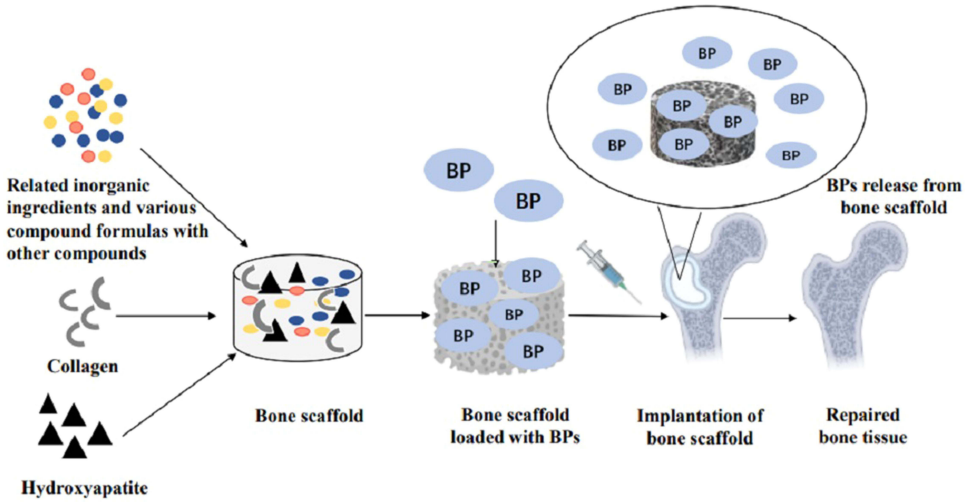



**Figure 2** Mechanism of bone tissue engineering combined with bisphosphonates to repair bones. The process shown here is to construct a bone scaffold with biomaterials and scaffold materials, load bisphosphonate on the bone scaffold, inject it into the bone defect site to repair the bone defect, and release BP molecules to inhibit osteoclast absorption and tumor growth.

on calcium phosphate (CAP)-modified sintered bioactive glass 45S5 (BG) scaffolds to investigate the effects of the scaffolds on the inhibition of osteoclasts and the stimulation of osteoblasts. By setting up a control group to observe its effect on cell proliferation, it was concluded that ALN-coated scaffolds inhibited the function of osteoclasts and promoted the activity of osteoblasts, thus promoting bone formation. More importantly, the study also found that low-dose ALN has a synergistic effect with bioactive glass-based materials.

Based on the fact that magnesium-strontium alloy has recently become a new bone substitute, Mei Li and other researchers[91] prepared magnesium-1.5wt.% strontium (Mg-1.5wt.%Sr) alloy loaded with ZOL and coated with calcium phosphate by coprecipitation method, and then studied the inhibitory effect and mechanism of ZOL-loaded alloy on tumors cells at cellular and molecular level, respectively. It has been found that the new alloy can inhibit the growth of tumor cells and osteolysis at both the cellular and molecular levels, which may be due to the alkaline environment produced by Mg-Sr alloy being conducive to the inhibition of ZOL on tumors. In general, this study confirmed that magnesium-strontium alloy loaded with ZOL has potential and broad application prospects in the treatment of bone defects caused by tumors.

In the study of Lu et al,[92] a multifunctional chitosan/nanohydroxyapatite-doped ZOL scaffold with excellent biocompatibility and bone induction was prepared. At the same time, the scaffold significantly inhibited the proliferation of bone giant tumor cells and decreased the osteoclast activity of tumor cells. In general, porous stents show a good effect in the repair of tumor-induced bone defects and the treatment of giant cell tumors of bone, which provides a new choice for the treatment of bone-related tumors.

Farbod et al[93] used PLGA microspheres, which can increase the degradation rate of materials as pore-forming agents, and hydroxyapatite nanoparticles loaded with platinum-bisphosphonate (Pt-BP) complexes were added to the powder phase of cement to accelerate the degradation of composite nanoparticles but did not affect the release rate of platinum species. The in vitro release kinetics study showed that the affinity and release rate of the modified Pt-BP complex with hydroxyapatite nanoparticles could be adjusted by changing its chemical structure and drug loading. In vitro experiments at the cellular level showed that they had stronger antiproliferative activity on human osteosarcoma cells than on human bone marrow mesenchymal stem cells. A series of studies have shown that hydroxyapatite nanoparticles loaded with PT-BP can cause injectable calcium phosphate cement to have chemotherapeutic activity.

The goal of bone tissue engineering is to produce materials with better properties than autogenous bone grafts and allogeneic bone grafts. When introducing biomaterials into the location of bone defects, clinical factors need to be taken into account. In the process of designing repair materials, some states of target bone tissue need to be considered, including defect-related and patient-related factors. In addition, when restoration is used in older patients, the natural

ageing of the bone microstructure and the decline in regenerative ability need to be taken into account.

## Conclusion

The main role of bisphosphonates is to prevent and treat bone metastasis and bone-related events, reduce bone loss caused by antitumor therapy, treat bone disease of primary bone tumors, and enhance the sensitivity of radiotherapy. However, due to the adverse reactions of the traditional administration of bisphosphonates, the compliance of patients is reduced. At present, research on novel methods to improve the delivery of bisphosphonates is booming, with various types of preparations, including nanodrug delivery systems, microspheres, microcapsules, prodrugs and bone tissue engineering. Among them, nanomedicine for bone tumors has made giant strides. In the current therapy of bone tumors, the emergence of nanodrugs has great potential. Nanomedicine has unique physical properties and can reach any part of the body accurately. Nanodrugs have the features of long blood circulation time, superior tumor selectivity, easy absorption by tumor cells, sensitivity to the tumor microenvironment, enhanced tumor inhibition effect and reduced side effects. It has emerged as a passionate spot in the research and development of new antitumor drugs. Combined with the research progress of the above new bisphosphonate preparations, a significant research and development trend is to combine clinical antineoplastic drugs or other small molecular drugs with BPs to target bones. At present, the combination drugs used by researchers are typically doxorubicin, bortezomib, paclitaxel, and so forth. Commonly used clinical antineoplastic drugs also include cytarabine, gemcitabine, and oestrogen. The associated application of bisphosphonates and these drugs is also very promising. The combined construction of bone tissue engineering and bisphosphonate can not only repair bone tissue defects but also inhibit tumor growth by local treatment. It can inhibit local bone resorption, promote local bone formation, and reduce the degree of bone metastasis and bone destruction.

However, it should be pointed out that most of the current understanding and prediction of the therapeutic effect of novel methods to improve the delivery of bisphosphonates are derived from in vitro and animal experimental data, and there are very few studies on transplanting these behaviours into humans. Moreover, there are few relevant data that can predict its safety and efficiency in humans based totally on the outcomes of animal experiments. A large variety of clinical trials have confirmed that external high-dose administration of bisphosphonates will bring about a series of adverse reactions, but no volatile, small-dose medication that can also cause similar adverse reactions has not been explored in depth. In addition, the currently developed new bisphosphonate formulations have not specifically investigated whether they can reduce the incidence of adverse reactions caused by traditional bisphosphonate administration methods and dosage forms.

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## Disclosure

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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