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Review Article

Pharmaceutical and clinical development of phosphonate-based radiopharmaceuticals for the targeted treatment of bone metastases

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ABSTRACT

Therapeutic phosphonate-based radiopharmaceuticals radiolabeled with beta, alpha and conversion electron emitting radioisotopes have been investigated for the targeted treatment of painful bone metastases for >35 years. We performed a systematic literature search and focused on the pharmaceutical development, preclinical research and early human studies of these radiopharmaceuticals.

The characteristics of an ideal bone-targeting therapeutic radiopharmaceutical are presented and compliance with these criteria by the compounds discussed is verified. The importance of both composition and preparation conditions for the stability and biodistribution of several agents is discussed. Very few studies have described the characterization of these products, although knowledge on the molecular structure is important with respect to *in vivo* behavior.

This review discusses a total of 91 phosphonate-based therapeutic radiopharmaceuticals, of which only six agents have progressed to clinical use. Extensive clinical studies have only been described for ^{186}Re-HEDP, ^{188}Re-HEDP and ^{153}Sm-EDTMP. Of these, ^{153}Sm-EDTMP represents the only compound with worldwide marketing authorization. ^{177}Lu-EDTMP has recently received approval for clinical use in India.

This review illustrates that a thorough understanding of the radiochemistry of these agents is required to design simple and robust preparation and quality control methods, which are needed to fully exploit the potential benefits of these theranostic radiopharmaceuticals. Extensive biodistribution and dosimetry studies are indispensable to provide the portfolios that are required for assessment before human administration is possible. Use of the existing knowledge collected in this review should guide future research efforts and may lead to the approval of new promising agents.

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1. Introduction

A serious complication of many solid and hematological cancers is the development of bone metastases, which often lead to severe pain, skeletal fractures, neurological symptoms and hypercalcaemia. These complications result in a significant decrease of the quality of life, increased health costs and shorter survival [1–3]. At this later stage of the disease, pain palliation with strong analgesics (typically opioids) or external beam radiation can have serious side effects, and are not always feasible or effective. Bone-seeking therapeutic radiopharmaceuticals constitute an attractive alternative treatment option for osteoblastic bone metastases - especially for multiple lesions - because of their specific targeting, effectiveness and good tolerability [4–6]. The development of therapeutic bone-seeking radiopharmaceuticals was first evaluated in the 1940s with the investigation of phosphorus-32 [7]. Because of the large patient population and effectiveness of this approach, significant research concerning bone metastases seeking radiopharmaceuticals has been pursued up to now [8–10].

Bone-seeking therapeutic radiopharmaceuticals can be divided into calcimimetic radiopharmaceuticals and phosphonate-based radiopharmaceuticals. The localization of calcimimetic agents is controlled by the same physiological and metabolic regulatory mechanisms as calcium and may thus be variable and unpredictable. Examples in this category include phosphorus-32 (^{32}P), strontium-89 (^{89}Sr) and radium-223 (^{223}Ra).

As an alternative strategy, the speed and efficacy of bone accumulation of radionuclides can be enhanced by coupling with phosphonates, for example the well-known bisphosphonates (also called diphosphonates). Phosphonates are non-hydrolyzable analogues of the natural occurring pyrophosphate (see Fig. 1), which has high affinity for bone mineral and regulates bone mineralization.

The general molecular structure of a bisphosphonate is shown in Fig. 2. Different non-radioactive bisphosphonates have been developed as bone resorption inhibitors and are applied clinically as pharmaceuticals for the treatment of several bone diseases [11–13].

These agents are adsorbed by attachment to the calcium atoms in hydroxyapatite (HA), and suppress osteolytic activation and bone resorption, inhibit the functioning of osteoclasts and the maturation of osteoclast precursors and stimulate skeletal osteoblasts. They are able to reduce pain by induction of apoptosis of osteoclasts, inhibition of proliferation of malignant cells, reduction of production of cytokines and secretion of metalloproteinase [14]. The HA affinity is strongly dependent on the molecular structures of these compounds. 'Small' bidentate bisphosphonates, like MDP (methylenediphosphonate, medronate), have the weakest binding capacity. HDP (hydroxymethylenediphosphonate, oxidronate), HEDP (1-hydroxyethylidene-1,1-diphosphonate, etidronate), pamidronate and alendronate act more or less like a tridentate because of the presence of a hydroxyl group [15]. Bisphosphonates with larger side chains, like ibandronate, risedronate and zoledronate, have the highest HA affinity.

Phosphonates are well-known chelators of radiometals and radiolanthanides [16,17]. For the diagnosis of skeletal metastases by using bone scintigraphy, gamma emitting ^{99m}Tc-based bisphosphonates are the most widely applied radiopharmaceuticals [18,19]. For the treatment of bone metastases, many therapeutic phosphonate-

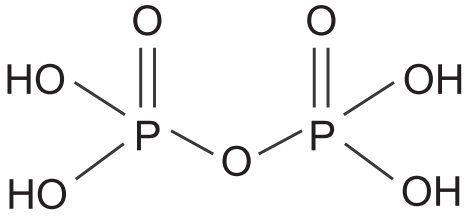

Fig. 1. Pyrophosphate.

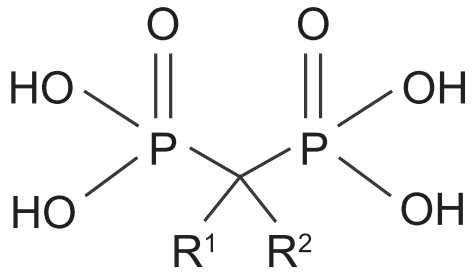

Fig. 2. General molecular structure of a bisphosphonate; R1 and R2 represent side-chains which determine the characteristics of the phosphonate analogue.

based radiopharmaceuticals have been developed and investigated, based on alpha and beta emitting radionuclides. The approach of selectively treating malignant lesions by using the same or closely related targeting ligands as for the diagnostic scans, also called theranostics, is an upcoming paradigm in nuclear medicine and medical oncology. The theranostic approach allows patient therapy planning by evaluation of individualized pharmacokinetics and dosimetric studies and is the basis of a personalized medical strategy.

When coupled to radionuclides, several aspects are important for their *in vivo* behavior. The complex should have a net charge to prevent solubility problems. Also, the ligands may compete with physiological ions like calcium, citrate and amino acids, which may lead to transchelation [20–22]. Furthermore, the complexes may dissociate resulting in altered characteristics and biodistribution of the formed species. It is assumed that the HA affinity of (bis)phosphonates determines biodistribution, even after coordination of a metal [23]. However, the nature of the phosphonate-metal complexes does not play a decisive role in HA binding and skeletal uptake [24].

The mechanism by which radionuclide therapy leads to pain palliation is not well understood. Although it is tempting to attribute this effect to inhibition or even killing of malignant cells, it is known that the absorbed radiation dose required for tumor growth inhibition is higher than for pain palliation [25]. In addition, the penetration of the radioactive emissions (*e.g.* alpha and weak beta particles) often does not reach the majority of tumor cells. Supposed mechanisms resulting in pain relief include decrease of pressure in the periosteal space, reduction of the osteoclast activity, decrease of the secretion of cytokines by specialized immune cells which are recruited and localize at the inflammatory sites and altered nociceptive transmission [25–27].

Although pain palliation is an important clinical goal, survival gain may be feasible as well. Survival benefit has been proven in large phase 3 studies only by ^{223}Ra-chloride [28]. For other bone-targeting radiopharmaceuticals the evidence on survival benefit is not yet as convincing [29–32].

It is generally recognized that, despite their undeniable potential, only a few radiopharmaceuticals gain wide clinical acceptance [33]. This is not different for phosphonate-based radiopharmaceuticals. Despite their recognized potential [4,6,8,10], only a few are used clinically. There may be several hurdles during pharmaceutical and preclinical development that may hamper their clinical introduction. Therefore, in this review we summarize the development of phosphonate-based radiopharmaceuticals for the palliative treatment of bone metastases. We focus on the pharmaceutical and preclinical development of these radiopharmaceuticals, which are of utmost importance for their clinical implementation. We discuss early human studies where available and illustrate which agents have reached clinical application. Furthermore, we identify compounds that have favorable properties for consideration for clinical evaluation.

To the best of our knowledge, this is the first review primarily focusing on the development aspects of phosphonate-based therapeutic bone-targeting radiopharmaceuticals. Use of existing knowledge collected in this review should guide future research efforts developing and optimizing radiopharmaceuticals for bone pain palliation.

2. Methods

In order to properly assess and discuss the literature on the development of phosphonate-based therapeutic bone-targeting radiopharmaceuticals, a reference framework was developed by listing the expected key properties of these agents. The characteristics of the ideal bone-targeting therapeutic radiopharmaceutical are summarized in Table 1.

The total energy of the emitted beta or alpha particles must be high enough to reach a sufficient number of the targeted inflammatory and tumor cells, but not too high to avoid bone marrow suppression. The energy should be deposited homogeneously to the malignant cells during their lifecycle. The emitted particles from targeted cells are most effective when they reach local tumor cells from different directions, which is called the "crossfire" effect. The targeting of the tumor cells is more effective when the radionuclide half-life is shorter, because the energy transfer takes place in a shorter time period [34,35]. A short half-life also allows for repeated treatment cycles and enables administration of other myelotoxic drugs (*e.g.* chemotherapy). Furthermore, a short half-life is advantageous to reduce the bother of radioactive waste handling and storage [36]. However, the half-life must be long enough for convenient logistics, for example when the radiopharmaceutical cannot be administered just after preparation or has to be transported to another center. The *in vitro* stability should be sufficiently long for logistical reasons. The *in vivo* stability should be sufficient to enable targeted radiation during the effective half-life, being the combined result of physical decay and biological processes. A high bone-to-soft tissue ratio and a high bone lesion-to-healthy bone ratio are very important to minimize unwanted irradiation of healthy tissues, most importantly the bone marrow. Furthermore, from a pharmacological/chemical point of view, a predictable and uniform molecular structure is desirable for predictable biodistribution. Lastly, when the final radiopharmaceutical

Table 1
Key characteristics of ideal therapeutic bone-targeting radiopharmaceuticals.

Criterion	Requirement
Total energy of the emitted particles of the radionuclide	Disposition of energy in soft tissue sufficient to reach as many malignant cells as possible (*i.e.* crossfire effect)
Physical half-life of the radionuclide	Relatively short (hours to 2 days)
Gamma radiation	Adequate to perform a post therapy scan and dosimetry studies
Availability of radionuclide	Fast and continuous supply or generator-derived
Phosphonate precursor quality	Available in adequate quality (according to published pharmacopoeia and Good Manufacturing Practice (GMP))
Preparation	Simple, preferably "kit" preparation, no concentration or purification steps needed, limited heating necessary, robust method
Quality control (QC)	Simple method (paper chromatography (PC) or (instant) thin layer chromatography ((i)TLC))
Stability	Stable *in vitro* at least for one working day (time from preparation to administration) Preferably stable under *in vivo* conditions during the effective half-life
Characterization	Uniform molecular structure, preferably one chemical species
Biodistribution	Rapid disposition and retainment in target tissues, rapid clearance from the blood, high bone-to-soft tissue ratio, high bone lesion-to healthy bone ratio
Human data	Experience with human administration: at least phase I/II-studies with maximum tolerated dose (MTD) established; if not: tracer dose administered
Authorization	Licensed as a drug

Table 2
Search terms used for literature search.

Biodistribution
Bisphosphonate, diphosphonate, phosphonate, tetraphosphonate, polyphosphonate
Bone accumulation, bone metastases, bone-seeking, bone-targeting, bone uptake
Clinical development, clinical trial, clinical use
Composition
Ligand (name)
Maximum tolerated dose (MTD)
Pain palliation
Phase I study, phase II study, phase III study
Preclinical development
Preparation
Radionuclide (name)
Radiopharmaceutical (name)
Therapeutic

preparation consists of a mixture of different compound structures, different *in vivo* biodistribution patterns for each component are conceivable, and this should be investigated. Identifying and isolating the most effective component should be an important goal during the radiopharmaceutical research and development stage and would provide the most effective and safe product with predictable properties.

The literature search for this review included PubMed, Google scholar and references in reviews and research papers published through December 2015. Table 2 summarizes the search terms used for identifying the publications for this review.

We screened the identified papers on the topics mentioned in Table 1.

3. Results from literature search

We identified 91 different phosphonate-based radiopharmaceuticals based on 16 different radionuclides, which have been investigated for the potential treatment of bone pain resulting from skeletal metastases. The principle physical characteristics of these radionuclides are summarized in Table 3 and the main research and development activities on the identified bone-seeking radiopharmaceuticals are provided as Supplementary material. The primary findings and results identified in the literature are discussed below and reviewed subsequently in alphabetical order of the radionuclide involved, in sections on radiopharmaceuticals based on beta emitting, conversion electron (Auger electron) emitting and alpha emitting radionuclides. The agent discussions are divided in two categories based on those agents that have progressed into the clinic and those which up to the current time have only been investigated to the preclinical research stage.

Table 3
Main physical characteristics of key radionuclides for phosphonate-based therapeutic bone-seeking radiopharmaceuticals. An extended version of this table is provided as Supplementary material.

Radionuclide	Physical half-life	Maximal range in soft tissue
Holmium-166 (^{166}Ho)	27 h (1.1 days)	9 mm
Iodine-131 (^{131}I)	8.0 days	2.3 mm
Lutetium-177 (^{177}Lu)	6.7 days	2 mm
Phosphorus-32 (^{32}P)	14.3 days	8 mm
Rhenium-186 (^{186}Re)	89 h (3.7 days)	5 mm
Rhenium-188 (^{188}Re)	17 h (0.7 days)	10 mm
Rhodium-105 (^{105}Rh)	35 h (1.5 days)	2 mm
Samarium-153 (^{153}Sm)	46 h (1.9 days)	4 mm
Thulium-170 (^{170}Tm)	128 days	5 mm
Ytterbium-175 (^{175}Yb)	4.2 days	2 mm
Yttrium-90 (^{90}Y)	64 h (2.7 days)	11 mm
Tin-117m (^{117m}Sn)	13.6 days	300 μm
Actinium-225 (^{225}Ac)	10.0 days	<100 μm
Astatine-211 (^{211}At)	7.2 h	70 μm
Bismuth-212 (^{212}Bi)	1.1 h	90 μm
Thorium-227(^{227}Th)	18.7 days	<100 μm

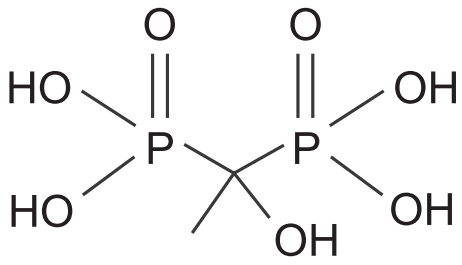

Fig. 3. HEDP (etidronate), a widely used first-generation bisphosphonate.

3.1. Phosphonates radiolabeled with beta emitting radioisotopes

In the 1970's it was shown that the bone accumulation in rats of the beta emitting radionuclide ^{32}P coupled to HEDP (etidronate, see Fig. 3), was higher than orthophosphate [$M_2(PO_4)_3$], whereas the distribution in normal bone marrow was lower. This result was repeated in dogs and humans [37], but the synthesis of ^{32}P-HEDP was difficult and the quality control unpredictable. Moreover, the results of an initial study in five patients with painful bone metastases demonstrated that only one patient experienced pain relief, while myelosuppression was observed in all patients [37]. In the years following, many other compounds were synthesized and investigated.

3.1.1. Holmium-166 (^{166}Ho)

^{166}Ho is a reactor-based radiolanthanide with chemical properties similar to the better-known ^{153}Sm. This trivalent radionuclide forms stable chelates with phosphonates.

3.1.1.1. Clinical use

3.1.1.1.1. ^{166}Ho-DOTMP. The ^{166}Ho complex of the cyclic 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra(methylene-phosphonate) (DOTMP, see Fig. 4) is the only ^{166}Ho-phosphonate that has been administered to humans. This compound can be prepared by simple incubation of ^{166}Ho-chloride and DOTMP at room temperature at a ligand-to-metal ratio of only 1.5:1 [38]. Bagheri et al. obtained radiochemical purity above 98% at a ligand-to-metal ratio of 30 or higher [39]. The optimized preparation showed a fast and prolonged skeletal uptake in rats (≈3% ID/g), with little soft tissue uptake. However, ^{166}Ho-DOTMP had been initially developed as part of a conditioning regimen for autologous stem cell transplantation in multiple myeloma and has not been evaluated for bone pain palliation [40,41].

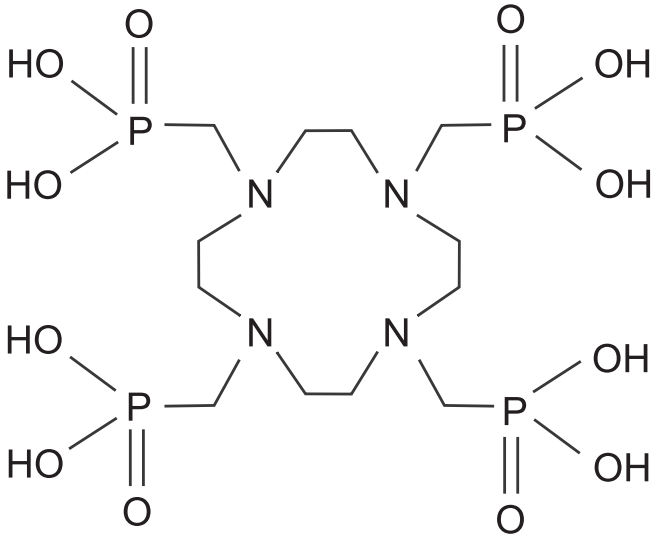

Fig. 4. DOTMP, a cyclic tetraphosphonate.

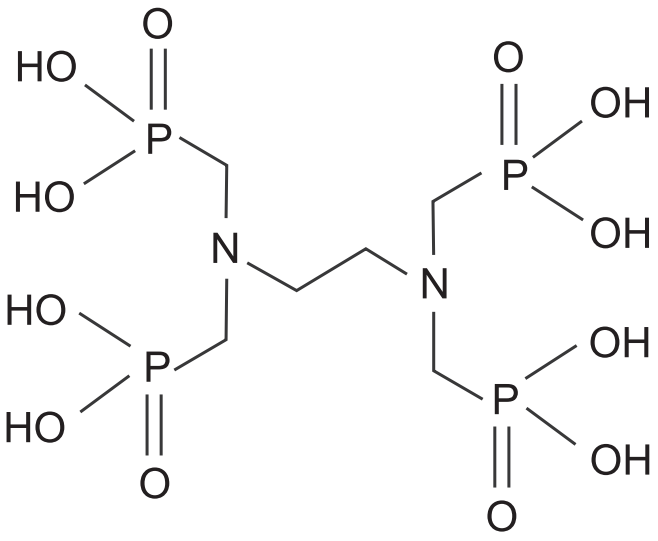

Fig. 5. EDTMP (lexidronam), a widely used tetraphosphonate.

3.1.1.2. Research and development

3.1.1.2.1. ^{166}Ho-EDTMP. Incubation at room temperature is sufficient for the formation of the complex of ^{166}Ho with ethylenediaminetetramethylenephosphonate (EDTMP, lexidronam, see Fig. 5) [20,42,43]. A biodistribution study in dogs showed high bone-to-normal tissue ratios, similar to ^{153}Sm-EDTMP [42]. However, ^{166}Ho-EDTMP was not developed for treatment of bone metastases, but was also investigated for marrow ablation purposes, similar to ^{166}Ho-DOTMP. In a baboon model ^{166}Ho-EDTMP showed pharmacokinetic and biodistribution properties inferior to data for ^{153}Sm-EDTMP in terms of blood-to-bone transfer rate and bone-to-background ratio. These findings may result from transchelation *in vivo* with endogenous citrate [20]. Biodistribution experiments in rats showed significant bone accumulation (>70%) within the first 48 h after administration [43].

3.1.1.2.2. ^{166}Ho-APD. The ^{166}Ho complex of 1-hydroxy-4-aminopropylidenediphosphonate (APD, pamidronate, see Fig. 6) can be prepared by room temperature incubation [44]. Identification of different species present *in vivo* at different ligand-to-metal ratios and pH-values was predicted by potentiometry and polarography and various species were identified. Experiments in a baboon and in rats showed lower bone uptake than ^{166}Ho-EDTMP and ^{153}Sm-EDTMP and a profound uptake and retention in the liver, probably due to colloid formation or precipitation of a neutral complex [44]. The preparation of ^{166}Ho-APD was optimized by Fakhari et al. [45]. The HA binding of the radiolabeled product was above 90% for all HA amounts used. Biodistribution studies in mice showed fast and prolonged bone uptake and little soft tissue uptake. However, the bone uptake (≈2%) was only half of that obtained with ^{153}Sm-EDTMP. Moreover, the latter compound has a higher bone-to-soft tissue ratio.

3.1.1.2.3. ^{166}Ho-APDDMP. In an attempt to reduce liver uptake a charged complex at physiological pH, *N,N*-dimethylene-phosphonate-1-hydroxy-4-aminopropylidenediphosphonate (APDDMP) was synthesized [21], and biodistribution studies of the ^{166}Ho complex in a baboon did show less liver uptake. However, the affinity of APDDMP for calcium ions was greater than for holmium and the affinity of holmium for citrate exceeded the affinity for APDDMP. As a result, bone uptake of ^{166}Ho-APDDMP was less than that of ^{153}Sm-APDDMP and ^{99m}Tc-APDDMP, and the authors stated that ^{153}Sm-APDDMP is a better candidate for possible further development [21].

3.1.1.2.4. ^{166}Ho-PDTMP. Experiments with the ^{166}Ho-labeled propylenediaminetetramethylenephosphonate (PDTMP) were reported by Zolghadri et al. [46]. Biodistribution studies in rats showed rapid and prolonged bone uptake (≈1% ID/g), with nearly no soft tissue uptake. The authors conclude that this compound might be a suitable candidate for the treatment of bone metastases.

3.1.1.2.5. ^{166}Ho-BPAMD. The ^{166}Ho complex of the DOTA-analogue (4-{[bis-(phosphonomethyl)) carbamoyl]methyl}-7,10-bis(carboxymethyl)-1,4,7,10-tetraazacyclododec-1-yl)acetic acid (BPAMD, see Fig. 7) was prepared by heating [47]. With ITLC one compound was visible. The radiochemical purity was >94%, while the HA binding of the complex exceeded 98%. The biodistribution was studied in mice and ^{166}Ho-BPAMD accumulated mainly in bone. The authors conclude that ^{166}Ho-BPAMD is a suitable compound to develop further for bone marrow ablation.

Our literature search did not identify any papers describing the use of ^{166}Ho-based therapeutic radiopharmaceuticals for bone pain palliation in humans.

3.1.2. Iodine-131 (^{131}I)

^{131}I is a readily available reactor-based radiohalogen that is widely used as a diagnostic and therapeutic radionuclide for thyroid malignancies. Since iodide is an anion, it cannot be attached to phosphonates by simple chelation, but must be covalently attached to moieties on substituted phosphonate analogues, such as electrophilic attachment to a phenyl ring.

3.1.2.1. Clinical use

3.1.2.1.1. ^{131}I-BDP3. Eisenhut et al. investigated six structurally related benzylidenediphosphonates (BDP) with *alpha* and *para* position substitutions based on the therapeutic radioisotope ^{131}I as potential therapeutic bone agents. Comparative pharmacokinetics and bone uptake studies in rats showed that α-amino-(3-^{131}I-iodo-4-hydroxy-

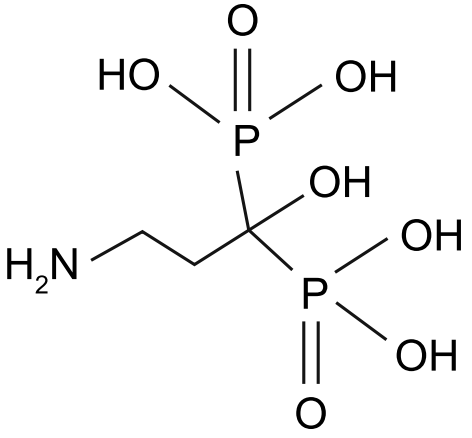

Fig. 6. APD (pamidronate), a second generation bisphosphonate.

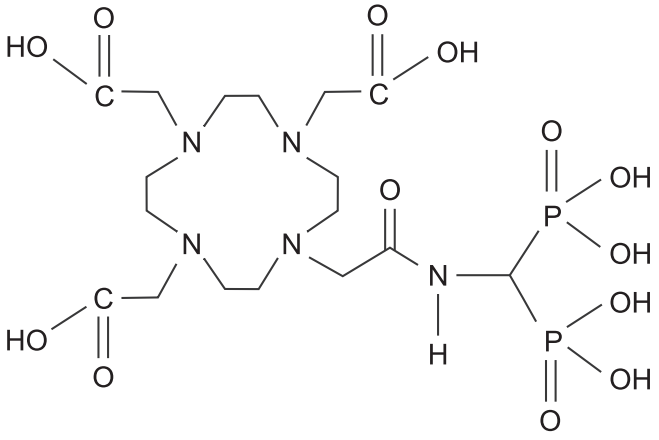

Fig. 7. BPAMD, a DOTA-conjugated bisphosphonate.

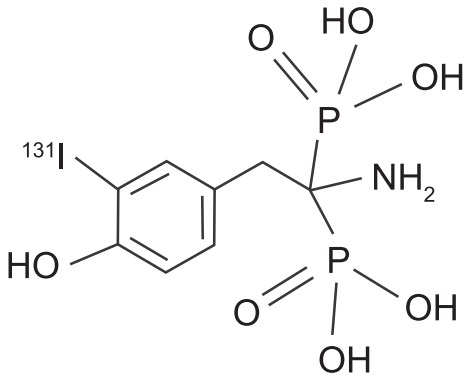

Fig. 8. Iodine-131-labeled benzylidenediphosphonate (^{131}I-BDP3).

benzylidene)-diphosphonate (^{131}I-BDP3, see Fig. 8) had the most attractive biological properties [48–51]. After synthesis and purification of the ligand, labeling with ^{131}I was performed by electrophilic aromatic substitution in the presence of IO_3^- [48]. Biodistribution was studied in rats where ^{131}I-BPD3 showed high bone affinity. The uptake in femur 24 h after injection was 1–2% of the injected dose per gram of tissue (ID/g) femur. After multiplying by the body weight (BW), to correct for the weight of the animals used, this value was 245%. Uptake in non-osseous tissues was low and in pharmacokinetic experiments rapid blood clearance and renal excretion were demonstrated [48].

The same group studied the pharmacokinetics, dosimetry data and pain response in a phase I trial with ^{131}I-BPD3 in 18 patients with painful bone metastases from prostate, breast and other primary carcinomas [50]. The blood clearance was rapid: 90% of the activity disappeared from the blood pool within 2 h and was excreted solely in the urine. The total body retention at 48 h after administration was found to be around 50%. The metastases-to-normal bone uptake ratio ranged from 2.5 to 7.4 and increased with time [50]. To prevent thyroid uptake of liberated radioiodine, Lugol's solution was used. 72% of the patients experienced pain palliation with the response in patients with prostate carcinoma being higher than in patients with other primary tumors. The duration of the response ranged from 1 to 8 weeks. Blood cell counts revealed no changes related to the therapy. After this study the dose was standardized to 800 MBq/m^2. However, no follow-up of these human studies was reported.

3.1.2.2. Research and development

3.1.2.2.1. ^{131}I-BPB and -PPB. Two amidobisphosphonates, 3-[-^{131}I]iodobenzamide-*N*-3-hydroxypropylidene-3,3-bisphosphonate (IBPB) and 5-[^{131}I]iodopyridine-3-amide-*N*-3-hydroxypropylidene-3,3-bisphosphonate (IPPB) were studied by Larsen and Murud [52,53]. The synthesis and purification of the bisphosphonates consisted of multiple steps. The radiolabeling required complex chemical reactions and purification steps. High Performance Liquid Chromatography (HPLC) analysis of the non-radioactive complex showed one peak. Biodistribution was investigated in mice [52], which established the complexes to exhibit rapid and stable bone accumulation. The uptake in femur 24 h after injection was calculated to be 20.3% ID/g for IBPB and 15.7% for IBPB, respectively. These figures were 406% and 315%, respectively, when corrected for BW. Low uptake values in thyroid and stomach indicated little dehalogenation *in vivo*. However, relatively high uptake in the spleen was reported [52]. Human administration of these two amidobisphosphonates has evidently not been reported.

3.1.2.2.2. ^{131}I-HPEB. Attempting to further improve bone affinity of these agents, Årstad et al. synthesized four different arylalkylidenebisphosphonates using complex chemistry and purification steps [54]. Of the compounds studied, 1-hydroxy-(m-iodo[^{131}I]-phenylethylidene)-1,1-bisphosphonate (^{131}I-HPEB) showed the best bone affinity in mice and negligible *in vivo* deiodination. The uptake measured in femur was reported to be 38% ID/g (850% corrected for BW) in mice and 16% ID/g (986% corrected for BW) in rats, respectively. These figures are favorable compared to the data in the above mentioned studies and to bone uptake values reported for ^{153}Sm-EDTMP (580% at 24 h in rats). Significant anti-tumor activity in terms of survival and disease-free latency time was shown in a mixed osteolytic/osteosclerotic bone metastasis model and in an osteosarcoma model in immunodeficient rats [54]. Although the reported uptake values are the highest mentioned in the literature, no further studies were identified.

3.1.3. Lutetium-177 (^{177}Lu)

The radiolanthanide ^{177}Lu can be both reactor and accelerator produced. The radiochemistry of this trivalent radionuclide is similar to that of ^{153}Sm. ^{177}Lu forms thermodynamically stable complexes with a coordination number of 6 to 9.

3.1.3.1. Clinical use

3.1.3.1.1. ^{177}Lu-EDTMP. The first investigated bone-targeting Lu-compound was ^{177}Lu-EDTMP [55]. The radiolabeling in this study was performed by heating ^{177}Lu-chloride and EDTMP in boiling water. The authors assume that the 1:1 complex is formed as one species. Biodistribution was assessed in rats with principal ^{177}Lu accumulation mainly in the skeleton with activity detected over a 10-day period. The uptake in femur at 24 h was 7.5% ID/g and the bone-to-organ ratios were very high. Chakraborty et al. performed the radiolabeling at room temperature [56,57]. The complexation yield was 99% at a ligand-to-metal ratio as low as 5:1. Biodistribution and imaging experiments in rats, rabbits and dogs showed rapid and stable skeletal accumulation, while essentially no activity was retained in other organs. The uptake in the rat tibia at 24 h was 7.7% ID/g, in rat femur 2.1% ID/g. Máthé et al. performed further biodistribution and imaging studies in mice, rats and rabbits, and a dose escalation study in dogs [58]. In all animal studies a stable and long-lasting binding of ^{177}Lu-EDTMP to bone was observed. The femur uptake at 24 h was 15.8% ID/g in mice and in rabbits 0.2% ID/g. The bone-to-muscle ratio in these species was assessed to be 130 and 730, respectively. A dose of up to 37 MBq/kg proved to be safe in dogs.

Although ^{177}Lu-DOTMP showed very promising results (see Research and development), ^{177}Lu-EDTMP was developed for human use, because EDTMP was already in use as the ligand in the approved ^{153}Sm-EDTMP agent.

The first study in man (phase I/II) of ^{177}Lu-EDTMP was published in 2012 [59]. Therapeutic doses were administered to 11 patients with bone metastases. After 24 h, the femur to muscle and lesion to normal bone uptake ratios were calculated to be 12.6 and 7.6, respectively. The same group investigated the efficacy and safety in a phase II study of 16 patients with bone metastases due to prostate or breast cancer [60]. A significant reduction in the pain score was seen, with complete palliation responses at 6 weeks after treatment of 55% and 80% in the low dose and high dose group, respectively. The Karnofsky score and mobility increased concomitantly. Only one patient experienced grade III hematological toxicity.

The formulation and preclinical and clinical evaluation of a freeze-dried kit identical to the commercial available product Quadramet® was published by Das et al. [61]. The authors showed that a high radiolabeling yield was achieved at a ligand-to-metal ratio from 5:1. In rats a fast and stable bone accumulation was established, with nearly no uptake in other tissues. ^{177}Lu-EDTMP in doses up to 3.7 GBq was administered to 10 patients. These patients all showed significant pain relief within a month and no patients experienced adverse effects. The dose of 3.7 GBq is the highest reported so far and may be considered as the maximum tolerated dose. Agarwal et al. published a phase II study of 44 patients with skeletal metastases due to prostate or breast carcinoma, who received kit-prepared ^{177}Lu-EDTMP in a dose of 1295 or 2590 MBq [62]. The overall response rate was 86%, with no significant

difference between the two dose groups or the cancer origin. The quality of life, as reflected by the Karnofsky score, improved significantly. 34% of the patients experienced grade I/II and 23% grade III/IV hematological toxicity, respectively. This myelosuppression was reversible and returned to baseline values within 8 weeks. A phase I study was performed in 30 patients by Alavi et al. [63] who showed a high bone-to-soft tissue ratio at whole body scans. Pain response was monitored by a brief pain inventory questionnaire. The total response rate (complete response or partial response) was reported to be 83%. A surprisingly high percentage (70%) of the patients experienced a flare reaction on the first day after injection. However, the occurrence of a flare reaction seemed to be correlated with a better performance. Other side effects were restricted to mild and transient drop of hematological parameters.

3.1.3.2. Research and development

3.1.3.2.1. ^{177}Lu-DTPMP, ^{177}Lu-TTHMP. In their study of ^{177}Lu-EDTMP, Chakraborty et al. investigated two other polyphosphonates, diethylene-triaminepentamethylenephosphonate (DTPMP) and triethylenetetraminehexamethylenephosphonate (TTHMP, see Fig. 9) [56]. The complexation was sufficient for a ligand-to-metal ratio from 5:1 for DTPMP and from 10:1 for TTHMP, respectively. The yield of these labeling reactions proved to be more pH-dependent than in case of ^{177}Lu-EDTMP. The results of biodistribution conducted in rats were comparable to similar data obtained with ^{177}Lu-EDTMP. The uptake in rat tibia at 24 h was 6.3% ID/g for ^{177}Lu-DTPMP and 7.3% ID/g for ^{177}Lu-TTHMP, respectively.

3.1.3.2.2. ^{177}Lu-DOTMP, ^{177}Lu-CTMP. Das et al. investigated the ^{177}Lu-complexes of two cyclic polyaminophosphonates: DOTMP and 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraaminomethylenephosphonate (CTMP) [64]. The complexation of ^{177}Lu with DOTMP showed excellent yield (99%) with a ligand-to-metal ratio of only 2:1 at room temperature. However, the labeling of CTMP required heating for 30 min at 100 °C and showed a maximum yield of only 85%. Therefore, no further experiments were performed with this ligand. Biodistribution studies with ^{177}Lu-DOTMP in rats showed a selective and long-lasting skeletal uptake, with minimal uptake in other organs. The uptake in rat femur at 24 h was 4.3% ID/g, in rat tibia 5.5% ID/g.

3.1.3.2.3. ^{177}Lu-HEDP, ^{177}Lu-TTHMP. The ^{177}Lu labeling with HEDP and TTHMP was studied by Lungu et al. [65]. Both preparations required heating. The TTHMP-complex tended to be more stable than the HEDP-complex. Animal studies were performed in rats, showing selective bone accumulation of both complexes. The uptake at 24 h was 82% ID/g of the remaining activity in the bone for TTHMP and 67% for HEDP. While the TTHMP-complex showed a higher bone uptake, the liver uptake values were higher than for the HEDP-complex.

3.1.3.2.4. ^{177}Lu-TTHMP. Zolghadri et al. carried out experiments with ^{177}Lu-TTHMP as well and showed that simple incubation at room temperature was sufficient for high radiochemical purity [66]. Biodistribution experiments in rats showed fast and stable bone uptake (≈3.5% ID/g at 24 h) and only little kidney uptake (≈0.5% ID/g at 24 h due to excretion of the compound.

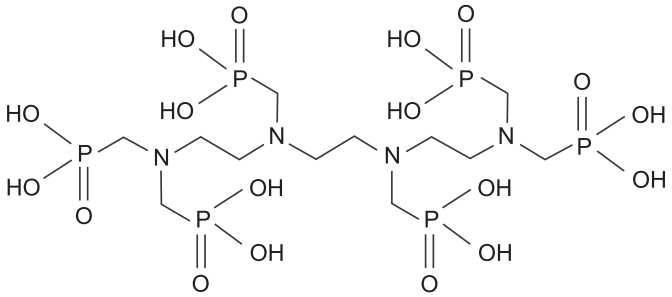

Fig. 9. TTHMP, a hexamethylenephosphonate.

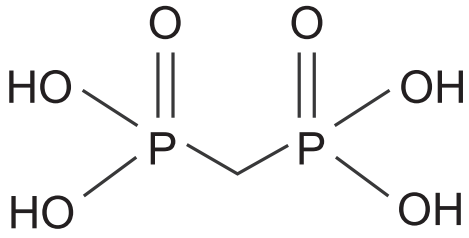

Fig. 10. MDP, a first-generation bisphosphonate.

3.1.3.2.5. ^{177}Lu-MDP. The complex of ^{177}Lu and the bisphosphonate MDP (see Fig. 10) was investigated by Abbasi [67]. In this study the optimal ligand-to-metal ratio proved to be 60:1. After injection of the complex in rats high skeletal uptake was measured. The femur uptake at 22 h was 2.3% ID/g (313% when corrected for BW). The uptake in other organs was low, except for the liver, which showed an uptake value even higher than that in the femur (6.1% at 22 h). Liver uptake was visible in imaging studies as well.

3.1.3.2.6. ^{177}Lu-PYP. Abbasi also studied the labeling of pyrophosphate (PYP) with ^{177}Lu [68]. From optimizing experiments it was concluded that a minimum ligand-to-metal ratio of 60:1 was required. Incubation of only one minute at room temperature was sufficient for a maximum labeling yield (>99%). An imaging study in a rabbit revealed activity in the skeleton, but a far too high liver uptake.

3.1.3.2.7. ^{177}Lu-zoledronate. Nikzad et al. published a study describing the complexation of ^{177}Lu with of 1-hydroxy-2-(imidazol-1-yl)-ethylidene-1,1-bisphosphonate (zoledronate) [69]. Optimization studies demonstrated that the ligand-to-metal ratio has to be at least 40:1 during incubation at room temperature. A hydroxyapatite binding assay showed high bone affinity *in vitro* (binding > 95%) and biodistribution investigations in mice demonstrated high bone uptake to soft tissue ratios. The bone uptake at 24 h was 6% ID/g. However, the bone-to-soft tissue ratios were considerably lower than those obtained with ^{177}Lu-EDTMP. Another group studied the same complex and obtained similar results [70]. The authors suggested that ^{177}Lu-zoledronate is formed as a 1:2 complex. From a rabbit imaging study the bone-to-soft tissue values of 12, 6 and 5 for kidney, bladder and lungs were calculated, however, the bone-to-liver ratio was not mentioned, although significant liver uptake is visible in the images obtained 1 and 2.5 days after injection.

3.1.3.2.8. ^{177}Lu-pamidronate, ^{177}Lu-alendronate. Experiments with two other ^{177}Lu-labeled bisphosphonates (pamidronate and alendronate) were reported by Fakhari [71]. Radiochemical purity was >98% and HA binding was >95%. HPLC analysis showed two separate peaks, indicating the presence of at least two different species, which were not further characterized. However, the two complexes demonstrated poor biodistribution characteristics in mice, with low bone uptake and high soft tissue uptake. The authors had no explanation for these findings.

3.1.3.2.9. ^{177}Lu-BPAMD. The development of a kit-based preparation method of the cyclic conjugate ^{177}Lu-BPAMD, using an automated synthesis module, was reported by Meckel et al. [72]. The ligand concentration and the use of buffers and scavengers were optimized. The optimal preparation showed radiochemical purity of at least 98%. No biodistribution data were presented.

3.1.4. Rhenium-186 (^{186}Re)

The chemistry of this transition metal closely resembles that for technetium, so rhenium can occur in several oxidation states. Although the chemistry of technetium and rhenium are similar, Deutsch et al. showed that there are differences in some aspects [73]. Firstly, the reduction of rhenium complexes is more difficult than their technetium analogues. The same reaction with ^{99m}Tc requires ten times less of the stannous ion (Sn^{2+}) reductant. Because reactions with ^{186}Re require a large amount of tin, a larger amount of ligand is needed to prevent precipitation of stannous salts at neutral pH. Once formed, rhenium

complexes are oxidized more readily than comparable technetium complexes. Secondly, no incubation time and heating are required with pertechnetate, whereas reduction of perrhenate requires heating and more time. Thirdly, ^{99m}Tc is carrier-free in radiopharmaceutical preparations, whereas ^{186}Re can often contain substantial amounts of carrier (^{185}Re and ^{187}Re from the reactor irradiated target material). Therefore, the total concentration of rhenium during preparation may be 10^4 times higher than the usual concentrations of technetium required for similar reduction of pertechnetate. The presence of carrier may result in the formation of polymeric complexes, which may exhibit different *in vivo* biodistribution patterns [74]. Although these species can often be visualized chromatographically and possible removed from the preparation prior to use, this adds considerably more time and QC issues.

3.1.4.1. Rhenium-186 bisphosphonates

3.1.4.1.1. Clinical use

3.1.4.1.1.1. ^{186}Re-HEDP. The first ^{186}Re-bisphosphonate investigated was ^{186}Re-HEDP [75]. The labeling was performed by electrolysis using tin electrodes and the radiolabeling yield was 95%. The biodistribution of ^{186}Re-HEDP in rats, investigated by scintigraphy, was similar to that of ^{99m}Tc-HEDP, although uptake in the liver and kidney was observed as well. The first kit-based preparation of ^{186}Re-HEDP was published by Deutsch et al. [73], and in this formulation a $^{186}ReO_4^-$ solution was added to a lyophilized kit containing HEDP, $SnCl_2 \cdot 2H_2O$ and sodium acetate. The solution was purified on a preparative HPLC column, and only those preparations which showed <10% of $^{186}ReO_4^-$ and <1% of ReO_2 were accepted. Because of the important role of tin, the investigators referred to the labeled complex as "^{186}Re(Sn)HEDP". HPLC showed that both ^{99m}Tc-HEDP and ^{186}Re-HEDP consisted of a mixture of components, which were not further identified. Biodistribution studies in rats indicated that ^{186}Re-HEDP is a bone-seeking radiopharmaceutical, with a femur uptake of 0.5–1.0% ID/g. Since soft tissue clearance was delayed, acceptable images by gamma camera imaging required long waiting times before acceptable images could be obtained.

Arano et al. investigated several optimization steps in their kit formulation containing tin and the antioxidant gentisic acid [76]. Biodistribution experiments in mice showed good bone-to-soft tissue ratios. The bone uptake at 24 h was 11.7% ID/g. In the first 24 h, 49% was excreted in the urine and 8% in the feces. Bai et al. describe the composition and quality control of a lyophilized cold kit for the preparation of ^{186}Re-HEDP, containing HEDP, stannous chloride and the antioxidant ascorbic acid [77]. The bone-to-soft tissue ratios in rats were found to be adequate and the bone uptake at 24 h was 11.6% ID/g. However, low levels of activity were detected in the thyroid during the first hours after administration.

The structures of the rhenium-HEDP complexes were investigated by X-ray spectroscopy by Elder et al. [78]. Depending on the stannous chloride concentration, various complexes of rhenium, tin and HEDP may be formed, like triangular trimers [proposed molecular formula: $Re_3Sn_3(HEDP)_8$] or linear tetramers [proposed formula: $Re_4(OH)_2Sn_4(HEDP)_{12}$] of rhenium, tin and HEDP may be formed, or a mixture of oligomers. So from these studies it is likely that the ^{186}Re-HEDP preparations that had been in clinical use up to then had represented a mixture of different species. Stability studies of ^{186}Re-HEDP were performed by Kothari et al. [79]. The results showed that both the composition and the preparation conditions were critical for adequate stability. Biodistribution studies in rats showed high and stable bone uptake (1.4% ID/g at 24 h), with little uptake in other tissues.

The first human administration of ^{186}Re-HEDP was reported by Maxon et al., using an improved kit-based labeling procedure [80]. A subtherapeutic dose was administered to five patients with bone metastases, within 1 h after preparation. The scintigrams were similar to those obtained after administration of ^{99m}Tc-MDP and high lesion-to-normal bone ratios were observed. This early comparison with ^{99m}Tc-biodistribution as a predictor of rhenium radioisotope distribution for therapy is an early example of theranostics. The authors predicted that the dose-limiting factor would be the bone marrow dose. The same group also reported the first experience with therapeutic doses (1225 MBq) of ^{186}Re-HEDP administered to 20 patients with painful bone metastases [81]. For the preparation they used a lyophilized kit containing ascorbic acid and the purification procedure was also further optimized. In 80% of the treatment group, a significant reduction in the pain index was demonstrated. Only a mild blood cell drop was reported. Maxon et al. evaluated this treatment in a placebo-controlled double blind trial of 20 patients [82] in a study with a "cross over" design in which ^{99m}Tc-MDP was used as the placebo. ^{186}Re-HEDP resulted in a significant greater decrease in pain than placebo ($p < 0.05$).

The pharmacokinetics of ^{186}Re-HEDP was also studied in 11 patients after injection of a therapeutic dose by de Klerk et al. [83]. The total excretion in the urine was around 70% and about 50% was excreted in the first 24 h after administration. The fraction of the skeleton showing metastases correlated well with the fraction of the dose that was not cleared *via* the kidneys. The authors concluded from this study that the levels of radioactivity taken up by the skeleton and hence the bone marrow dose can be predicted from a diagnostic scintigram. This is another example of theranostics. de Klerk et al. also investigated the MTD in a phase I dose escalation study in 24 patients with bone metastases due to prostate cancer [84]. The five dose levels ranged from 1295 to 3515 MBq and thrombocytopenia proved to be the dose-limiting toxicity. In the highest dose group two patients showed grade III thrombocytopenia. Therefore, the authors concluded that the maximally tolerated dose of ^{186}Re-HEDP would be 2960 MBq.

3.1.4.1.2. Research and development

3.1.4.1.2.1. ^{186}Re-MDP. After the first publication of the preparation of ^{186}Re-HEDP Eisenhut and co-workers prepared ^{186}Re-MDP from $^{186}ReO_4^-$ by the tin-reduction method [85]. The complex formation was visible by the appearance of an olive-brown color. After injection of the unpurified preparation in a rabbit, the scintigram showed uptake in the skeleton, but in soft tissues as well. By using HPLC the presence of at least three different components was demonstrated. This radiopharmaceutical has not been further explored for clinical application.

3.1.4.2. Rhenium-186 acyclic tetraphosphonates

3.1.4.2.1. ^{186}Re-EDTMP, ^{186}Re-PDTMP, ^{186}Re-DMPDTMP. Goeckeler et al. showed that multidentate ligands form more stable complexes with less structural variation than bisphosphonates, while exhibiting comparable or higher bone affinity [86]. With this knowledge in mind, Banerjee et al. explored the ^{186}Re-complexes of the tetraphosphonate ligands EDTMP and its higher homologues PDTMP, dimethylpropylenediaminetetramethylenephosphonate (DMPDTMP) and butylenediaminetetramethylene-phosphonate (BDTMP) [87]. The main impurity found during quality control was $^{186}ReO_4^-$ for three of the ligands studied. However, the labeling with BDTMP showed hydrolyzed reduced rhenium ($^{186}ReO_2$) to be the main impurity. Biodistribution studies in rats pointed out that bone uptake of these four tetraphosphonates was higher than for ^{186}Re-HEDP. The bone uptake at 24 h was 3.1%, 3.0%, 2.8% and 1.3% *vs.* 1.1% ID/g, respectively. In addition to the bone uptake, the PDTMP-complex showed rather high renal retention, while the BDTMP-complex showed the highest liver uptake, probably due to the presence of colloidal rhenium. The overall skeletal uptake of ^{186}Re was lower than that of comparable complexes of ^{153}Sm. The authors contributed this result to the known tendency of *in vivo* oxidation of ^{186}Re-complexes [87]. These tetraphosphonate complexes of ^{186}Re have not been further investigated.

3.1.4.3. Rhenium-186 cyclic tetraphosphonates

3.1.4.3.1. ^{186}Re-CTMP. Knowing that cyclic phosphonates form complexes with metal ions at a lower ligand-to-metal ratio than non-cyclic tetraphosphonates, Kothari et al. investigated the cyclic tetraphosphonate CTMP-complex of ^{186}Re [88]. CTMP is an analogue of DOTMP, a cyclic phosphonate that does not form complexes with ^{186}Re, probably because of the mismatch between the ionic radius of

the metal and the cavity size of the cyclic phosphonate. The ^{186}Re-CTMP-complex was found to be stable for 6 days at room temperature, even after adjustment of the pH to 7. Biodistribution in rats showed that the skeletal uptake was a little higher (1.3% ID/g at 24 h) than that of ^{186}Re-HEDP (1.1% ID/g at 24 h) and the activity in the skeleton remained stable for 48 h. Scintigraphic images in rabbits 24 h and 48 h after injection of ^{186}Re-CTMP showed better bone-to-soft tissue ratios than those obtained with ^{186}Re-HEDP [88]. Further studies with this cyclic complex have not been published.

3.1.4.4. Bifunctional complexes of rhenium-186

3.1.4.4.1. ^{186}Re-MAMA-BP. In an attempt to improve the biodistribution and *in vivo* stability of rhenium-186 complexes, Ogawa et al. synthesized the bifunctional chelator monoaminemonoamidedithiol-bisphosphonate (MAMA-BP) [89]. In this conjugate the MAMA forms a stable complex with ^{186}Re, leaving the bisphosphonate available for bone binding. The synthesis of ^{186}Re-MAMA-BP with a radiochemical yield of only 32% required multiple steps, including a ligand exchange procedure. After purification with RP-HPLC the radiochemical purity was >95%. Incubation at 37 °C (pH 7.0) showed that ^{186}Re-MAMA-BP had a higher stability than ^{186}Re-HEDP, prepared according to Arano et al. [76]. However, this bifunctional complex was never studied *in vivo*.

3.1.4.4.2. ^{186}Re-MAMA-HBP. ^{186}Re-MAMA-HBP is an analogue of ^{186}Re-MAMA-BP with a hydroxyl group at the central carbon atom of the bisphosphonate which was synthesized to further improve the bone binding properties [90]. The synthesis resulted in a radiochemical yield of 54% and the radiochemical purity was improved to >95% by HPLC. Stability at 37 °C and pH 7.0 were similar to values for ^{186}Re-MAMA-BP. The *in vitro* affinity of ^{186}Re-MAMA-HBP for hydroxyapatite was significantly higher than that of ^{186}Re-MAMA-BP, probably due to the influence of the central hydroxyl group. The *in vivo* bone binding of ^{186}Re-MAMA-HBP in mice was higher as well, as reflected by the higher femur-to-blood ratio compared to that of ^{186}Re-MAMA-BP. The bone uptake at 24 h was 25% ID/g for the HBP-compound and 21% for the BP-compound *vs.* 13% for ^{186}Re-HEDP. When corrected for body weight, the bone uptake after 1 h was 694% ID/g, which is similar to ^{153}Sm-EDTMP. When compared to ^{186}Re-HEDP, gastric uptake was lower. However, hepatic uptake was higher, probably due to higher lipophilicity [90].

3.1.4.4.3. ^{186}Re-MAG3-HBP. Ogawa et al. also synthesized several other bifunctional complexes of ^{186}Re. The multiple step synthesis of the complex of ^{186}Re and mercaptoacetylglycylglycylglycine (MAG3)-HBP resulted in a radiochemical yield of 76% [91]. The stannous reduction method was used to couple ^{186}Re to the MAG3-part of the complex. The radiochemical purity was >95% after purification by RP-HPLC. ^{186}Re-MAG3-HBP showed no decomposition after 24 h during incubation at 37 °C (pH 7.0) and was more stable than ^{186}Re-HEDP. Biodistribution studies in mice showed rapid uptake and long residence time in the skeleton. The bone uptake at 24 h was 25% ID/g (711% corrected for BW) for ^{186}Re-MAG3-HBP *vs.* 12% ID/g (342% ID/g × BW) for ^{186}Re-HEDP. Faster clearance from the blood and lower stomach uptake compared to ^{186}Re-HEDP were demonstrated as well. In another study by Ogawa et al., biodistribution was established in rats [92]. The femur uptake of ^{186}Re-MAG3-HBP at 24 h was 4.1% ID/g *vs.* 1.8% ID/g for ^{186}Re-HEDP. The therapeutic efficacy was studied in a rat model of bone metastasis. After inoculation with tumor cells, the tumoral bone-to-normal bone ratios of both agents did not differ significantly. However, with ^{186}Re-MAG3-HBP both dose-independent inhibition of tumor growth and pain palliation were achieved, while ^{186}Re-HEDP only showed palliation of pain. In a third study the influence of competitive inhibition of the protein binding of ^{186}Re-MAG3-HBP by the antibiotic ceftriaxone was investigated, hoping this strategy could positively influence the biodistribution [93]. In rats, this approach enhanced the clearance of the radiopharmaceutical from blood and non-target tissues while not effecting accumulation in bone.

3.1.4.4.4. ^{186}Re-CpTR-Gly-APD. Uehara et al. synthesized the complex-conjugate of tricarbonyl-^{186}Re-cyclopentadienyl-carbonylaminoacetic acid with APD (CpTR-Gly-APD) [94]. After multiple synthesis and purification steps, the radiochemical yield was 25% with a purity of >95%. Hydroxyapatite (HA) binding was a little higher for ^{186}Re-CpTR-Gly-APD compared to ^{186}Re-HEDP. ^{186}Re-CpTR-Gly-APD showed better plasma stability than ^{186}Re-HEDP. The biodistribution of this agent was investigated in mice and its bone uptake after 6 h was higher (23% ID/g) than for ^{186}Re-HEDP (12% ID/g). Co-injection with free HEDP led to a decrease in bone accumulation of ^{186}Re-CpTR-Gly-APD (uptake 14% ID/g) and blood clearance, while pre-treatment with free HEDP did not impair bone accumulation.

3.1.5. Rhenium-188 (^{188}Re)

^{188}Re is the daughter radionuclide formed by radioactive decay of reactor-produced tungsten-188 (^{188}W) and can be obtained on demand from an alumina-based generator [95] or from direct reactor production from irradiation of ^{187}Re. Concentration of the eluate (sodium ^{188}Re-perrhenate) with ion-exchange columns is required for use of some generator types and for extending the useful generator shelf-life [95]. The radiochemistry of ^{188}Re (carrier-free) is identical to that of ^{186}Re and resembles the chemistry of technetium likewise (See Introduction ^{186}Re). However, ^{186}Re is often reactor-produced and thus contains cold rhenium (carrier) originating from the target. In contrast, ^{188}Re is almost exclusively derived from a radionuclide generator and is thus obtained carrier-free. For syntheses of ^{188}Re phosphonates, in most studies a perrhenate salt is added as cold rhenium to reduce the ^{188}Re specific activity and to thus optimize complex formation and *in vivo* bone accumulation.

3.1.5.1. Rhenium-188 bisphosphonates

3.1.5.1.1. Clinical use

3.1.5.1.1.1. ^{188}Re-HEDP. Knapp et al. first reported experiments with kit formulations to prepare ^{188}Re-HEDP [95]. They describe a kit preparation method using ion exchange-concentrated eluate, tin and carrier. Lin et al. demonstrated that the stability of their kit-prepared ^{188}Re-HEDP was independent of the presence of carrier [96,97]. Biodistribution studies in rats using carrier-added ^{188}Re-HEDP showed good uptake in bone (1.4% ID/g in spine after 24 h) and low uptake in soft tissues (bone-to-muscle ratio 79 after 24 h). In a rabbit bone-lesion model the lesion-to-normal bone ratio was found to be 4.2, identical to that of ^{99m}Tc-MDP. About 60% of the activity appeared in the urine within 24 h, reflecting the ^{188}Re not bound to the skeleton. The bone uptake of Re-HEDP prepared with carrier-free ^{188}Re was low (0.8% ID/g) and most of the activity was excreted *via* the urine. Increasing the carrier amount to 10^{-3} M resulted in a dramatically increased bone uptake value of 2.8% ID/g. The authors provided no explanation for the carrier effect, and because the product with this carrier concentration turned a brown color, they stated the optimal carrier concentration to be 10^{-4} M. Hsieh et al. prepared ^{188}Re-HEDP in the same composition and under the same conditions and studied the biodistribution in rabbits, with and without carrier [98]. While the labeling yield was found to be >90% with or without carrier (10^{-4} M), high uptake was only visible on scintigrams when carrier was added, indicating a different ratio of specific complexes formed on the basis of the rhenium concentration. When carrier was added, the bone-to-soft tissue ratio was 25, in the carrier-free formulation this value was below unity. Verdera et al. performed similar experiments [99] and concluded that the highest tested carrier amount was required for optimal labeling yield and adequate stability. An inert atmosphere was found to be critical as well. When the optimal formulation was used in mice studies, 80% of the injected activity was retained in the skeleton. Imaging studies in rats and rabbits showed adequate bone-to-soft tissue ratios.

In addition to the effects of rhenium carrier, the reaction conditions were investigated by Hashimoto [100]. The amounts of HEDP and tin were optimized, as well as the pH value, which was found to be less

critical than for preparation of ^{188}Re-MDP, which is contributed to the presence of a hydroxyl group in HEDP. Although complexation took place at room temperature (pH 0.6), the reaction proceeded more rapidly at 100 °C. The labeling yield was increased by a higher ionic strength. Adding carrier resulted in a higher labeling yield, even at neutral pH. The stability of the product was investigated by diluting and by changing the pH and was found to be higher when carried was added. Furthermore, the stability of the carrier added product was better when the complex was formed at low pH and high temperature. Knowing that the product consists of a mixture of complexes, the author concludes that the stability of each component must be different and that the reaction conditions influence the overall stability. No biodistribution experiments were performed. Other groups have reported further investigations on the preparation and preclinical evaluation of ^{188}Re-HEDP. Faintuch et al. describe experiments with a carrier-free formulation [101] and although a complexation rate of above 95% was obtained, the complex formed was found to be stable for only 1 h. Biodistribution data in mice showed a value for bone uptake at 24 h of only 0.4% ID/g, actually lower than the uptake in other organs evaluated. For example, the stomach uptake was 1.9%, indicating low stability of the complex. Kohlickova-Koudelkova et al. investigated the influence of different variables on the labeling at room temperature and found that the relative amount of tin required for reduction of rhenium was less at higher concentrations of rhenium [102]. Also the amount of ligand needed for sufficient labeling could be lowered at higher rhenium concentrations. However, no experiments on the effects of heating during labeling and on animal biodistribution studies were reported.

A liquid kit without using carrier was developed by Marczewski et al. [103]. These investigators reported adequate labeling at room temperature. However, the stability of the complex was not reported, and unfortunately, no animal studies were performed. In another study, Lungu et al. studied a carrier-added kit-based formulation and performed the labeling under inert atmosphere and heating [65]. Biodistribution was investigated in rats and showed adequate bone accumulation (uptake at 24 h 80% ID/g of the remaining activity in the bone) and low soft tissue uptake. In elegant optimization experiments, Nassar et al. confirmed that ^{188}Re-HEDP has better stability when prepared under heating and when carrier rhenium is added [104]. At room temperature, the radiochemical purity of the carrier-free complex reached only 80% and the complex was found to be stable for 6 h. Heating for 10 min at 100 °C resulted in a radiochemical purity of 91%. With carrier addition, the purity could be increased to 97%. Only this preparation method resulted in a product that was stable for 24 h at room temperature. The complexes prepared in this manner showed the highest resistance upon increasing the pH and diluting the product with NaCl 0.9%. A biodistribution study in mice showed a bone uptake of 46% of ID after 3 h and an adequate bone-to-soft tissue ratio (maximum uptake in soft tissue 1.5% of ID after 3 h).

The group of Shiryaeva et al. investigated the carrier-free and carrier-added preparation of the ^{188}Re-complex of the monopotassium salt of HEDP at 20 °C and 100 °C, and reported a radiochemical purity of at least 95% for all formulations. The pharmacokinetics and biodistribution of ^{188}Re-HEDP were studied in rats [105,106]. The biodistribution experiments pointed out that the best pharmacokinetic properties and highest bone uptake were reached with the carrier-added product prepared at 100 °C. Moreover, significant higher uptake in the thyroid was measured in the carrier-free preparation, indicating less *in vivo* stability. Also Yang et al. demonstrated that carrier is needed for a good radiochemical purity and optimized their kit preparation [107]. The HPLC chromatogram suggested that only one complex was formed. The bone uptake of the product prepared with different carrier amounts was assessed in biodistribution experiments in mice. High bone uptake (27% ID/g after 6 h) and low soft tissue uptake (maximum 1.5%) were demonstrated. This was confirmed by SPECT imaging of a rabbit. An important recent publication by ter Heine et al. described the development of GMP grade ^{188}Re-HEDP, including their constituents, and can be used as a roadmap for local routine use of this radiopharma-ceutical [108]. The composition of this kit was identical to the Re-Bone®-kit (Mallinckrodt, The Netherlands), which was until recently used for the commercially available ^{186}Re-HEDP. These workers investigated the *in vitro* hydroxyapatite affinity and bone-to-soft tissue ratio in mice of preparations with different carrier amounts [109]. They investigated several preparation conditions as well, applying boundary testing [110]. Using the optimized composition, complexation proved to be independent on the reaction temperature, provided the reaction time was long enough. The undiluted end product was stable for 24 h at room temperature. Routine clinical use of the ^{188}Re-HEDP prepared under these GMP conditions has been initiated in a hospital in The Netherlands.

The first administration of ^{188}Re-HEDP to patients was reported by Maxon et al. [111] using reactor-produced ^{188}Re and a kit preparation. Carrier rhenium was needed for good radiochemical yields. After biodistribution studies in rats and rabbits, dosimetry was performed in five patients with prostate cancer after injection with a subtherapeutic dose. All results were found to be comparable to those with ^{186}Re-HEDP. Thereafter, 8 patients with painful skeletal metastases due to prostate cancer received a therapeutic dose and five of these patients experienced pain relief. The authors concluded that same-day, on demand outpatient therapy with ^{188}Re-HEDP may be feasible.

The maximum tolerated dose (MTD) of ^{188}Re-HEDP was later investigated by Palmedo et al. [112]. This dose escalation study used ^{188}Re-HEDP prepared with a carrier added kit formulation. A total 22 patients with bone metastases from prostate cancer were included in this study. The MTD was established to be 3.3 GBq and a higher dose of 4.4 GBq (8 patients) resulted in transient thrombocytopenia WHO grade III in 1 patient and grade IV in 2 patients, respectively. The nadir was determined to occur 4 weeks after administration. Pain palliation was reported by 64% of the patients. The response rate increased from 33% in the lowest dose group (1.3 GBq) to 75% in the group receiving 4.4 GBq. The response started about 12 days after injection and lasted for 8 weeks on average. Several studies with larger patient numbers were performed subsequently [31,32,113,114]. These studies not only demonstrated high palliation response rates and transitory mild hematologic toxicity, but also suggested survival benefit when the treatment was repeated [31,32].

3.1.5.1.2. Research and development

3.1.5.1.2.1. ^{188}Re-MDP. The first study of a ^{188}Re-based phosphonate was published by Hashimoto et al. [115], who labeled MDP with ^{188}Re. The influence of different reaction conditions was studied. The labeling reaction proceeded well at room temperature at a pH just below 1. Increasing the tin concentration a higher yield was obtained. When the tin concentration was too high, precipitation was induced. Ascorbic acid was found to be the best antioxidant, slightly better than gentisic acid and much better than citric acid. The labeling yields were higher in experiments with a higher ionic strength, using sodium acetate as a pH regulator. This was contributed to a change in the average charge of the ligand. Experiments with two concentrations of carrier showed that the labeling yields increased with a higher carrier concentration. Finally, the product prepared with carrier-added ^{188}Re showed a better stability after increasing the pH. However, the radiochemical purity was only 80% 1 h after adjustment of the pH to 4. Hsieh et al. prepared ^{188}Re-MDP with a tin-based kit at 100 °C and studied the biodistribution in rabbits [98]. While the labeling yields were found to be >90% with or without carrier (10^{-4} M), no significant skeletal uptake was visible on the scintigrams. The bone-to-soft tissue ratio was <2 with both formulations. This result has also been observed by other authors, although no explanation has been provided. Further investigations of ^{188}Re-MDP were performed by Faintuch et al. [101]. No carrier was used in these experiments. Although the complexation yields were above 95% and the pH-adjusted complex was found to be stable for 4 h in the presence of ascorbic acid, the biodistribution in mice showed poor results. The bone uptake was only 1% ID/g 4 h after injection and most of the

activity was accumulated in soft tissue. As a result of the collective studies showing poor results, ^{188}Re-MDP has apparently not been developed further.

3.1.5.1.2.2. ^{188}Re-HDP. Hsieh et al. studied a kit preparation containing tin for the ^{188}Re-labeling of HDP and investigated the biodistribution in rabbits of preparations with and without carrier (10^{-4} M) [98]. The results of these studies showed no significant uptake with the bone-to-soft tissue ratios being <3 with both formulations, while the radiochemical purity was >90%. The authors provided no explanation for these poor results.

3.1.5.1.2.3. ^{188}Re-SEDP. In an attempt to improve the *in vivo* characteristics of bisphosphonates Lisic et al. studied the ^{188}Re-complex of 2-sulfonatoethylidene-1,1-diphosphonic acid (SEDP) [116]. HPLC analysis demonstrated that the formulation with tin and carrier resulted in a mixture of components. The biodistribution in rats was comparable to ^{188}Re-HEDP. However, the bone accumulation of ^{188}Re-SEDP after 24 h was found to be somewhat better (2.5% ID/g) than that of ^{188}Re-HEDP (1.9% ID/g), probably due to additional anionic character of the sulfonic acid moiety.

3.1.5.1.2.4. ^{188}Re-risedronate. Erfani et al. investigated ^{188}Re-labeled 2-(3-pyridinyl)-1-hydroxyethane diphosphonic acid (risedronate), a third generation bisphosphonate with a >1000-fold potency compared to HEDP [117]. After optimizing the composition and preparation, the radiochemical purity was above 98% for the carrier-added formulation. Biodistribution studies in mice showed a bone uptake of 4.2% ID/g and a kidney uptake of 1.9% after 24 h. The uptake in other soft tissues was <0.5% at that time point.

3.1.5.2. Rhenium-188 aminophosphonates

3.1.5.2.1. ^{188}Re-AEDP. The preparation of the complex of ^{188}Re with 1-aminoethylenediphosphonic acid (AEDP) by heating for 15 min at 50 °C under different conditions has been published by Li et al. [118]. The radiochemical purity and stability of carrier added ^{188}Re-AEDP were higher than was the case with the carrier-free compound. The bone uptake in rats 24 h after injection was higher for the carrier-added preparation (11% ID/g) than for the carrier-free product (ID/g 2.3%). The soft tissue uptake was relatively low.

3.1.5.2.2. ^{188}Re-alendronate. Experiments with the ^{188}Re labeling of alendronate were reported by Arteaga de Murphy et al. [119]. The concentration of the reductant (stannous fluoride), antioxidant (gentisic acid) and carrier ($KReO_4$) were varied and the reaction conditions were optimized. When little to moderate amounts of carrier were used, the solution remained colorless. At higher carrier concentrations, however, yellow to dark brown solutions with lower radiochemical purity were formed. HPLC analysis demonstrated that polymerization of the chelate might contribute to the decrease of the radiochemical purity after storing for 24 h. Biodistribution studies in rats showed high bone uptake (35%/g tissue) after 24 h, with little soft tissue uptake.

3.1.5.2.3. ^{188}Re-EDTMP, ^{188}Re-EDBMP and ^{188}Re-NTMP. The labeling of the aminomethylenephosphonate derivatives EDTMP, ethylenediamine-*N*,*N*′-bismethylene-phosphonate (EDBMP) and nitrilotrimethylenephosphonate (NTMP) with ^{188}Re was investigated by Hashimoto [120]. The labeling at room temperature yielded similar radiochemical purity to that at 100 °C. However, the stability of the complex upon pH change and dilution was higher when the reaction was conducted under optimal conditions. The addition of carrier had no influence on the labeling yield of EDTMP and EDBMP, but increased the labeling yield of ^{188}Re-NTMP. The carrier-added formulations had a greater stability than the carrier-free preparations. However, the actual stability of the products was not reported and unfortunately no biodistribution experiments were performed with these compounds. The preparation and properties of ^{188}Re-EDTMP were also investigated by Oh et al. [121]. Several preparation conditions were evaluated, including the method of heating. The reaction proceeded optimal at pH 1, and at room temperature the highest radiochemical purity was found after 30 min. Thereafter, the purity decreased with time. Heating for 15 min at 100 °C or 15 s in a microwave oven were adequate to provide a high purity product. Although the addition of carrier did not affect the labeling yield, after pH adjustment the stability of the carrier-added preparation heated at 100 °C was the highest. Dilution did not influence the stability. Biodistribution data in rats were comparable to those obtained with ^{188}Re-HEDP. Bone uptake after 24 h was about 0.8% ID/g and the bone-to-soft tissue ratios were adequate. The effect of carrier on biodistribution was not studied.

The preparation conditions for the labeling of ^{188}Re-EDTMP were investigated and optimized by Pervez et al. [122] who found that the radiochemical purity and stability of the carrier-added formulation were equal to the carrier-free preparation. Adding ascorbic acid resulted in better stability. Biodistribution experiments in rats showed that the bone uptake of carrier-added ^{188}Re-EDTMP was almost twice as high (2.0% ID/g after 24 h) as the carrier-free product (1.1% ID/g after 24 h). Comparable experiments were carried out by Faintuch et al. [101]. Biodistribution data in mice showed that bone uptake after 24 h was 1.6% ID/g. However, relatively high stomach uptake was also detected (1.2% ID/g) and the moderate bone-to-soft tissue ratios may be explained because these data were determined using a no carrier added preparation. The preparation of ^{188}Re-EDTMP using a commercial available kit (Multibone®, Izotop, Hungary) has also been reported by Mitterhauser et al. [123]. Surprisingly, the radiochemical purity was better in the carrier-free formulation (97%) than in the carrier-added preparation (81%). The main impurity was found to be colloidal $^{188}ReO_2$, indicating a suboptimal tin-to-metal ratio. The presence of this impurity could be reduced by filtrating the final product. Unfortunately, data on stability were not reported and no biodistribution experiments were performed with these preparations. In another study the *in vitro* bone binding of ^{188}Re-EDTMP was investigated in human cortical bone and spongiosa [124]. The non-carrier-added preparation showed better bone binding than the carrier-added formulation. The authors concluded that bone binding is mainly restricted to the inorganic compartment and that human bone allografts yield comparable results to artificial bone. They suggest the use of hydroxyapatite as a useful tool to evaluate bone-seeking radiopharmaceuticals.

3.1.5.2.4. ^{188}Re-DTPMP. Nassar et al. studied the ^{188}Re-labeled pentaaminomethylenephosphonate DTPMP [125]. The radiochemical purity of the carrier-added formulation was slightly higher than when prepared carrier-free. The carrier-added product showed better stability than the carrier-free product. Moreover, the stability upon pH-change and dilution proved to be better in the carrier-added preparation. The carrier-free preparation was stable for only 1 h at a pH up to 4, while the carrier-added product was stable at a pH up to 7. The maximum dilution rate of the carrier-free product was 1:3, being 1:8 for the carrier-added formulation. Biodistribution experiments in mice demonstrated adequate bone-to-soft tissue ratios with a bone uptake of 27% ID/g after 3 h (uptake in other organs ≤ 1.5% ID/g at 3 h).

3.1.5.2.5. ^{188}Re-TTHMP. The hexaaminomethylenephosphonate TTHMP, was labeled with ^{188}Re by Lungu et al. [65], who optimized the reaction conditions and showed adequate stability at room temperature for the carrier-added formulation. However, in biodistribution experiments in rats the ^{188}Re-TTHMP showed somewhat less bone accumulation (uptake at 24 h 51% ID/g of the remaining activity in the bone) than ^{188}Re-HEDP (77%).

3.1.5.3. Conjugate complexes of rhenium-188

3.1.5.3.1. ^{188}Re-DTPA-BP and ^{188}Re-5FU-BP. Two ^{188}Re-labeled bisphosphonate conjugates, diethylenetriaminepentaacetic acid-bisphosphonate (DTPA-BP) and 5-fluorouracil-bisphosphonate (5FU-BP) were studied by El-Mabhouh and Mercer [126]. The biodistribution of carrier-free and carrier-added preparations were investigated in mice and compared to ^{188}Re-HEDP. The carrier concentration was critical for favorable biodistribution. These novel compounds exhibited similar behavior to ^{188}Re-HEDP. However, the bone uptake of ^{188}Re-DTPA-BP was somewhat lower (1.4% ID/g after 8 h) than that of ^{188}Re-5FU-BP

(2.7%) and ^{188}Re-HEDP (3.2%). The liver and stomach uptake of ^{188}Re-5FU-BP were a little higher than with ^{188}Re-DTPA-BP and ^{188}Re-HEDP. The uptake in knee joints of all three agents was higher than in the axial bone, indicating increased accumulation at sites with high bone turnover. The authors concluded that these novel agents provide new possibilities for combining therapeutic radionuclides and locally released chemotherapy.

3.1.5.3.2. ^{188}Re-Gem-BP. The same group studied another ^{188}Re-labeled conjugate bisphosphonate including the chemotherapeutic agent gemcitabine [127]. Although no optimization experiments were reported, the authors mentioned that adding carrier was essential. Bone affinity was investigated by measuring binding to hydroxyapatite and bovine bone, and the binding was found to be about 60% in both assays and compared well to the control (^{188}Re-HEDP). In mice, bone accumulation was found to be somewhat lower for ^{188}Re-Gem-BP (1.7% ID/g after 8 h) than for ^{188}Re-HEDP (3.2%). The uptake in the distal femur joint was measured to be higher than in the whole tibia for both compounds. Whether gemcitabine is released from the conjugate at the tumor site was not investigated.

3.1.5.3.3. ^{188}Re(CO)$_3$-dipicolylamine-alendronate. In an attempt to improve the radiochemical properties and biodistribution of ^{188}Re-HEDP, Torres Martin de Rosales et al. designed the novel biconjugate, ^{188}Re(CO)$_3$-dipicolylamine-alendronate [128,129]. These authors hypothesized improvement of the properties by separation of the metal chelation site from the bone-binding group. Neither stannous ion nor carrier was required during labeling. Using HPLC only one species was detected. Imaging studies in mice showed accumulation in areas with a high bone turnover. Biodistribution experiments in mice showed significantly higher bone uptake for the biconjugate compound (21.2% ID/g after 24 h) than for ^{188}Re-HEDP (13.4% ID/g). However, higher uptake of the compound in soft tissue was found, especially in the liver, when compared to ^{188}Re-HEDP. This result might be due to the higher lipophilicity of the biconjugate. The thyroid uptake was higher for ^{188}Re-HEDP, possibly due to partial decomposition and release of ^{188}Re-perrhenate. It is difficult to draw conclusions to these differences, because the preparation method and optimization for ^{188}Re-HEDP were not described in this paper.

3.1.6. Rhodium-105 (^{105}Rh)

^{105}Rh is a reactor-produced radionuclide and radiochemical reactions with this transition metal take place in the 3^+-oxidation state.

3.1.6.1. Research and development

3.1.6.1.1. ^{105}Rh-EDTMP. The only paper describing a phosphonate-based radiopharmaceutical labeled with ^{105}Rh was published by Ando et al. [130], who investigated complexation of carrier-free ^{105}Rh with EDTMP. No dissociation of the complex was detected after 5 days storage. Biodistribution was studied in mice and bone uptake after 24 h was about 12% (ID/g), which was somewhat lower than ^{99m}Tc-MDP. However, while non-skeletal uptake was negligible, the bone-to-soft tissue ratio of ^{105}Rh-EDTMP was superior. Despite these promising features, no further studies of ^{105}Rh-compounds have evidently been reported.

3.1.7. Samarium-153 (^{153}Sm)

^{153}Sm is a reactor-produced radiolanthanide, and most radiochemical reactions, including coupling to phosphonates, are conducted in the 3^+-oxidation state.

3.1.7.1. Clinical use

3.1.7.1.1. ^{153}Sm-EDTMP. Goeckeler et al. initially explored the complexation of ^{153}Sm with ten different bidentate and multidentate phosphonates [86,131], which included the bisphosphonates MDP, HDP, HEDP and 2,3-dicarboxypropane-1,1-diphosphonic acid (DPD) and the polyphosphonates NTMP, EDTMP, DTPMP, hydroxyethylethylene-diaminetrimethylenephosphonate (HEEDTMP), dicyclopentadienyltetramethylene-phosphonate (DCPDTP) and bis-(aminomethyl)-norbornyltetramethylenephosphonate (NBTP). All complexes were prepared in high yields. However, complexation with the multidentate phosphonates proceeded under less rigorous conditions and required a lower ligand-to-metal ratio than with the bisphosphonates. HPLC demonstrated the presence of a single peak for some of the phosphonates (*e.g.* EDTMP), indicating single species being 1:1 complexes. Stability was not studied extensively, but was at least 2 h for all complexes. For some phosphonates, for example MDP, two or more peaks were detected, suggesting the presence of different species. Biodistribution experiments in rats and rabbits showed that complexes with the multidentate ligands generally had higher bone uptake than the bisphosphonate complexes. Of all complexes evaluated, ^{153}Sm-EDTMP had the most favorable characteristics, with high skeletal uptake, low liver uptake and rapid blood clearance. The bone-to-muscle ratios for this agent were the highest (1460 after 2 h in rats, 1200 after 3 h in rabbits), which were confirmed by scintigraphy. The percentage of injected radioactivity detectable in the skeleton was about 50% during 72 h. Using the drill hole model in rabbits, the lesion-to-normal bone ratio values were comparable to that of ^{99m}Tc-MDP.

After encouraging results from treatment of dogs with primary bone cancer, a subtherapeutic dose was initially administered to five patients with metastatic bone cancer [36,132]. ^{153}Sm-EDTMP was prepared at room temperature using a lyophilized kit. Post-therapy scintigraphy showed that ^{153}Sm-EDTMP behaved like ^{99m}Tc-HDP. The lesion-to-normal bone, lesion-to soft tissue and the normal bone-to-soft tissue ratios of both compounds were nearly identical. ^{153}Sm-EDTMP showed rapid blood clearance; after 8 h 50% of the injected activity was excreted in the urine, leaving most of the remaining activity in the skeleton. No changes in serum or urine chemistry were detected.

Turner et al. performed several animal studies with ^{153}Sm-EDTMP [133]. In biodistribution studies in rats a fast bone uptake and long-term retention were shown. About 40% of the injected dose was excreted in the urine, while <1% of activity was retained in non-osseous tissues. Bone uptake experiments in sheep demonstrated preferential surface accumulation in cortical bone. Comparison of activity in trabecular and cortical bone and imaging demonstrated a 7:1 uptake ratio. The bone marrow toxicity was investigated in rabbits. A transient marrow depression was seen, with nadir in thrombocyte and leukocyte counts after 2 to 8 days and full recovery within 25 days. After evaluating the animal data, a clinical study was initiated, which was reported in two papers [133,134]. ^{153}Sm-EDTMP was administered at a fixed dose of 740 MBq to investigate pharmacokinetics and dosimetry. The blood clearance was complete within 1 h. In all patients, nearly all non-skeletal activity (5 to 60%) was cleared in the urine within 6 h. The retained skeletal activity varied between 40 and 95% of the injected dose. The retention of activity was greatest in patients with extensive bone metastases. The efficacy was evaluated at doses up to 2400 MBq. Pain relief was experienced within 2 weeks, was maximal at 4–6 weeks and lasted for 4 to 35 weeks. Pain palliation was reported in 65% of the evaluable patients. In prostate and breast carcinoma patients, the pain response was 83%. No relationship between dose and pain response could be assessed. However, myelotoxicity was found to be dose-related. The nadir in the granulocyte counts occurred at 2 weeks, in the platelet counts at 6 weeks. However, no interventions were required. Thrombocytopenia was the dose-limiting toxicity and the MTD was determined to be 24–31 MBq/kg. In nearly half of the patients stabilization or regression of skeletal metastases was seen at follow-up bone scans.

A dose escalation study was performed with ^{153}Sm-EDTMP by Farhanghi et al. in 22 patients [135]. Complete concordance with diagnostic ^{99m}Tc-HDP scintigrams was demonstrated, illustrating the theranostic use of radiopharmaceuticals for bone pain palliation long before use of this term became popular. The skeletal uptake was related to the number of metastatic sites and inversely related to the plasma activity at 30 min post-injection. Pain palliation was reached in 65% of patients. Reversible thrombocytopenia was observed in 34% of patients

and the authors state that 37 MBq/kg is a feasible dose. Several groups performed further phase I/II studies. Biodistribution and dosimetry were investigated in a phase I, dose escalation study by Eary et al. [136]. In total 52 patients received ^{153}Sm-EDTMP, at 5 dose levels, the highest dose being 11 GBq. On average 37% of the injected dose was excreted *via* the urine in the first 10 h after injection. No difference between the dose groups was observed. The mean lesion-to-normal bone ratio was about 4 in all dose groups, similar to that of ^{99m}Tc-MDP. Dosimetry studies showed that the absorbed dose was highest at the bone surface, followed by the red marrow and the bladder. Radiation dose in other tissues were negligible. In the same patients the toxicity and efficacy were evaluated by Collins et al. [29]. Bone marrow suppression was dose related and determined the dose limiting toxicity. The MTD was assessed to be 92.5 MBq/kg. Pain palliation was reached in 74% of patients, with a mean response duration of 2.6 months. In the 92.5 MBq/kg dose group, a trend towards longer survival was observed in comparison with the 37 MBq/kg group. A detailed study of dosimetry and toxicity was performed by Bayouth et al. in 19 patients, receiving two dose levels [137]. The skeletal uptake varied between patients from 14 to 83% (mean value about 50%). The nadir values in the platelet and white blood counts were found to take place 24 days after injection. A good correlation between marrow radiation dose and decrease in platelets was established. No toxicity in other organs was observed.

Following these early studies, several phase III randomized trials were performed, including double-blind studies [30,138,139]. ^{153}Sm-EDTMP was licensed in 1997 in the USA under the brand name Quadramet® (Lantheus Medical Imaging, USA) and in 1998 in Europe (CIS bio international, France). The product is delivered as a ready-to-use solution for injection. The standard dose is 37 MBq/kg. Only recently the molecular structure of the 1:1 Sm-EDTMP complex was characterized by using several spectroscopic techniques [140].

3.1.7.2. Research and development.

3.1.7.2.1. ^{153}Sm-pamidronate, ^{153}Sm-alendronate, ^{153}Sm-neridronate. Neves et al. [24] synthesized three different bisphosphonates: pamidronate, alendronate, and neridronate and coupled them with ^{153}Sm, using the procedure described by Zeevaart et al. for ^{166}Ho-pamidronate [44]. A good correlation was demonstrated between *in vitro* hydroxyapatite binding and *in vivo* skeletal uptake. Alendronate showed the highest HA binding, followed by neridronate and pamidronate. The skeletal uptake was investigated in mice. Neridronate showed the highest uptake values (20% of ID) at 3 h after injection, whereas the highest values at 24 h were observed for alendronate (9% of ID). The authors considered ^{153}Sm-alendronate the best candidate for further development.

3.1.7.2.2. ^{153}Sm-APDDMP. Experiments with ^{153}Sm-labeled APDDMP were performed by Zeevaart et al. [21]. Identification of different species present *in vivo* was predicted by potentiometry. 63% of ^{153}Sm was bound to ligand and 35% to citrate. These results were less favorable than those obtained with ^{153}Sm-EDTMP. Scintigraphy in a baboon showed selective bone uptake.

3.1.7.2.3. ^{153}Sm-BPAMD. Experiments with ^{153}Sm labeled BPAMD were reported by Rabie et al. [141]. Preparation conditions were optimized and the complex was prepared with a radiochemical purity of above 98%. HA binding was above 99% when BPAMD was available in adequate amounts. Biodistribution experiments in mice showed a high bone to soft tissue ratio. The maximal bone uptake of ^{153}Sm-BPAMD was 10.8% ID/g (24 h after injection), which renders ^{153}Sm-BPAMD a promising compound.

3.1.8. Thulium-170 (^{170}Tm)

^{170}Tm is a reactor-produced radioisotope and has similar radiochemistry as other trivalent radiolanthanides, such as ^{153}Sm, to form stable complexes with phosphonates. No clinical studies have yet been reported.

3.1.8.1. Research and development

3.1.8.1.1. ^{170}Tm-EDTMP. The radiolabeling of EDTMP with ^{170}Tm was reported by Das et al. [142]. Simple incubation at room temperature was sufficient for preparing a highly stable complex. Biodistribution studies in rats showed high uptake (56% of ID after 24 h) that was similar to ^{153}Sm-EDTMP. The bone-to-muscle ratio was about 250 after 24 h. Imaging studies in rats showed skeleton uptake from 3 h after injection until 60 days thereafter. From 3 h, no uptake in other tissues could be detected. Dosimetry calculations also showed insignificant radiation dose to non-osseous tissues and the authors proposed the use of ^{170}Tm-EDTMP as a cost-effective alternative to $^{89}SrCl_2$.

3.1.8.1.2. ^{170}Tm-DTPMP, ^{170}Tm-TTHMP, ^{170}Tm-DOTMP, ^{170}Tm-CTMP. The ^{170}Tm-labeled complexes of four aminophosphonates, including the acyclic DTPMP and TTHMP and the cyclic DOTMP and CTMP, have been evaluated by Vats et al. [143]. Incubation at room temperature proved to be sufficient for obtaining very stable complexes of high radiochemical purity. ^{170}Tm-DOTMP showed the best properties since complexation occurred instantaneously in a wide pH-range, requiring only a very small amount of ligand (ligand-to-metal ratio of 2.5:1). Biodistribution experiments in rats showed good skeletal accumulation (2.4% ID/g after 24 h) and insignificant uptake in other organs (<0.15% ID/g). The authors designated ^{170}Tm-DOTMP as an excellent candidate for clinical development.

3.1.9. Ytterbium-175 (^{175}Yb)

^{175}Yb is a reactor-produced radionuclide with similar radiochemistry as other radiolanthanides such as ^{153}Sm and ^{170}Th, and forms stable complexes with phosphonates in the 3^+-oxidation state. No clinical studies have yet been reported.

3.1.9.1. Research and development

3.1.9.1.1. ^{175}Yb-EDTMP, ^{175}Yb-PDTMP, ^{175}Yb-DTPMP, ^{175}Yb-TTHMP. Mathew et al. investigated several ^{175}Yb-labeled complexes polyaminomethylenephosphonates which included EDTMP, PDTMP, DTPMP and TTHMP [144]. Incubation at room temperature was found sufficient to obtain complexes of high radiochemical purity and stability. Biodistribution in rats demonstrated significant bone uptake within 3 h (around 4% ID/g). Soft tissue uptake was low for all complexes except PDTMP, which showed moderate liver and kidney uptake. This may be due to the relatively poor complexation, requiring the highest ligand-to-metal ratio (86:1) and pH (9.0).

3.1.9.1.2. ^{175}Yb-DOTMP. Experiments with the ^{175}Yb-labeled cyclic tetraphosphonate DOTMP were performed by Safarzadeh et al. [145]. Labeling took place at room temperature and the yields increased with higher ligand concentrations. Biodistribution was studied in rats. Bone accumulation was fast (uptake 4% ID/g at 2 h) and remained stable for 4 days. Liver uptake was negligible.

3.1.9.1.3. ^{175}Yb-TTHMP. The polyaminomethylenephosphonate ^{175}Yb-TTHMP was studied by Safarzadeh as well [146]. The reaction was found to proceed well at room temperature at a ligand-to-metal ratio of 25:1 and yielded a radiochemical purity of >95% and a stable complex, even in physiological conditions. Biodistribution experiments were performed in rats and showed high bone uptake and retention (uptake around 2.5% ID/g from 4 h post injection up to 8 days). Uptake in soft tissue was measured to be <0.2% at all time points.

3.1.9.1.4. ^{175}Yb-pamidronate, ^{175}Yb-alendronate. Experiments with the ^{175}Yb-labeled bisphosphonates pamidronate and alendronate were carried out by Fakhari et al. [147]. Simple incubation at room temperature resulted in high radiochemical purity and stable complexes. HA binding assays showed >95% binding, even at low ligand concentrations. Biodistribution studies in rats demonstrated good bone uptake (around 4% ID/g at 24 h) for both compounds. Due to the high kidney uptake of ^{175}Yb-alendronate, the authors conclude that ^{175}Yb-pamidronate would be a better candidate for further development.

3.1.10. Yttrium-90 (^{90}Y)

^{90}Y is a reactor- or generator-produced radionuclide, formed by decay of ^{90}Sr. It is a transition metal and can form stable complexes with phosphonates. It decays by β^- emission without emitting gamma photons. No patient studies with ^{90}Y-labeled phosphonates have been reported.

3.1.10.1. Research and development

3.1.10.1.1. ^{90}Y-DPD. Djokić et al. investigated the complex of ^{90}Y with DPD [148]. Surprisingly, the stability at 37 °C was much higher than at room temperature. By using molecular modeling techniques two structural formulas were proposed: $[^{90}Y\text{-}(LH_4)_2(H_2O)_2]^-$ and $[^{90}Y\text{-}(LH_2)(H_2O)_2]^-$ (L = ligand). The preferential structure was found to be dependent on the reaction pH. *In vivo* experiments were performed in rats and the bone uptake of ^{90}Y-DPD was measured to be 11.3% ID/g 24 h after injection, with a bone-to-muscle ratio of 470. The bone accumulation was found to be higher than that of ^{99m}Tc-DPD and ^{90}Y-DPD prepared with a labeling kit containing tin. The bone uptake profile was demonstrated to be dependent on the pH. The authors contribute this phenomenon to the different molecular structures formed.

3.1.10.1.2. ^{90}Y-DOTA-HBP. The ^{90}Y-labeled conjugate of the bisphosphonate 4-amino-1-hydroxybutylidene-1,1-bisphosphonate (HBP) with 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetracetic acid (DOTA) was studied by Ogawa et al. [149]. Using RP-HPLC the authors concluded that ^{90}Y is chelated by the DOTA-part of the conjugate. Biodistribution was studied in mice and compared to ^{90}Y-citrate. The measured femur uptake after 3 h was higher (38% ID/g) for ^{90}Y-citrate compared to ^{90}Y-DOTA-HBP (20% ID/g). However, because of fast blood clearance of ^{90}Y-DOTA-HBP and slow soft tissue clearance of ^{90}Y-citrate, the biodistribution of the conjugate was superior. The authors concluded that ^{90}Y-DOTA-HBP has similar properties to ^{153}Sm-EDTMP and ^{177}Lu-DOTMP.

3.1.10.1.3. ^{90}Y-EDTMP. The complex of ^{90}Y with the tetradentate ligand EDTMP was investigated by Khalid et al., who prepared the carrier-added complex using reactor-produced ^{90}Y and the carrier-free complex from ^{90}Y obtained from a $^{90}Sr/^{90}Y$-generator system [150]. Several parameters were optimized. At a ligand-to-metal ratio of 5:1 complexation proceeded fast at room temperature, both for the carrier-added and the carrier-free preparation and yielded stable products. Bone uptake (about 50% of ID after 4 h) and bone-to-soft tissue ratio (>100 at 4 h) in rats were similar for both preparations as well.

3.2. Phosphonates radiolabeled with conversion electron (CE) emitting radioisotopes

Although bone pain palliation has been mostly based on the use of beta emitting radioisotopes, interest has also been focused on the use of low energy emitters, *e.g.* conversion electron (Auger electron) emitting radioisotopes, because of the decreased tissue penetration and the potential benefits of dose reduction to non-target tissue (*i.e.* bone marrow).

3.2.1. Tin-117m (^{117m}Sn)

The conversion electron emitting radionuclide ^{117m}Sn can be both reactor (low specific activity, LSA) – and accelerator (high specific activity, HSA)-produced. Bone pain palliation agents based on ^{117m}Sn can be prepared with reactor-produced ^{117m}Sn since high specific activity is not required for this application. This post-transition radiometal occurs in the $^{2+}$- and $^{4+}$-oxidation state. The skeletal localization of $^{117m}Sn(IV)$ was initially described by Yano and co-workers [151]. Early animal studies showed that osseous localization of ^{117m}Sn-(IV)-DTPA (stannic form) was far superior to bone localization of $^{117m}Sn(II)$-DTPA (stannous form). Later studies demonstrated that the stannic form was rapidly localized in bone and exhibited quick urinary excretion and absence of marrow activity [152,153]. Phase I and II studies were performed demonstrating pain palliation in 75% of patients at doses of 1 to 10 MBq/kg [154–157]. Pain palliation was reported as early as 1 week after treatment [157]. Since ^{117m}Sn-(IV)-DTPA is not a phosphonate-based radiopharmaceutical, further details are not discussed in this review.

3.2.1.1. Research and development

3.2.1.1.1. $^{117m}Sn(II)$–APDDMP. The $^{117m}Sn(II)$-labeled *N,N*-dimethylenephosphonate-1-hydroxy-3-aminopropylidene-phosphonate (APDDMP) has been evaluated by Zeevaart et al. [158]. These studies used reactor-produced LSA ^{117m}Sn, which was added as $SnCl_2$ to the APDDMP dissolved in dilute NaOH, yielding high radiochemical purity. Biodistribution experiments in rats showed relatively high kidney (≈6.5% ID/g) and bladder (≈1% ID/g) uptake after 4 h, which exceeded uptake in bone (<1% ID/g). Sequential gamma camera imaging showed that high kidney and bladder activity persisted over a 30–240 minute period. The data demonstrated the weakness of the Sn-chelate bound in the APDDMP-complex and for this reason this agent has not been investigated further.

3.2.1.1.2. $^{117m}Sn(IV)$-PEI-MP. In a series of physical chemistry experiments, Jansen and co-workers have reported the preparation and evaluation of the ^{117m}Sn-labeled *N,N′,N″*-trimethylenephosphonate-polyethyleneimine (PEI-MP) [22]. This ligand was chosen because of the expectation that this macromolecule would enable accumulation within tumors. The stability constants of Sn^{4+} complexed with different amino acids were measured by potentiometry. A thermo-dynamic blood plasma model predicted that the ^{117m}Sn-(IV)-PEI-MP complex readily dissociates in plasma, followed by complexation of the Sn^{4+} by glutamine. Computer simulation suggests that the PEI-MP ligand becomes complexed with Ca^{2+} subsequently. The ^{117m}Sn-Sn(IV)-PEI-MP complex is thus unsuitable for further evaluation as a bone-seeking radiopharmaceutical.

3.3. Phosphonates radiolabeled with alpha emitting radioisotopes

Alpha-particle emitting radioisotopes were the subject of some of the earliest radiochemical studies when naturally actinides were first available for chemical analysis after isolation from pitch blend as early as 1798. In more modern times, the broad emergence of interest in alpha emitters in the clinical community has focused on their high linear energy transfer (LET), minimal tissue radiation penetration and expected effective use for targeted therapy, primary for cancer treatment. Several alpha emitters (^{211}At, ^{225}Ac, ^{212}Bi, ^{213}Bi, ^{212}Pb, ^{227}Th) have been attached to bone targeting agents, or in one case, used directly as the targeting cation ($^{223}Ra^{2+}$ dichloride). A variety of approaches have been evaluated for attaching these radioisotopes to metabolically active osteoblastic sites. The approval and market entry in 2013 of ^{223}Ra-chloride (Xofigo®, Bayer Pharma AG, Germany; Bayer Healthcare Pharmaceuticals, USA) for the treatment of castration resistant metastatic prostate cancer reflects the increasing interest in the use of alpha emitting radioisotopes. However, this radiopharmaceutical is not phosphonate-based and will therefore not be discussed in this review.

3.3.1. Actinium-225 (^{225}Ac)

Thorium radioisotopes are naturally occurring and are generated by radioactive decay of the corresponding uranium series (*i.e.* $^{233}U \rightarrow {}^{229}Th$; $^{232}U \rightarrow {}^{228}Th$). The ^{225}Ac is the decay product of ^{229}Th and is of interest as a therapeutic radioisotope. This trivalent radioactinide can be coupled to phosphonates.

3.3.1.1. Research and development

3.3.1.1.1. ^{225}Ac-EDTMP. EDTMP is a strong chelator for actinides and the ^{225}Ac-labeled agent was initially prepared by Beyer et al. for potential tumor therapy [159]. These studies included EDTMP complexes of ^{141}Ce, ^{143}Sm, ^{149}Gd, ^{167}Tm and ^{225}Ac, and were directed at evaluation of the effects of the ionic radii of radiolanthanides on nude mice tissue localization and excretion data after 15 h. Although tumor and liver

uptake were dependent on the EDTMP concentration, there was no effect of ligand concentration on bone uptake. An examination of tissue distribution data in mice clearly indicates that ^{225}Ac-EDTMP shows high osseous localization, with osseous uptake values of 10–11% ID/g and high bone/blood ratio after 15 h, but this agent was not further evaluated because of the improved properties of ^{225}Ac-DOTMP described below.

3.3.1.1.2. ^{225}Ac-DOTMP. The cyclic phosphonate DOTMP has been radiolabeled with ^{225}Ac by Henriksen et al. [160]. Early studies evaluated the preparation and mice biodistribution of the ^{228}Ac chelate of DOTMP which showed high bone uptake and low soft tissue localization. ^{228}Ac was eluted from a ^{228}Ra generator and was used as a surrogate for ^{225}Ac because of low availability of ^{225}Ac during the experiments. After purification, aliquots were administered to mice. The 4 h post injection biodistribution results demonstrated adequate *in vivo* stability, high bone uptake (20% ID/g) and high bone-to-soft tissue ratios. The femur-to-kidney ratio was measured to be 7.6 at that time point. ^{228}Ac-DOTMP showed a liver uptake of 1%, probably due to some demetallation. The results of these studies demonstrated that DOTMP is a more effective ligand than EDTMP for stable chelation of Ac^{3+}. There have not yet been any human studies reported with these agents, but these data suggest that ^{225}Ac-DOTMP may be an attractive agent.

3.3.2. Astatine-211 (^{211}At)

^{211}At is an artificially produced alpha radionuclide that decays *via* a two-branched pathway, where each decaying ^{211}At atom contributes to the production of one alpha particle per decay to stable lead. Because it is the last member and least electronegative of the halogen family, astatine (as $^{211}At^{1-}$) thus exhibits some metallic properties, and no stable isotopic forms exist. For this reason, non-radioactive At-compounds cannot be prepared and characterized. The carbon-astatine bonds are the weakest in the halogen series and At-compounds are thus labile to de-astatination. Electrophilic astatodemetallation of organostannanes is often pursued to prepare ^{211}At-tracers that are more stable. ^{211}At is difficult to produce and handle and requires halogen chemistry rather than the more subtle and easier binding of cations to chelating agents.

3.3.2.1. Research and development

3.3.2.1.1. ^{211}At-BPB and ^{211}At-PPB. Both ^{131}I- and ^{211}At-labeled amidobisphosphonate analogues were prepared and evaluated by Murud et al. [53]. Two precursors were astatinated with ^{211}At by an electrophilic route, and then coupled with the commercially available bisphosphonate pamidronate (APB) in 60–97% final product yields. After purification, the lipophilic properties and stability were studied. *In vitro* stability in sera from mouse, fetal calf and humans was evaluated by HPLC, which demonstrated high stability and only one peak, indicating one species. In another study these ^{211}At-labeled agents were evaluated in mice in conjunction with the ^{131}I-labeled analogues by Larsen et al. [52]. With regard to the ^{211}At agents, rapid blood clearance and high, stable skeletal localization were observed, although the ABPB analogue showed higher bone uptake and higher bone-to-soft tissue ratios than the APPB agent. Evidently, none of these agents were evaluated further in human studies.

3.3.3. Bismuth-212 (^{212}Bi)

Another generator-derived alpha emitting radioisotope that has been evaluated for bone pain palliation and other therapeutic applications is ^{212}Bi, which is obtained from decay of the lead-212 (^{212}Pb) parent from the ^{212}Pb/^{212}Bi generator system [161]. The ^{212}Pb is obtained from decay of ^{224}Ra, which is generated from the decay chain of long-lived ^{228}Th. This generator is being further commercially developed, primarily for peptide radiolabeling for Targeted Alpha Therapy (TAT) for treatment of some cancers [162,163]. Disadvantages of using radioisotopes such as ^{212}Bi are the high beta energies and very high-energy gamma emission from the ^{208}Tl decay product (2.6 MeV).

3.3.3.1. Research and development

3.3.3.1.1. ^{212}Pb/^{212}Bi-EDTMP. ^{212}Pb/^{212}Bi-radiolabeling of EDTMP was accomplished by Hassfjell et al. in 90% yield [164]. Biodistribution experiments were conducted in mice and showed good bone uptake (10% ID/g for ^{212}Pb and 8% ID/g for ^{212}Bi after 24 h). However, high kidney uptake was also observed with retention even after 24 h, and further studies with these agents have not been reported.

3.3.3.1.2. ^{212}Bi-DOTMP, ^{212}Pb/^{212}Bi-DOTMP. As an alternative, phosphonate complexes of both ^{212}Pb and its ^{212}Bi decay product have been evaluated for osseous targeting by the same investigators [165]. In these studies, the biodistribution of ^{212}Bi-DOTMP and DOTMP labeled with a ^{212}Pb/^{212}Bi mixture were evaluated in Balb/c mice and exhibited prominent bone localization (*e.g.* uptake 22% ID/g for ^{212}Bi-DOTMP after 2 h). Bone-to-soft tissue ratios were higher in young mice than in old mice, indicating enhanced uptake in areas with high bone turnover. However, these compounds also exhibited some kidney uptake (1% ID/g after 2 h). The biodistribution data are similar to that exhibited by ^{153}Sm-EDTMP. The results have shown that DOTMP chelates ^{212}Pb and ^{212}Bi more effectively than EDTMP. The same group used the longer lived ^{205}Bi radioisotope ($t_{1/2}$ 6.2 days) permitting autoradiographic (ARG) evaluation of tissue samples post injection [166]. The ^{205}Bi-DOTMP analogue was evaluated in young Balb/C mice and tissues analyzed by ARG. Although these studies showed high osseous localization of activity, inhomogeneous skeletal biodistribution of the tracer was detected with higher uptake in the bone matrix, endosteum and growth zones. Chromatographic analysis of the urine showed excretion of the intact administered agent, suggesting high *in vivo* stability. No human studies have yet been reported with the ^{212}Bi- and ^{213}Bi-labeled agents.

3.3.4. Thorium-227 (^{227}Th)

^{227}Th is also of interest for bone pain palliation, as a sort of "*in vivo*" generator system - since it is obtained from decay of actinium-227 (^{227}Ac). The long-lived and highly prized ^{227}Ac β^- decays to ^{227}Th, which is the key intermediary radioisotope for alpha decay to ^{223}Ra.

3.3.4.1. Research and development

3.3.4.1.1. ^{227}Th-DOTMP, ^{227}Th-DTMP. Henriksen et al. evaluated the biodistribution of ^{227}Th as chelates of DOTMP and diethylenetriaminepentamethylenephosphonate (DTMP) [160]. Anion exchange chromatography of a ^{227}Ac decay mixture was used to extract ^{227}Th. The thorium polyphosphonate chelates were prepared by heating mixtures of the chelating agent and solutions containing the radionuclide, followed by isolation of the complexes by cation-exchange resin columns. >80% of the ^{227}Th eluted with both DTMP and DOTMP. Femur uptake in mice 4 h after administration was comparable to that of ^{228}Ac-DOTMP (20% ID/g). The femur/kidney values from biodistribution of ^{227}Th-DOTMP and ^{227}Th-DTMP evaluated were comparable to ^{228}Ac-DOTMP (6.0 for ^{227}Th-DOTMP and 14.2 for ^{227}Th-DTMP). However, the femur/liver ratio of the two ^{227}Th-complexes was higher, probably due to less demetallation.

3.3.4.1.2. ^{227}Th-EDTMP. In a study of Washiyama et al., the ^{227}Th-EDTMP complex exhibited good and stable bone accumulation evaluated in mice [167]. Femur uptake of after 24 h ^{227}Th-EDTMP was 8.7% ID/g and remained stable for 14 days. Uptake in other tissues was negligible. Since ^{227}Th continually decays to ^{223}Ra as an "*in vivo*" generator, this phenomenon provides even longer target tissue exposure to alpha rays. Evidently, no human studies have yet been reported with these ^{227}Th-labeled phosphonates.

4. Discussion and conclusion

Targeted skeletal radiotherapy using phosphonate-based therapeutic bone-seeking radiopharmaceuticals is an elegant and established treatment option in therapy-resistant painful bone metastases based on a theranostic approach and is well tolerated and effective in 60–80% of the patients [4,6,8,10]. >35 years of research has been conducted

on the development of phosphonate-based therapeutic bone-seeking radiopharmaceuticals. Despite all research efforts, of the 91 identified radiopharmaceuticals, only six of these radiolabeled therapeutic phosphonate agents have been reported in patient studies. It is very difficult to introduce newly developed radiopharmaceuticals into clinical trials, even after many years of research. This reality reflects the economical and legal hurdles on the path to marketing authorization [33]. In this review we unlock the literature on development and early clinical application of phosphonate-based bone-targeting therapeutic radiopharmaceuticals.

4.1. Meeting selection criteria: are there ideal phosphonate radiopharmaceuticals?

The characteristics of an ideal therapeutic bone-targeting radiopharmaceutical that are pursued are mentioned in Table 1 (see section Methods) and below we summarize these criteria with regard to the various compounds discussed in this review.

4.1.1. Energy and range in tissue.

An indirect comparison of success in palliation without complications with the radionuclides that have been clinically evaluated indicates that the energy of the emitted particles is not critical for their efficacy. Similar palliative results can be obtained with high (^{186}Re, ^{188}Re), medium (^{131}I, ^{153}Sm) and low energy (^{177}Lu) beta emitting radionuclides, similar to both CE- and with alpha emitting radionuclides. However, because direct comparative studies are lacking, no definitive conclusion can be drawn on the influence of radiation energy and its range in tissue on efficacy. For future research, we propose to develop radiopharmaceuticals that deliver their radiation dose over a short period of time, to reduce possible myelotoxicity and allow serial treatment with other cytotoxic drugs. For a similar radiation dose in a shorter period, preferably a high-energetic radionuclide (*e.g.* ^{188}Re) is used.

4.1.2. Physical half-life

Of the 16 radionuclides investigated for the development of bone-seeking phosphonate-based radiopharma-ceuticals, only ^{170}Tm does not meet the criteria in Table 1 due to its very long half-life of 128 days. The use of radioisotopes for bone palliation which have relatively short half-lives of a few hours to a few days has the potential advantage of a high dose rate and enables less expensive outpatient treatment. In addition, dose cycles with repetitive administration and serial treatment with other myelotoxic drugs (*e.g.* chemotherapy) are feasible. Furthermore, the use of short-live radioisotopes is advantageous from the perspective of reducing radioactive waste. Radionuclides evaluated for bone pain palliation which have short half-lives (<2 days) include ^{166}Ho, ^{188}Re, ^{105}Rh and ^{153}Sm, and those categorized with a medium half-life (2 days–14 days) include ^{131}I, ^{177}Lu, ^{32}P, ^{186}Re, ^{117m}Sn, ^{90}Y and ^{175}Yb. For the alpha emitters the situation is more complex, because the radioisotopes are often part of a decay system consisting of several radionuclides with different half-lives, making it meaningless to apply the criterion of a short half-life for the radionuclide itself.

4.1.3. Gamma radiation for imaging

Most radionuclides discussed in this review that have been evaluated for bone pain palliation emit gamma photons suitable for imaging, which allows dose prescription and correlation of effectiveness of therapy in a personalized manner (theranostic approach). The exception is ^{90}Y that does not decay with emission of gamma photons and imaging must be conducted with detection of bremsstrahlung radiation. Some of the alpha decay chains emit photons.

4.1.4. Availability

Some of the therapeutic radioisotopes required for preparation of radiolabeled phosphonates for bone pain palliation are commercially available. Generator-based radionuclides are available on demand, which is a distinct advantage in terms of availability, cost and continuity of patient care. The only generator-derived beta emitting radionuclides that have been used in the context of preparation of phosphonate-based agents for bone pain palliation are ^{166}Ho, ^{188}Re and ^{90}Y. The alpha emitters ^{212}Bi and ^{227}Th are also generator-derived. Some of these generator systems are GMP-produced, which is a major advantage for clinical application.

4.1.5. Precursor quality

The chemical quality of precursor molecules that are used for attachment of the therapeutic radionuclides have strangely been rarely mentioned in the literature. Moreover, Pharmacopoeial monographs lack for most ligands studied. However, given the current requirements to insure that agents for human use are manufactured under GMP conditions, the quality aspects of radionuclides, ligands and other chemicals, including procedures, storage conditions, QC issues, specifications, as well as the supply chain, must be clearly documented [168]. Due to unavailability, quality issues or other reasons some researchers have often synthesized the ligands in house. In such situations, all quality assurance activities are the responsibility of the investigators. All quality issues have to be solved before human studies can be started.

4.1.6. Preparation aspects

Our review of the literature revealed large differences in the preparation aspects of phosphonate-based radiopharmaceuticals. The most simple preparation method (incubation at room temperature) is sufficient for the labeling of phosphonates with many cationic radiolanthanides, such as ^{166}Ho, ^{177}Lu, ^{153}Sm, ^{170}Tm and ^{175}Yb. Coupling of some radionuclides (^{90}Y, ^{117m}Sn and ^{225}Ac) to phosphonates, however, requires heating to accelerate chelation by some ligands, while incubation at room temperature is enough for other ligands. ^{105}Rh, ^{212}Pb/^{212}Bi and ^{227}Th require heating for adequate labeling of all phosphonates identified. In contrast, the coupling of ^{186}Re and ^{188}Re with phosphonates requires an initial reduction step as well as heating. Furthermore, radiolabeling with generator-derived ^{188}Re requires the addition of carrier rhenium and optimization of the composition and reaction conditions for the formation of a stable complex and to maximize *in vivo* bone accumulation of the labeled species. The biconjugate complexes of ^{186}Re and ^{188}Re and of the radiohalogens ^{131}I and ^{211}At cannot be synthesized by using simple preparation methods.

4.1.7. Quality control (QC)

For most compounds discussed in this review, simple QC techniques such as paper chromatography and (instant) thin layer chromatography are generally sufficient for routine evaluation of the radiochemical purity of the final products. In some cases, however, complexes have also been analyzed by (paper) electrophoresis, ion exchange chromatography, HPLC or gel permeation chromatography.

4.1.8. Stability

Large differences in stability data were reported in the reviewed studies, not only between different compounds, but for the same compound as well. In general, lanthanide-based phosphonates are very stable, up to a week or a month at room temperature. Also, the complexes of some transition metals (^{105}Rh and ^{90}Y) and the radiohalogens ^{131}I and ^{211}At have been reported to be stable up to a week at RT. On the other hand, the stability of some phosphonate-complexes of ^{186}Re and ^{188}Re has been found to be as short as only a few hours. Moreover, for the rhenium-HEDP-complexes divergent stabilities were reported, which may indicate suboptimal composition and/or reaction conditions. Although in some studies the stability under *in vivo* conditions, but measured *in vitro*, is reported, virtually no data on the *in vivo* stability have been reported. Rhenium-complexes are known to oxidize readily *in vitro* and *in vivo* and this disadvantage results in possible perrhenate

accumulation in non-target tissues. However, *in vivo* oxidation might proceed more slowly in metastatic bone lesions, due to their hypoxic nature, leading to higher retention of the radionuclide than in normal bone tissue [169].

4.1.9. Characterization

Our analysis of the literature clearly indicates that characterization of the molecular species has unfortunately not been a goal in most studies. Some general comments on this topic can be made. As a key example, rhenium can exist in a variety of oxidation states. Complexes of rhenium and other metals with bisphosphonates consist of different species and the exact molecular structures of these complexes are not known. Some experiments suggest that oligomeric mixed metal complexes (including rhenium and tin) are formed [78]. Because of the availability of chelate species that very effectively and tightly bind metallic cations – especially in the 3^+ oxidation state – the chelates of radiolanthanides with multidentate ligands generally form single well-defined uniform species. Complexes of radiometal cations with multidentate phosphonates probably form single species as well [140]. Because radiohalogens must be covalently attached at specific molecular positions during reaction with phosphonates, single species are usually formed.

It is interesting to note that traditional strategies such as preparation of the non-radioactive complexes have in general not been pursued, even when the non-radioactive congeners of the radioisotope are available. This strategy involves preparation of the complex with non-radioactive or very low specific activity species, followed by chromatographic comparison with the high specific activity radioactive preparations. If identical chromatographic data are obtained for both preparations, than the non-radioactive species can then be fully characterized by various traditional methods and will then provide guidance concerning the molecular species formed during the radioactive preparation. In addition, the literature search has also demonstrated that the fate of the administered complexes *in vivo* is not at all well understood. As an example, competition can take place between phosphonates and physiological ligands, like citrate and amino acids [20–22]. Multiple species that are formed during radiochemical preparation, resulting from decomposition before or after administration or due to other *in vivo* mechanisms may have different biodistribution patterns. Extraction of tissues and chromatographic analysis of the radioactive species present *in vivo* after various time periods after administration would provide useful information on the metabolism of the administered agent to active or non-targeting species.

4.1.10. Biodistribution in animals

Significant research has been conducted on the biodistribution of administered radioactive phosphonate species in several animal species, which has been mainly focused on bone and soft tissue uptake and imaging. However, no uniform methods for performing these experiments have been applied, in particular measuring bone uptake and bone-to-soft tissue calculations and for these reasons it is very difficult to compare the study outcomes. Although animal studies are of course mandatory and have provided many useful data that have served as the basis for further research and for introduction of agents for human studies, standardization of preclinical biodistribution experiments of bone-targeting radiopharmaceuticals is warranted.

4.2. Clinically used compounds

Of the compounds used in humans, ^{166}Ho-DOTMP was specifically developed for bone marrow ablation [40,41]. ^{131}I-BPD3 was only studied in a small phase I study, but the relatively poor results and the development of more promising agents precluded further clinical evaluation of this agent [50].

The development and evaluation of ten ^{186}Re-labeled phosphonate analogues identified in this review resulted in the clinical introduction of only ^{186}Re-HEDP for bone pain palliation [80–84]. Although ^{186}Re-HEDP has been investigated for >35 years, worldwide approval and marketing authorization was never achieved for several reasons. Evidently, appropriately designed and controlled clinical trials had not been completed prior to the attempts for market approval. The product Re-Bone® had been licensed in some European countries for a short time period in the 1990s, but the marketing was discontinued due to economic reasons. Since then, the application of this unlicensed radiopharmaceutical has likely come to a complete stop.

At the current time, ^{188}Re-HEDP is the only one of sixteen ^{188}Re-phosphonates studied which has entered the clinical arena [111,112]. Although significant research on the preparation and evaluation of ^{188}Re-HEDP over nearly 20 years has led to encouraging results, ^{188}Re-HEDP is not yet available as a licensed product. This ^{188}Re-labeled phosphonate has been used in many countries, but well-designed protocols and a defined strategy for its application are not yet widely accepted and applied, except in the Netherlands [108].

^{153}Sm-EDTMP is the only one of fifteen ^{153}Sm-labeled phosphonates that is approved and used extensively for bone pain palliation. It has gained worldwide marketing authorization and is currently probably the most widely used phosphonate-based radiopharmaceutical on a worldwide basis.

Up to now, of twelve lutetium-compounds investigated, only ^{177}Lu-EDTMP has reached the stage of clinical evaluation [59,60]. Recently, phase I/II-studies with ^{177}Lu-EDTMP have been reported and although experience with this new agent is still limited, it has been approved recently for clinical use in India [61,62].

For clinicians, choosing the best treatment option depends on several criteria, and may turn out differently due to local conditions. Although one might prefer an approved agent, the availability of ^{153}Sm-EDTMP is not guaranteed for all nuclear medicine departments due to high costs or logistical issues. ^{177}Lu-EDTMP may turn out to be a better option in some situations, but the clinical experience with this compound is still limited. Of the clinical used agents, ^{188}Re-HEDP is the only generator-based radiopharmaceutical, which enables cheap and on demand preparation. Despite the wide clinical experience, this compound has some disadvantages. Kits are not available commercially, so the composition and preparation method must be optimized to ensure proper biodistribution.

4.3. Compounds in research

Despite auspicious *in vitro* results and *in vivo* data from animal studies, most of the phosphonate-based radiopharmaceuticals described in the radiopharmaceutical literature have not yet progressed to human studies. These include complexes of multidentate phosphonates of ^{186}Re, ^{188}Re, ^{105}Rh, ^{170}Tm and ^{175}Yb and bifunctional phosphonate complexes of ^{186}Re, ^{188}Re and ^{90}Y. Of these new agents, ^{175}Yb-TTHMP may represent one of the most promising compounds. Preparation of this agent is simple and provides a stable complex, but a disadvantage is the low specific activity of reactor-produced radioisotope ^{175}Yb. Furthermore, the TTHMP ligand is evidently not routinely available, and would probably have to be synthesized by any investigator interested in studying this agent further.

5. Conclusion

In conclusion, phosphonate-based radiopharmaceuticals are effective and safe agents for the treatment of painful bone metastases. Some compounds allow for theranostic approach. Significant research with these compounds has been conducted over the past 35 years, however, clinical introduction has proven to be very difficult. Of 91

compounds identified in this review, only six have been clinically used, and of these, only three compounds, ^{188}Re-HEDP, ^{153}Sm-EDTMP and ^{177}Lu-EDTMP are currently in clinical use. Their pain palliation response is similar (60–80%) and their side effects are limited. A key point which has been distilled from this literature search is that thorough understanding of the radiochemistry is required to fully exploit the potential benefit of these radiopharmaceuticals. In addition, simple and robust preparation and quality control methods are essential. Moreover, studies focused on the molecular characterization of these compounds are very important to identify species present in the radiopharmaceutical preparations. Extensive biodistribution and dosimetry studies are also required to complete the dossier required for approval before administration to humans is possible. Focusing on the desired properties and use of existing knowledge should guide future research.

Conflicts of interest

The authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bone.2016.08.002.

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