General summary, discussion and future perspectives
1. Introduction

Due to a lack of awareness and misconceptions regarding toxicity and costs radionuclide therapy of bone metastases is an underutilized treatment modality [1–5]. However, the targeted treatment with phosphonate-based radiopharmaceuticals in advanced cancer patients with a positive bone scintigram is an appealing example of theranostics with a high probability of improving quality of life [6–8]. Rhenium-188-HEDP (\(^{188}\text{Re}\)-HEDP) might be a preferable compound for the targeted treatment of painful bone metastases. It can be prepared in a typical hospital pharmacy department, enabling fast and tailored therapy upon presentation of the patient [9,10].

As explicated in the General introduction (chapter 1), the central goal of the research described in this thesis was the bench-to-bedside development of \(^{188}\text{Re}\)-HEDP. We performed the pharmaceutical development, preclinical studies and clinical evaluation of this bone-seeking therapeutic radiopharmaceutical. In this chapter the main outcomes of our investigations are summarized and discussed. Furthermore, some future perspectives are provided.

1.1 Phosphonate-based therapeutic radiopharmaceuticals

Chapter 2 is a review of the development of therapeutic phosphonate-based radiopharmaceuticals and their investigation for the targeted treatment of painful bone metastases across several decades. We performed a systematic literature search to unlock research data on the pharmaceutical development, preclinical research and early human studies of these radiopharmaceuticals. A total of 91 phosphonate-based therapeutic radiopharmaceuticals were identified, from which only six agents have been clinically used. Only \(^{186}\text{Re}\)-HEDP, \(^{188}\text{Re}\)-HEDP and \(^{153}\text{Sm}\)-EDTMP have been investigated in extensive clinical studies. \(^{153}\text{Sm}\)-EDTMP is the only compound that received marketing authorization in many countries. \(^{188}\text{Re}\)-HEDP is generator-based, enabling on demand preparation. In India, \(^{177}\text{Lu}\)-EDTMP has recently been approved for clinical use.

The topics we explored in reviewing the literature on the development of these theranostic radiopharmaceuticals included their energy and range in tissue, physical half-life, suitability for imaging, precursor availability and quality, preparation and quality control aspects, stability, characterization and biodistribution.

To obtain a comparable radiation dose in a shorter time period, a high-energetic radionuclide (e.g. \(^{188}\text{Re}\)) is preferable. In view of availability, continuity of patient care and healthcare costs generator-based radionuclides, like \(^{188}\text{Re}\), are advantageous. In a recent review comparing 11 radionuclides for palliative treatment of metastatic bone pain, \(^{188}\text{Re}\) is proclaimed the best option for medium to large sized metastases [11].

The composition and preparation conditions may be crucial for product quality and biodistribution of phosphonate-based radiopharmaceuticals. Although the molecular structure is important for the \(\text{in vivo}\) behaviour of these radiopharmaceuticals, characterization has been
the subject of only very few studies. Complexes of rhenium with phosphonates like $^{188}$Re-HEDP consist of different species of which the exact molecular structures and spatial orientation are not known. Therefore, extensive research on the preparation and stability is required in each centre that prepares this radiopharmaceutical.

In order to exploit the full potential of therapeutic phosphonate-based radiopharmaceuticals, simple and reproducible preparation and quality control methods are essential. Proper understanding of the radiochemistry of these agents is indispensable to design these methods. Moreover, extensive biodistribution and dosimetry studies are required before these products can be administered to humans. The knowledge provided in this review should guide research on the development and potential approval of new promising agents.

This review demonstrates the difficulty of introducing newly developed radiopharmaceuticals into clinical trials. Legal and economical hurdles may contribute to the challenging route to marketing authorization [12].

1.2 Regulations for the preparation of radiopharmaceuticals

To enable clinical studies and introducing new pharmaceuticals adherence to appropriate regulations is essential. In Chapter 3 European legislation and guidelines on the preparation of radiopharmaceuticals are reviewed. The strict regulations on production and radiation safety may constitute a hurdle for their development and clinical use. Moreover, they may have a large budget impact and are not always appropriate for their production, due to some particularities of radiopharmaceuticals.

Therefore, we propose a risk-based approach for the implementation of these regulations and provide guidance on interpreting and applying Good Manufacturing Practice (GMP) for the small-scale production of radiopharmaceuticals, including cleanroom classification, air pressure regime, cleanroom qualification and microbiological monitoring. We present an algorithm to assess the risk of microbiological contamination of a radiopharmaceutical preparation process and propose corresponding GMP classification levels. We argue that the risk of carry-over of radiopharmaceuticals cannot be contained by pressure differences and that a simple cleanroom design is sufficient in most radiopharmacy departments. We propose a sterility assurance level of $10^{-2}$ for the preparation of radiopharmaceuticals that are administered within a working day, and suggest adopting less strict limits for environmental monitoring of microbial contamination than those provided in GMP Annex 1. Recent regulatory documents seem to be more liberal than current legislation and appear to recognize the use of risk-assessment [13]. We state risk-assessment as the gold standard for implementation of appropriate quality assurance standards for the preparation of unlicensed radiopharmaceuticals.
1.3 Development of GMP-grade rhenium-188-HEDP

As $^{188}$Re-HEDP might be a preferable therapeutic radiopharmaceutical for the treatment of painful bone metastases, we developed a simple method for the preparation and quality control of GMP-grade $^{188}$Re-HEDP for its application in routine clinical practice and for support of clinical studies. In chapter 4 the entire bench-to-bedside development is presented. When we started to develop $^{188}$Re-HEDP in our hospital, no standardized preparation method was available. Standardization is crucial, since drug composition and preparation conditions are known to affect product quality, stability and efficacy. Also the production of sterile non-radioactive starting materials is described. These materials constitute a cold kit (containing the ligand HEDP, the reductant stannous chloride and the antioxidant gentisic acid), an ammonium perrhenate solution (carrier rhenium) and a sodium acetate solution (for pH correction). Furthermore, validation, stability and microbiological data are provided. Finally, hydroxyapatite binding assay results and biodistribution data of $^{188}$Re-HEDP in mice and in patients with bone metastases are presented.

We showed that our composition and preparation process result in a product with requisite quality, stability and biodistribution in mice. Scintigrams acquired 3 hours after administration of $^{188}$Re-HEDP to patients demonstrated only uptake in the predicted localizations. Product quality review during the first year of routine use of $^{188}$Re-HEDP proved that the preparation method was robust. This completely validated method to prepare GMP-grade $^{188}$Re-HEDP may be transferred to other centres with sufficient numbers of patients who are eligible for bone-targeted radionuclide therapy.

1.4 Influence of carrier concentration

The above-mentioned development of $^{188}$Re-HEDP included investigations on the influence of non-radioactive (carrier) rhenium concentration. The presence of carrier rhenium in the reaction mixture is known to be required for adequate product quality and bone affinity of $^{188}$Re-HEDP, but the optimal carrier concentration was unknown when we started our research. In chapter 5 investigations on the influence of carrier concentration on radiochemical purity, in vitro hydroxyapatite affinity and in vivo bone accumulation of $^{188}$Re-HEDP in mice are presented.

The carrier concentration proved to influence both hydroxyapatite binding in vitro and bone accumulation in vivo. Hydroxyapatite binding was around 95% at carrier amounts between 0.1 and 20 $\mu$mol. The radiochemical purity of the complex was sufficient for clinical use at carrier amounts from 0.01 $\mu$mol up to 20 $\mu$mol. In the in vivo experiments in mice no bone accumulation was observed for the $^{188}$Re-HEDP formulation without carrier, and only soft tissue uptake was visible. A carrier amount of 0.01 $\mu$mol showed both bone and soft tissue uptake. With 10 $\mu$mol of carrier only bone uptake was detected and soft tissue uptake was absent.

Since sufficient radiochemical purity and hydroxyapatite affinity not always resulted in
acceptable \textit{in vivo} bone accumulation of $^{188}$Re-HEDP, we hypothesize that the $^{188}$Re-HEDP-complex is only stable \textit{in vivo} at higher carrier concentrations. We conclude that human administration of phosphonate-based radiopharmaceuticals should only be initiated after evaluation of animal biodistribution experiments.

\section*{1.5 Quality by design of the preparation method}

Since preparation conditions may influence the product quality and \textit{in vivo} behaviour of $^{188}$Re-HEDP, we investigated the effect of critical process parameters on product quality and stability. The methods and results are described in chapter 6. We adopted the quality by design (QbD) concept of the ICH Q8 (Pharmaceutical Development) guideline and followed a stepwise approach.

First potential critical process conditions were identified. Subsequently, these conditions were varied, investigating the impact of each variable on radiochemical purity and stability of the product upon dilution and storage. The acceptable ranges of the process conditions were established by boundary testing.

Only two boundary tests: starting with a larger eluate volume than in the standard preparation method and a reaction time of 10 minutes resulted in inadequate radiochemical purity and stability. Furthermore, diluting the end product and storage at elevated temperature had a strong negative influence on product stability.

Our standard preparation method proved to fall well within the acceptable ranges, demonstrating the intrinsic robustness of both composition and preparation method. We showed that applying QbD principles is feasible for the small-scale preparation of radiopharmaceuticals. Mapping the acceptable ranges by validating the preparation process for each parameter while keeping all other variables constant is meaningful for assuring product quality.

\section*{1.6 Combination with taxanes}

In order to investigate the potential added value of combining chemotherapy and $^{188}$Re, exploiting the radiosensitization concept, we explored the combined treatment of the taxanes docetaxel and cabazitaxel with $^{188}$Re in prostate carcinoma cell lines. In chapter 7 we report on the methods and results of this research. We investigated the cytotoxic effects of single and combined treatment with taxanes and $^{188}$Re in three human prostate carcinoma cell lines, using the colony-forming assay (CFA).

All CFA experiments showed significant dose-dependent cell growth inhibition for both the taxanes and $^{188}$Re. The half maximum effective concentration (EC50) values of the individual agents were similar to results from earlier studies. However, the EC50 values of $^{188}$Re were not reported before.

The combined treatment was studied at 0.25, 0.5, 1, 2 and 4 times the EC50 value of the agents.
The interaction was investigated with a regression model, which was well capable of explaining the data. The model confirmed large negative effects on cell growth for the individual agents and proved significant additive effects of combination of the two taxanes and $^{188}\text{Re}$. Although synergistic action might have been expected, this was not demonstrated. However, additivity is a good basis for further research. The type and extent of interaction may be less important for optimizing the therapeutic strategy due to other factors contributing to the final treatment effectiveness.

The outcome of this proof-of-mechanism study encourages the design of (pre)clinical in vivo studies (e.g. in an animal bone metastasis model) and may contribute to the optimization of the treatment of advanced prostate carcinoma.

1.7 Clinical benefit in routine clinical care

$^{188}\text{Re}$-HEDP is an unlicensed radiopharmaceutical. Therefore, after introduction in the clinic, we evaluated the clinical benefit of treatment of prostate or breast cancer patients with painful bone metastases with this radiopharmaceutical in routine clinical care. This prospective, observational practice study is discussed in chapter 8.

Clinical benefit was defined as a response in pain palliation (using visual analogue scale (VAS)-scores and corrected for opioid intake) or quality of life (QoL, using the EORTC QLQ-C30 questionnaire), along with acceptable haematological toxicity. In this study, 45 patients were evaluable for pain palliation and 47 for QoL.

A single injection of $^{188}\text{Re}$-HEDP resulted in an overall pain response rate of 69% and a significant and relevant decrease of mean VAS-scores. The pain palliation efficacy of $^{188}\text{Re}$-HEDP in this study is comparable to that of external beam radiotherapy (EBRT).

This study is the first to evaluate the quality of life upon treatment with $^{188}\text{Re}$-HEDP. The overall QoL response rate was 68%, and a significant and relevant increase of the mean Global health status/QoL-scores was demonstrated.

Since haematological side effects were mild and transient, this study proves the clinical benefit of treatment of painful bone metastases with $^{188}\text{Re}$-HEDP and supports its application in routine clinical care.

The results in this ‘real world’ study may be influenced by the relatively large proportion of missing data. However, the measured pain palliation efficacy nicely corresponds to previous research. Moreover, a sensitivity analysis showed a pain response between 51% and 77%. This satisfactory outcome may be predictive of the value of this treatment in routine clinical practice.
2. Future perspectives

As we have shown in chapter 2, a bone-targeting therapeutic radiopharmaceutical that is ideal in every aspect still does not exist. For future research, development of radiopharmaceuticals delivering their radiation dose over a short period of time, reducing possible myelotoxicity and allowing repeated administration and serial or even concurrent treatment with other cytotoxic drugs, should be stimulated. To obtain a comparable radiation dose in a shorter time period, use of a high-energetic radionuclide is preferable.

Although $^{188}$Re-HEDP has several advantages over other agents, the complexation of rhenium with phosphonates may result in different species with unknown molecular structures and spatial orientation. The species present in the final product may exhibit different biodistribution patterns. Future research should include characterization of complexes that are formed in vitro and their in vivo behaviour. These investigations may involve preparation of the non-radioactive complexes, followed by chromatographic comparison with the radioactive preparations. If the chromatogram is identical for both preparations, the non-radioactive species can be characterized by various methods, providing information regarding the molecular species formed during the radioactive preparation. In addition, the stability and fate of the administered complexes in vivo have only rarely been explored. Chromatographic analysis of the species present in blood, tissues, urine and stool at different time points after administration would provide useful information on the pharmacokinetics of targeting and non-targeting species.

With respect to the clinical application of $^{188}$Re-HEDP, some recommendations for optimizing the use and for future research can be made.

First of all, dose individualization could be explored. Up to now, fixed doses or doses based on total body weight are applied. However, it is known that renal function may impact elimination of $^{188}$Re-HEDP, as it is mainly renally excreted [14] and it is unlikely that in obese patients total body weight correlates well with muscle mass. It might be worthwhile to investigate a dosing algorithm that is based on the number of lesions [15]. Moreover, a higher dose may be feasible and safe in patients with adequate platelet levels. In their dose finding study Palmedo et al. showed that the maximum tolerated dose (MTD) might be 4.4 GBq in patients with a thrombocyte count above $200 \times 10^9$/l [16]. A higher administered dose leads to a higher radiation dose to the metastases, which might improve the clinical outcome.

Secondly, repeated administration, e.g. every 8 weeks, should be pursued as long as this is feasible for the patient. The limited and transient side effects of $^{188}$Re-HEDP, maybe due to the short half-life of $^{188}$Re, make $^{188}$Re-HEDP suitable for iterated treatment. Furthermore, since the pain response is decreasing after 8 weeks, repeated injections could extend palliation. In our practice study, we observed similar pain responses in patients with repeated administration compared to the first treatment effect. Whether repeated treatment is able to extend survival, which is suggested in previous studies in relatively small numbers of
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patients [17,18], should be investigated in large prospective studies. These studies should preferably be carried out in a head-to-head fashion compared to $^{223}\text{Ra}-\text{chloride}$, since this is the only radiopharmaceutical with proven overall survival benefit in a large phase III trial [19]. Although pain palliation was demonstrated in some studies with $^{223}\text{Ra}-\text{chloride}$, this was not investigated as one of the endpoints in the registration study. Therefore, this indication is not included in the registration label. Furthermore, a slight decline in quality of life was observed in the treatment period. We are currently planning to conduct a large randomized multicentre study comparing $^{188}\text{Re}-\text{HEDP}$ and $^{223}\text{Ra}-\text{chloride}$ at two endpoints: palliative effectiveness (pain palliation and quality of life) and overall survival. This study should provide a major contribution to determining the real value of $^{188}\text{Re}-\text{HEDP}$ and to its positioning in the arsenal of radiopharmaceuticals for the treatment of bone metastases.

Thirdly, potential integration with other modalities should be explored. Combining $^{188}\text{Re}-\text{HEDP}$ with chemotherapy (e.g. taxanes) may augment the treatment efficacy, which was demonstrated previously for the combination of $^{153}\text{Sm}-\text{EDTMP}$ and docetaxel [20,21]. In a phase I study, the feasibility and safety of sequential therapy with docetaxel and $^{188}\text{Re}-\text{HEDP}$ was showed [22]. Results of phase II studies of the administration of docetaxel and cabazitaxel alternated with $^{188}\text{Re}-\text{HEDP}$ are pending. However, no in vivo data on concurrent application of taxanes and $^{188}\text{Re}-\text{HEDP}$ are available. Since significant additivity was clearly demonstrated in our study in prostate carcinoma cell lines, the design of (pre)clinical in vivo studies is warranted to explore the potential added value of concomitant treatment of taxanes and $^{188}\text{Re}-\text{HEDP}$. These studies could be carried out using $^{188}\text{Re}-\text{HEDP}$ in an animal bone metastasis model. However, as satisfactory results have been demonstrated in phase I and II studies combining $^{153}\text{Sm}-\text{EDTMP}$ and docetaxel using different schedules [20,21,23,24], the design of phase I/II studies exploring the safety and efficacy of concurrent administration of $^{188}\text{Re}-\text{HEDP}$ and taxanes may be considered as well. Also combination with EBRT, treating the largest lesions selectively with EBRT and smaller or invisible lesions with $^{188}\text{Re}-\text{HEDP}$, may be worthwhile. In order to optimize treatment effectiveness, even combination with other radiopharmaceuticals (e.g. $^{223}\text{Ra}-\text{chloride}$) might be considered and investigated.

Fourthly, next to examining pain palliation, in future studies additional (surrogate) response parameters should be monitored, like prostate specific antigen (PSA), alkaline phosphatase (ALP) and bone markers, which may provide valuable information on the treatment effectiveness of $^{188}\text{Re}-\text{HEDP}$. The use of recently introduced PET-tracers, like $^{18}\text{F}$-methylcholine, $^{18}\text{F}$-PSMA and $^{68}\text{Ga}$-PSMA, may be of added value for treatment response monitoring as well [25–28].

Lastly, treatment in an earlier stage of the disease should be studied [3]. Usually, radionuclide therapy is applied as a last resort in the end stage of advanced cancer. From studies with $^{89}\text{Sr}-\text{chloride}$ it is known that early treatment may result in a better outcome than a later start of the therapy [15,29]. This could be explained from a dosimetric point of view: small metastases are ablated better than larger lesions [30,31]. Complete eradication of small metastases
or prevention of lesions to grow might improve survival [31]. Moreover, patients may sustain (multiple cycles) of radionuclide therapy better in an earlier phase of their disease. Due to the short half-life of $^{188}$Re-HEDP, early administration does not preclude other treatment options in later stages of the disease. Since treatment with $^{188}$Re-HEDP is very safe, this opens possibilities to extend its range of use and to investigate its application earlier in the course of the disease.

Next to the optimization of the clinical use of $^{188}$Re-HEDP, it would be interesting to compare its application with the use of therapeutic radiopharmaceuticals based on the PSMA-motif. $^{27}$Lu-PSMA has already been applied in clinical studies [32]. Although the labelling of PSMA with $^{188}$Re has been published [33], no clinical use of this agent has been reported up hitherto. Again, rapid preparation of this radiopharmaceutical would be possible, when a $^{188}$W/$^{188}$Re-generator is already present in the radiopharmacy department. No literature on the labelling of PSMA with the alpha emitting radionuclide $^{223}$Ra has been identified up to now, but a case report on treatment with the alpha emitter $^{225}$Ac-PSMA was published recently [34]. When PSMA-based therapeutical radiopharmaceuticals become available for clinical use, they might be combined with bone-targeting agents as well, since they are directed to additional targets. This might further improve the clinical benefit of radionuclide therapy in patients with metastatic bone disease.

References are listed after the Dutch summary (page 222).
References / Literatuur


