User project report

$^{203/212}$Pb-mcp-D-PSMA for an improved tumor therapy: Preclinical evaluation, automatization and translation to clinical application. Marc Pretze

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2. Context of the project (800 characters max. including spaces)

^{212}Pb is a promising radionuclide for TAT, which emits only one α-particle per β^--decay to ^{212}Bi and then either 64% to ^{212}Po or 36% to ^{208}Tl. Therefore, a ^{212}Pb-labelled radiopharmaceutical, once accumulated to the tumor tissue, deposit its highest dose in form of the α-particle specifically within the tumor cells, with lower probability of further impairment of healthy organs. It has already been proven that ^{225}Ac-mcp-Di-PSMA exhibit higher tumor uptake (Figure 1) compared to ^{225}Ac-PSMA-617. Precursor and optimized radiolabeling protocols for $^{203/212}$Pb are already established.
The automation will be implemented on a commercial GAIA synthesis module, which is suitable for the radiolabeling of peptides and small molecules in aqueous medium with radiometal nuclides, and in particular with $^{68}Ga^{3+}$.
Patient application could also be performed in the coverage of an individual theranostic treatment according to German Law ("Heilversuch" = compassionate care).

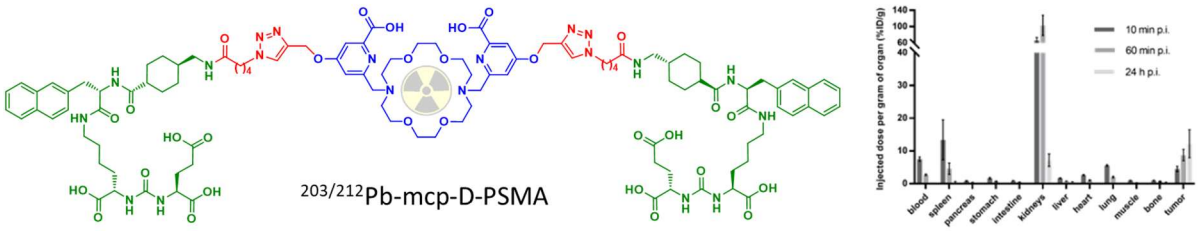

Figure 1. Chemical structure of $^{203/212}$Pb-mcp-D-PSMA and results of the biodistribution of the appendant ^{225}Ac-mcp-D-PSMA radioconjugate.

3. Results and discussion (confidential unpublished data)

Results on automation of syntheses (Mannheim)

Within the framework of PRISMAP, the radionuclides ^{203}Pb and ^{212}Pb were utilized to investigate the automation of corresponding radiopharmaceuticals. Specifically, experiments aimed to assess the feasibility of monitoring radioactivity within the module (GAIA) over the synthesis period and to evaluate the potential for real-time reaction control. Furthermore, the study examined the feasibility of automating the separation of ^{212}Pb from ^{224}Ra using the module, as opposed to manual methods, with a view to establishing a fully automated synthesis protocol being applicable in a clinical context. Further, a FAP-specific peptide (FAP: fibroblast activation protein) developed in our research group was radiolabeled with these nuclides. The radioanalytical characterization of the resulting products was established, and *in vitro* studies were conducted to investigate target affinity. Prospectively, the potential of these compounds for human imaging and therapy of FAP-specific tumors will be assessed in a human tumor-bearing mouse model.

Preclinical results (HZDR)

The new macropa-containing ligands mcp-PSMA, mcp-D-PSMA, and an albumin-binding(alb) derivative mcp-alb-PSMA were evaluated in comparison with standard ligands PSMA-617 and PSMA-11. The use of lesser substance (10 µg versus 50–100 µg) for radiolabelling led to higher molar activity. Radiolabelling was

performed at room temperature. Rechelation of free ^{212}Bi was observed when using higher amount of mcp-ligand (50 µg). Promising preclinical imaging results were obtained.

Following these promising imaging results, a therapy study was performed with LNCaP xenografts (PSMA-positive cell line). Animals received different doses 200, 400 and 800 kBq of ^{212}Pb-ligands and their overall survival was evaluated. From these results the following key points can be taken:

- [^{212}Pb]Pb-mcp-alb-PSMA performed better compared to PSMA-617 in all three doses
- [^{212}Pb]Pb-mcp-D-PSMA performed better than PSMA-617 with the doses of 200 and 400kBq/mouse
- Increasing of the dose of ^{212}Pb had a positive effect on survival – with highest dose (800 kBq) all mice had higher survival and lower tumour volumes than control - saline group

Clinical translation (NUK Dresden)

Mcp-[^{123}I]alb-PSMA and [^{203}Pb]Pb-mcp-D-PSMA were tested first-in-human. The preclinical most promising mcp-alb-PSMA was first radiolabeled with ^{123}I at the alb-moiety, eventually for enabling a new therapy with ^{131}I. However, the mcp-[^{123}I]alb-PSMA had a very unfortunate biodistribution with long-lasting accumulation in the blood pool, but as well a significant tumor accumulation. Due to the long accumulation in the blood pool, mcp-alb-PSMA was excluded from further clinical testing. Maybe a less binding alb-moiety could help to further proceed with albumin-binding radioligands.

Next, mcp-D-PSMA was tested in April 2025 with one last ^{203}Pb delivery from Arronax. The unpublished data in Figure 4 show similar target accumulation for [^{203}Pb]Pb-mcp-D-PSMA in bigger lesions in comparison with [^{68}Ga]Ga-HBED-PSMA. However, higher off-target accumulation was seen in blood, bone and liver for 25 h p.i.. This would lead to a higher toxicity with ^{212}Pb compared to ^{177}Lu-PSMA-I&T. Therefore, the higher lipophilicity and different charge of the ^{203}Pb-mcp-complex in comparision to ^{225}Ac/^{133}La was mainly the reason, for this unfortunate biodistribution.

4. Conclusions (800 characters max. including spaces)

The automation of the radiosynthesis with $^{203/212}$Pb was developed and first radioligands were produced by this methods and evaluated *in vitro*. While the preclinical and translational results were promising with $^{203/212}$Pb and produced at least two publications to date, the clinical evaluation of the new compounds lacked of performance, since two of the three tested compounds proofed to have a different biodistribution in patients compared to the preclinical results. Clinical diagnostics were performed with ^{203}Pb and ^{123}I to evaluate their effectiveness as ^{212}Pb-therapeutics. However, none of the compounds were further applicated clinically labelled with ^{212}Pb. The third compound will be further investigated clinically by ^{133}La and ^{225}Ac. If this evaluation proves as feasible, the application with ^{212}Pb will be overthought.

5. Involvement of the PRISMAP services (600 characters max. including spaces)

With the many deliveries of ^{203}Pb (7x) and ^{212}Pb (4x) the preclinical stage of the new radiotracer could be driven up to clinical translation and first-in-human application at least for diagnostic purposes as well as for automated ^{224}Ra workup for radiation safety and automated radiosyntheses.

6. Feedback to PRISMAP (600 characters max. including spaces)

Outlook for a further application: First we want to compare the biodistribution of the substance tested with ^{203}Pb^{2+} with a macropa-radionuclide^{3+} and look, whether the biodistribution is better then. If this turns out to be better, we might switch back to ^{203}Pb with the mcp-M-PSMA in order to get a ^{212}Pb-theranostic.

At the moment, no further Pb-evaluations are planned with the macropa-molecule up to the test with the radionuclide^{3+}. Publications are currently in progress for the preclinical evaluation.

It turned out that the Pb^{2+}macropa-complexes have a different unfortunate biodistribution compared to the preclinical results. This might be of the different charge of the complex with Pb^{2+} compared to cation^{3+}-metals.

7. Publications and other dissemination activities (conferences etc.)

„Blaue Bohnen für den Krebs – Blei-Isotope für die FAPI-Theranostik“ Henning Rudolph, Patrick A. Cieslik, Carmen Wängler, Björn Wängler, *31. Jahrestagung der Arbeitsgemeinschaft Radiochemie/Radiopharmazie, Davos, Schweiz* **2025**, Vortrag.

„Patientensicherheit bei TAT - Entdeckung eines Rechelatisierungseffekts in Injektionslösungen“ Marc Pretze, Magdalena Blei, Florian Brandt, Charlotte Duchemin, Thierry Stora, Constantin Mamat, Klaus Kopka, Ralph A. Bundschuh, *31. Jahrestagung der Arbeitsgemeinschaft Radiochemie/Radiopharmazie, Davos, Schweiz* **2025**, Vortrag.

“Preclinical Evaluation of ^{203}Pb-/^{212}Pb-labeled mcp-PSMA Ligands for Imaging and Therapy of Prostate Cancer and PSMA-positive Cancer Cells” Magdalena K. Blei, Katarína Hajduová, Zbyněk Nový, Miloš Petřík, Santiago A. Brühlmann, Marc Pretze, Sven Stadlbauer, Klaus Kopka, Constantin Mamat, *26th International Symposium on Radiopharmaceutical Sciences, Australia* **2025**, Vortrag.

“Proof-of-Concept-Studie zur One-Pot Synthese zur simultanen Radiomarkierung und Verknüpfung des Chelators macropa mit dem PSMA-Liganden via Click-Reaktion” Linda Belke, Magdalena K. Blei, Klaus Kopka, Sven Stadlbauer, Constantin Mamat, *63. Jahrestagung der Deutschen Gesellschaft für Nuklearmedizin, Bremen* **2025**, Vortrag.

„Präklinische Bewertung von $^{203/212}$Pb-markierten mcp-PSMA-Liganden für die Bildgebung und Therapie von Prostatakrebs und PSMA-positiven Krebszellen“ Magdalena K. Blei, K. Hajduová, Z. Nový, M. Petřík, Santiago S. Brühlmann, Marc Pretze, Klaus Kopka, Sven Stadlbauer, Constantin Mamat, *63. Jahrestagung der Deutschen Gesellschaft für Nuklearmedizin, Bremen* **2025**, Vortrag.

“Lead-it-EAZY - GMP-compliant synthesis of [^{212}Pb]Pb-VMT-α-NET and challenges in quality Control” Marc Pretze, Enrico Michler, David Kästner, Falk Kunkel, Edwin Sagastume, Michael K. Schultz, Ralph Bundschuh, Jörg Kotzerke, *SNMMI-Mid—Winter Meeting Anaheim, CA, USA* **2025**, Poster.

“Different ^{212}Pb Generators and Its Radiation Safety Concerning ^{220}Rn (Thoron) Emanation” Marc Pretze, Holger Hartmann, Charlotte Duchemin, Thierry Stora, Muhammad Inzamam, David Kästner, Edwin A. Sagastume, Michael K. Schultz, Jörg Kotzerke, Ralph A. Bundschuh, Robert Freudenberg, *Toxics* **2025**, 13, 462. DOI: 10.3390/toxics13060462.

“Comparison of ZnS(Ag) Scintillator and Proportional Counter Tube for Alpha Detection in Thin-Layer Chromatography” Marc Pretze, Jan Wendrich, Holger Hartmann, Robert Freudenberg, Ralph A. Bundschuh, Jörg Kotzerke, Enrico Michler, *Pharmaceuticals* **2025**, 18,26. DOI: 10.3390/ph18010026.

“Präklinische Evaluierung von $^{203/212}$Pb-markierten mcp-PSMA-Liganden“ Magdalena K. Blei, Zbynek Novy, Milos Petrik, Santiago S. Brühlmann, Marc Pretze, Sven Stadlbauer, Klaus Kopka, Constantin Mamat, *30. Jahrestagung der Arbeitsgemeinschaft Radiochemie/Radiopharmazie, Mannheim* **2024**, Vortrag.

Appendix 1. Dissemination guidelines for user projects as agreed in the signed User Agreement

Dissemination rules

Only user groups that are allowed to disseminate the results which they have generated under the project may benefit from the access, unless they are working for SMEs.

For each user group project, a publishable project summary and a publishable summary of the results will be published on the European Union Horizon 2020 PRISMAP project website www.prismap.eu. The publication of results in journals or at conferences is strongly encouraged.

To ensure the long-term sustainability of the PRISMAP initiative, proper recognition of the contributing facilities, their services and the involved persons is necessary. All participating PRISMAP facilities shall be acknowledged in the publication. Acknowledgement and co-authorship of PRISMAP staff members who participated in the experiment shall be considered according to the research field best practices and verified with the PRISMAP Technical Manager before any publication.

The user group shall contact the PRISMAP Technical Manager 30 days prior to submission of publications or other communications of results that were obtained by making use of services provided by PRISMAP (radionuclides delivered or medical services provided). The Technical Manager will communicate to the user group the list of PRISMAP facilities and persons that have contributed to each specific project and the way this contribution must be acknowledged in the publication/communication or where co-authorship is required to reflect specific scientific contributions.

Users must comply with Horizon 2020 dissemination rules (i.e. acknowledge that their work was financially supported by the European Union's Horizon 2020 Research and Innovation Programme by including the following acknowledgement: "This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101008571 (PRISMAP)"), and grant open access to resulting publications and related data.

Dissemination shall take place only once legitimate interests regarding intellectual property have been safeguarded. A maximum publication delay of 90 days may be granted for this purpose.

Acknowledgements

The list of name(s) to be mentioned in the acknowledgment section is sent to the technical manager by the main contact of the involved facilities.

A general sentence will be added by the corresponding author of the article (user side):

"The authors would like to thank the members of the PRISMAP consortium and of the PRISMAP user selection panel, coordination and management team for their advice and support."

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