User project report

Added Value Using Terbium-161 over Lutetium-177 in Combination with the Metabolically more Stable GRPR Ligand AMTG for Targeted Radiotherapy of GRPR-Expressing Malignancies? – A Preclinical Evaluation

Thomas Günther, PhD

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101008571 (PRISMAP). This document reflects only the view of the author(s). The Agency is not responsible for any use that may be made of the information it contains.

1. Authors

Thomas Günther - TUM School of Natural Sciences, Department of Chemistry, Chair of Pharmaceutical Radiochemistry, Technical University of Munich, Garching, Germany; Molecular Imaging Program at Stanford (MIPS), Department of Radiology, School of Medicine, Stanford University, Stanford, CA, USA

Nadine Holzleitner, Tatjana Cwojdzinski, Roswitha Beck, Nicole Urtz-Urban, Angela Casini - TUM School of Natural Sciences, Department of Chemistry, Chair of Pharmaceutical Radiochemistry, Technical University of Munich, Garching, Germany

Stijn Ramaekers, Michiel Van de Voorde, Maarten Ooms - Nuclear Medical Applications, Belgian Nuclear Research Centre, Mol, Belgium

Bernard Ponsard - BR2 Reactor, Belgian Nuclear Research Centre, Mol, Belgium

Abraham S Moses, Elisabeth Günther, Madeleine R Landry, Corinne Beinat - Molecular Imaging Program at Stanford (MIPS), Department of Radiology, School of Medicine, Stanford University, Stanford, CA, USA

Colin C Hillhouse, Pascal V Grundler, Nicholas P van der Meulen, Zeynep Talip - Center for Radiopharmaceutical Sciences ETH-PSI, Paul Scherrer Institute, Villigen, Switzerland

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

Based on the favourable in vivo stability and thus, overall pharmacokinetics of the gastrin-releasing peptide receptor (GRPR) ligand AMTG, which has already been translated to the clinics for imaging and treatment of prostate and breast cancer, the aim of this project represented the preclinical comparison of AMTG with the structurally similar yet less stable RM2, both labelled either with ^{177}Lu or ^{161}Tb. As these two radionuclides comprise nearly identical physical properties, with the abundance of Auger and conversion electron emissions the main difference in favour of ^{161}Tb, we wanted to investigate a potential beneficial impact of ^{161}Tb-labelled GRPR ligands on treatment efficacy, as well as an additional positive effect due to the improved in vivo stability of the AMTG peptide.

3. Results and discussion (1000 characters max. including spaces)

While most in vitro properties (affinity, lipophilicity, membrane-bound activity, etc.) were very similar between the ^{177}Lu- or ^{161}Tb-labelled AMTG and RM2, respectively, a substantially higher in vivo stability was observed for both AMTG analogues. Biodistribution studies over time revealed a significantly higher activity retention in the PC-3 tumour xenograft for [^{161}Tb]Tb-AMTG over [^{177}Lu]Lu-AMTG, and both higher than their RM2 counterparts, while most of the initial high uptake in the pancreas (endogenously expressing GRPR) was cleared within the first 24 hours. Treatment with two injections (day 0 and day 7) of 13-15 MBq of either [^{177}Lu]Lu-RM2, [^{177}Lu]Lu-AMTG, [^{161}Tb]Tb-RM2, or [^{161}Tb]Tb-AMTG, each at a molar activity of 50 MBq/nmol, resulted in an overall survival of 26.8±1.9 d ([^{177}Lu]Lu-RM2), 39.5±2.6 d ([^{177}Lu]Lu-AMTG), 44.9±3.8 d ([^{161}Tb]Tb-RM2), and 58.1±5.4 d ([^{161}Tb]Tb-AMTG), respectively (untreated group: 22.8±1.2 d), while no apparent organ toxicity was observed.

4. Conclusions (800 characters max. including spaces)

Due to the higher number of Auger and conversion electron emissions per decay for ^{161}Tb compared with ^{177}Lu, and the substantially higher in vivo stability of the GRPR ligand AMTG compared to RM2, [^{161}Tb]Tb-AMTG outperformed each of the other compounds in this study and thus, has a high potential to improve treatment efficacy of patients suffering from GRPR-expressing malignancies such as prostate and breast cancer, which is why clinical translation of [^{161}Tb]Tb-AMTG is warranted. We could also show that combination of short-ranged Auger and conversion electrons additionally emitted by ^{161}Tb (compared to

^{177}Lu) with membrane-bound GRPR antagonists led to a higher tumour damage, which strengthens the "membrane effect" theory previously observed only for somatostatin-2 receptor antagonists.

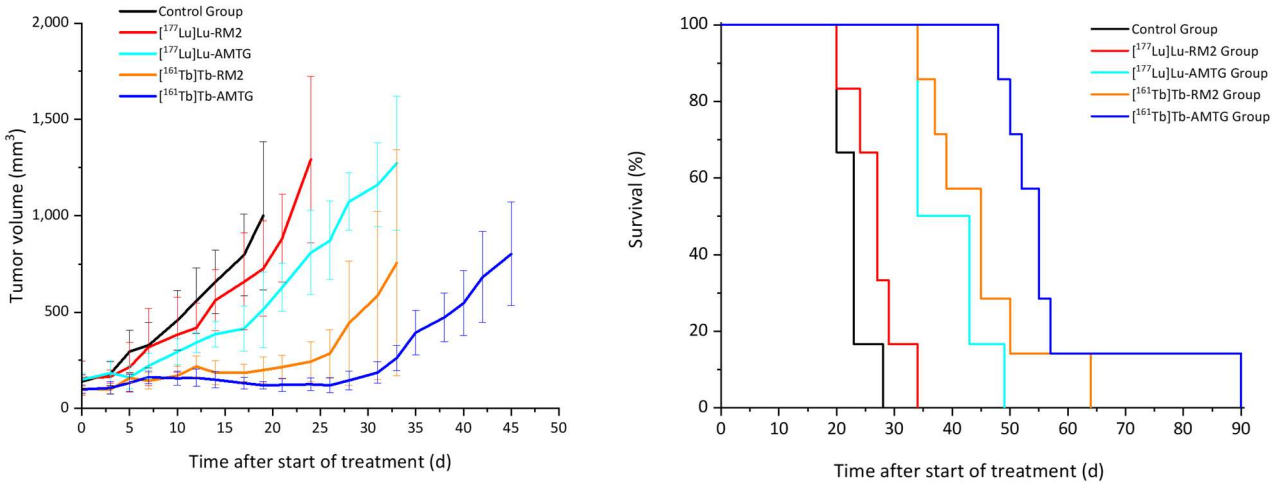

Figure 1. Tumour growth (left) and overall survival (right) of animal groups treated with radiolabelled GRPR ligands, as well as the untreated control group.

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

PRISMAP provided access to the still relatively rare and difficult to obtain radionuclide ^{161}Tb, and covered all production costs. PRISMAP also helped to extend my network, and connected me to peers at SCK CEN and PSI. After I moved from TUM (Germany) to Stanford University (CA, USA), PRISMAP continued to support the project and coordinated the ^{161}Tb shipments from SCK CEN to the United States. Moreover, I could always count on the support of PRISMAP whenever I encountered problems during the project e.g., low labelling yields or delayed ^{161}Tb shipments, and help was provided immediately.

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

I am very grateful for the chance provided by PRISMAP to carry out this exciting project and the opportunity to work with ^{161}Tb. The application process was smooth and I will always be appreciative for the permission to continue the project at Stanford University despite all the regulatory hurdles in the United States after I left TUM. I would like to particularly thank Charlotte Duchemin for all her organizational help over the course of my project, and Uli Köster for his support when I encountered ^{161}Tb-labelling issues that prevented the completion of the treatment studies.

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

1) Holzleitner et al., Preclinical Evaluation of Gastrin-Releasing Peptide Receptor Antagonists Labeled with ^{161}Tb and ^{177}Lu: A Comparative Study, J Nucl Med 2024; 65:481–484, DOI: 10.2967/jnumed.123.266233
2) Günther et al., Comparison of the Treatment Efficacy and Toxicity of double injection of ^{161}Tb- and ^{177}Lu-Labeled GRPR Antagonists in PC3 Tumor-Bearing Mice, in preparation (intended submission to the Journal of Nuclear Medicine)
3) Holzleitner et al., Preclinical Comparison of the GRPR Antagonists AMTG and RM2 Labelled with Terbium-161 and Lutetium-177 – A PRISMAP Project, oral presentation (OP-141) at the 2023 Annual Congress of the European Association of Nuclear Medicine (Vienna, Austria)

4) Günther et al., Comparison of the Treatment Efficacy of ^{161}Tb- and ^{177}Lu-Labeled GRPR Antagonists in PC3 Tumor-Bearing Mice, abstract submitted to the SNMMI 2025 Annual Meeting (New Orleans, LA, USA) for oral presentation
5) Günther et al., Comparison of the Treatment Efficacy and Toxicity of double injection of ^{161}Tb- and ^{177}Lu-Labeled GRPR Antagonists in PC3 Tumor-Bearing Mice, abstract will be submitted to the 2025 Annual Congress of the European Association of Nuclear Medicine (Barcelona, Spain) for oral presentation
6) Oral presentation on the recent findings of P12 (Added value using Tb-161 over Lu-177 in combination with the metabolically more stable GRPR ligand AMTG for targeted radiotherapy of GRPR-expressing malignancies? – A preclinical evaluation) during the PRISMAP Consortium Meeting (Denmark, May 31, 2023)

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."

Funding acknowledgement

"This work was supported by the European Union's Horizon 2020 research and innovation programme as a user project of PRISMAP – The European medical radionuclides programme (GA 101008571)".