User project report

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Dual 152Tb/149Tb radiolabeling and preclinical validation of an AAZTA-FAPi ligand for diagnostic and theranostic applications

Call ID: PRISMAP-2022-1

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1. Authors

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

Fibroblast activation protein-α (FAPα) is transmembrane protein overexpressed by cancer-associated fibroblasts (CAFs) in over 90% of epithelial tumors. CAFs promote tumor progression, angiogenesis, and metastasis. In recent years, several FAP inhibitors (FAPis), such as FAPI-02, FAPI-04, and FAPI-46, have shown promising results in PET imaging. While most FAPis use DOTA for radiometal chelation, the AAZTA chelator allows for efficient labelling under milder conditions. This project aims to explore the AAZTA-FAPi-46 conjugate labelled with the theranostic pair 152Tb/149Tb. Despite no prior data on Tb(III)-AAZTA complexes, chelation of nearby lanthanides supports its feasibility. This innovative approach may improve radiolabelling efficiency and expand clinical options for FAP-targeted theranostic.

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

Following the suggestion of user selection panel, AAZTA-FAPI-46 (1nmol), whose chemical structure is reported in Figure 1, was radiolabelled with 50 MBq of 161Tb ($TbCl_3$) in sodium acetate buffer 0.5M pH=8 to adjust the labelling pH to 5.4-5.8. The solution was heated at 90° C for 10 minutes and the labelling efficiency was then evaluated by radio-TLC measurements using sodium citrate 0.1M pH=5 as solvent. The results showed a radiolabelling efficiency (RLE) of 96.55%, making purification unnecessary. The stability of the radiotracer was evaluated in formulation buffer, saline, and human serum (HS) at 37 °C, with RCP evaluated at 1h, 4h, 24h, and 72h post-end-of-synthesis (EOS). For stability studies purposes, 5 nmol of AAZTA-FAPI-46 was labelled under identical conditions, achieving 98% RLE. While RCP remained above 90% up to 24h, a significant decrease was observed at 72h in both saline and HS (<90%). Due to reduced stability, no further cell binding assays were conducted.

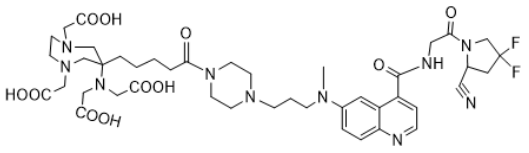

Figure 1. AAZTA-FAPI-46 ligand chemical structure.

4. Conclusions (800 characters max. including spaces)

AAZTA-FAPI-46 was successfully labelled with 161Tb, reaching RLE >98% without purification. Stability remained acceptable up to 24 h in all tested media, but RCP dropped below 90% at 72 h in saline and human serum, indicating partial decomplexation. As therapeutic applications require prolonged in vivo stability, these results currently limit further development.

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

To test 161Tb labelling of our AAZTA-FAPi-46 ligands, the support provided by PRISMAP through the SCK CEN centre in Belgium was fundamental. For our project, we have used one batch of 161Tb delivered to the Department of Nuclear Medicine of TUM MRI. The test labeling and stability tests have been carried out by both the PhD student Adriano Bolognani and Prof. Calogero D'Alessandria. Despite the discontinuation of the project, the TUM MRI team is willing to support our group toward further development, if requested, beyond the completion of PRISMAP.

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

The overall experience with the PRISMAP call was very positive. The submission and evaluation procedures were clear and efficiently managed. The communication with the coordination team was prompt and supportive. We greatly appreciated the assistance provided by the TUM MRI group, whose help was essential to overcome the issues related to the supply of Tb-based radioisotopes.

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

We are committed to acknowledge PRISMAP in future publications that will eventually originate upon completion of the project.

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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