User project report

Project title: Improved FAP-radiotheranostics for personalized cancer treatment.
Leading author: Filipe Elvas

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

Filipe Elvas[1], Maarten Vanermen[1], Sigrid Stroobants[1,2], Pieter Van Der Veken[3], Ingrid De Meester[4].

[1] University of Antwerp, Molecular Imaging Center Antwerp (MICA), 2610 Wilrijk, Belgium

[2]Antwerp University Hospital (UZA), Nuclear Medicine / Radiopharmacy

[3]University of Antwerp, Medicinal Chemistry (UAMC)

[4]University of Antwerp, Medical Biochemistry (LMB)

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

Fibroblast activation protein (FAP) is a serine protease found on stromal cells in most epithelial cancers but rarely in normal tissues, except transiently during wound healing or fibrosis. FAP-positive cancer-associated fibroblasts (CAFs) correlate with poor prognosis, making FAP a promising diagnostic and therapeutic target. Building on the success of FAP-targeted PET tracers, we aim to improve FAP-targeted radionuclide therapy limited by rapid tumor washout and poor pharmacokinetics. Using a pretargeting strategy, we aim to enhance tumor retention and reduce blood exposure by developing radiolabeled trans-cyclooctenes (TCOs) with the $^{18}F/^{211}At$ theranostic pair and dimeric FAP ligands containing a tetrazine moiety. These tracers will be evaluated in vitro and in a pancreatic cancer mouse model for imaging and therapy.

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

The initial research phase focused on synthesizing d-TCO cores as reference molecules and precursors. Starting from cyclooctene, a sequence of oxidation, mesylation, azidation, and reduction yielded cis-cyclooctene-amine (89%), followed by protection and photoisomerization to obtain trans-cyclooctene-amine (TCO-amine 1, 70%). This intermediate enabled the synthesis of reference molecules using 4-iodobenzoic acid and 2-(4-iodophenyl)acetic acid, producing two analogs (20% and 34% yield). Iodine served as a stable surrogate for astatine due to similar reactivity. Two organometallic precursors—boronic acid and stannyl derivatives—were synthesized (48% and 35% yield) for direct astatination; boronic acids were preferred for lower toxicity. Direct labeling with astatine-211 achieved up to 75% RCC and 85% purity. A two-step labeling approach using N-succinimidyl-astatobenzoate intermediates improved conversion (up to 78%) and product purity. *In vitro* binding evaluation and *in vivo* biodistribution studies were performed using two lead astatinated TCOs, ^{211}At-CREANT-205 and -206.

4. Conclusions (800 characters max. including spaces)

We have evaluated the optimal astatination method of d-TCOs using the most efficient precursor, either $Sn(Bu)_3$ or $B(OH)_2$. Two lead radiotracers, ^{211}At-CREANT-205 and -206, were evaluated for labeling efficiency, purity, lipophilicity, stability, and tetrazine reactivity. These results guided the selection of the most promising candidate for future theranostic development. Biodistribution experiments using ^{211}At-TCOs in healthy mice preceeded *in vivo* evaluation in tumor models with FAPI-Tz compounds.

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

PRISMAP services allowed us to practically execute our research proposal, which would otherwise be impossible since there is no access widespread access to ^{211}At, including in Belgium. The ^{211}At delivery from Arronax allowed us to optimize the radiolabeling conditions, leading to the development of **[^{211}At]CREANT-**

205 and -206 as a potential therapeutic candidates for pretargeted radioligand therapy. Furthermore, we were able to investigate **[^{211}At]CREANT-305** preclinically, evaluating its metabolic stability and biodistribution.

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

PRISMAP has provided us full support for the project application. Additionally, the timely deliveries of ^{211}At were ensured so that our experiments could be carried out. Very pleased with the collaboration with Arronax. Professionalism, good communication and timely deliveries.

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

An abstract describing the astatination and in vitro characterization of novel TCOs was presented in April 2024 in the European Symposium on Radiopharmacy & Radiopharmaceuticals (ESRR) 2024 (Coimbra, Portugal). Currently, a manuscript is “in preparation” phase.

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)".