User project report

Development and preclinical evaluation of a mesothelin-targeting theranostic agent

Émilien N'guessan, Sandrine Bacot, Ulli Köster and Alexis Broisat

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

Émilien N'guessan[1], Sandrine Bacot[1], Ulli Köster[2], Alexis Broisat[1]
[1] *Univ. Grenoble Alpes, INSERM U1039, LRB, Grenoble, France*
[2] *Institut Laue-Langevin, Grenoble, France*

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

The Laboratoire Radiopharmaceutiques Biocliniques (UMR S1039) in Grenoble has previously evaluated and validated ^{99m}Tc-A1, a single domain antibody (sdAb) targeting mesothelin, a 40kDa GPI-anchored membrane protein which expression is limited in healthy tissues but that is overexpressed in nearly 40% of solid tumors. The aim of this project was to engineer A1 so that it can be radiolabeled with ^{68}Ga for PET imaging and with the therapeutic radioisotopes ^{177}Lu and ^{161}Tb, for theranostic applications. The main objectives were to validate the radiolabeling of A1-based tracers, evaluate their biodistribution and evaluate their efficacy *in vitro* and *in vivo* in mice bearing human breast or pancreatic tumours.

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

A1 was reengineered by directed mutagenesis to produce four single-lysine variants (A1K1, A1K2, A1K3, and A1K4). Each was site-specifically conjugated with p-SCN-Bn-DOTA, radiolabeled with ^{68}Ga, and evaluated by PET imaging in mice. These findings identify DOTA-A1K2 as the lead candidate based on the DOTA coupling efficiency, its radiochemical purity and biodistribution. It was successfully radiolabelled with ^{177}Lu and ^{161}Tb with radiochemical purity >95% after 24h in murine and human blood. Biodistribution in mice was evaluated up to 168 hours. Therapeutic efficacy was then evaluated *in vitro* and *in vivo* using MDA-MB-231 cells overexpressing mesothelin. *In vivo*, a single intravenous injection of 5 or 10 MBq of either ^{177}Lu-DOTA-A1K2 or ^{161}Tb-DOTA-A1K2 inhibited tumor growth compared to untreated controls ($p<0.001$ Vs vehicle; p=NS between ^{161}Tb and ^{177}Lu labelling).

4. Conclusions (800 characters max. including spaces)

The anti-mesothelin sdAb A1 was engineered and the lead compound A1K2 was then radiolabelled with ^{68}Ga, ^{177}Lu and ^{161}Tb for theranostic application. While *in vitro* ^{161}Tb-DOTA-A1K2 efficacy was found to be higher than that of ^{177}Lu-DOTA-A1K2, no such superiority was observed *in vivo*. This might be attributed to mesothelin shedding, that has recently been described by other groups, resulting in suboptimal distribution of the sdAb in the tumor microenvironment. Nevertheless, *in vivo*, a single injection of either ^{161}Tb-DOTA-A1K2 or ^{177}Lu-DOTA-A1K2 successfully inhibited tumor growth. This work provides the first demonstration of a theranostic approach using sdAbs against mesothelin-overexpressing tumors. Moreover, to our knowledge, it is also first time that a sdAb is radiolabelled with ^{161}Tb.

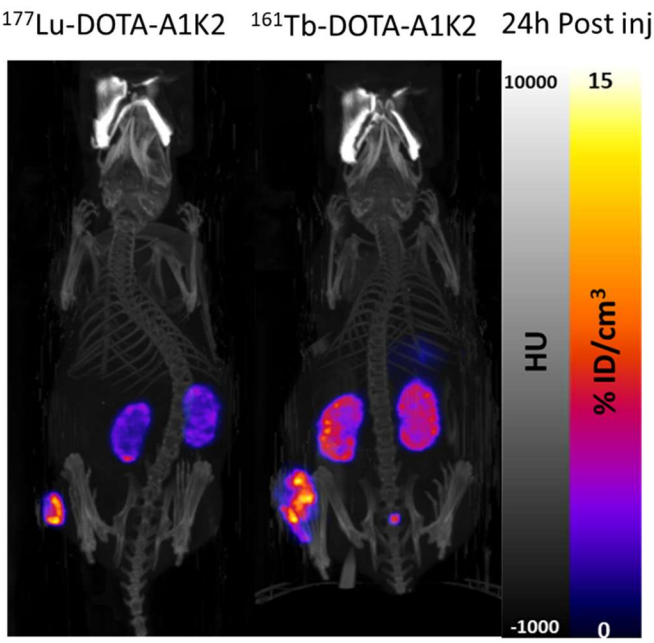

Figure 1. *In vivo* tumor therapy study on mice bearing MDA-MB-231 hMSLN+ xenografts, using ^{161}Tb-DOTA-A1K2 or ^{177}Lu-DOTA-A1K2.

Representative SPECT/CT images of mice 24 hours after intravenous co-injection of ^{177}Lu-DOTA-A1K2 or ^{161}Tb-DOTA-A1K2, with gelofusin.

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

PRISMAP was involved in producing, purifying and providing ^{161}Tb, an innovative radioisotope for theranostic applications, that combines emission of beta minus, conversion and Auger electrons for vectorized internal radiotherapy. Irradiations of Gd-160 targets have been performed at the high-flux reactors BR2 at SCK-CEN and RHF at ILL respectively, and radiochemical Tb/Gd separations have been performed at SCK-CEN (Michiel van de Voorde et al.).

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

PRISMAP provided us with the opportunity to use ^{161}Tb, an innovative radioisotope with unique characteristics for theranostic applications. We remained in close contact during all the duration of the project, and were able to obtain ^{161}Tb with optimal characteristics such as elevated purity and specific activity. Deliveries were always on schedule. Besides, we also benefited for the PRISMAP community. For example, we had the opportunity to present our work during one online event and one in person meeting in Lisboa and to interact closely with the PRISMAP community.

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

The first part of this project (selection of the lead compound) has just been accepted for publication in EJNMMI Radiopharmacy and Chemistry. The article related to the second part, involving PRISMAP and the use of ^{161}Tb, is currently in preparation and will be submitted in the coming weeks. It is included in the thesis manuscript of Emilien N'Guessan whose PhD defense is scheduled on June 24th 2025. Emilien had the

opportunity to present these results twice as oral communications in international meetings. A first one during the congress « New modalities in cancer imaging and therapy » in Erquy, France on 11 October 2024, and a second one during the European Molecular Imaging Meeting 2025, in Bilbao, Spain on 12 March 2025. Those 2 communications both acknowledged PRISMAP funding.

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)".