User project report

Identification and assessment of A20FMDV2-derived compounds for a theranostic approach to $\alpha_V\beta_6$-targeted alpha therapy with lead-203 and lead-212

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2. Context of the project

This project was designed to identify and develop conjugates of the RGD peptide A20FMDV2 (A20), which targets the integrin $\alpha_v\beta_6$ with high affinity and selectivity, for a theranostic approach using Pb-203 for imaging and Pb-212 for targeted alpha therapy (TAT). $\alpha_v\beta_6$ is an important target for imaging and therapy in epithelial cancers. A20 has previously been assessed after radiolabelling with imaging radionuclides, but was shown to have over 100 % injected dose per gram when labelled with radiometals. Modifying the structure of the peptide could influence the peptide's pharmacokinetics and biodistribution profile including significant changes in kidney uptake. The kidney uptake must be reduced from the values seen previously if A20 is to be a viable candidate for TAT.

3. Results and discussion

DOTA-A20, DOTA-RK-A20 (RK = kidney reducing modifier), DOTA-RK2-A20 (RK2 = second generation kidney reducing modifier), DOTAM-A20 and DOTAM-RK2-A20 were labelled with a specific activity of 66.7 MBq/µg. DOTA-A20 was also labelled up to 133.3 MBq/µg. Human serum stability of [^{203}Pb]Pb-DOTA-A20, [^{203}Pb]Pb-DOTA-RK2-A20, and [^{203}Pb]Pb-DOTAM-RK2-A20 was measured by HPLC. [^{203}Pb]Pb-DOTAM-RK2-A20 had 99% stability up to 4 hours, and 90% stability up to 24 hours. The DOTA-conjugated peptides had > 95% stability up to 4 hours, and 80-90% stability up to 24 hours. All ^{203}Pb-A20 conjugates showed high specific binding to $\alpha_v\beta_6$ through *in vitro* saturation binding assays to A375.B6.puro cells and were deemed suitable for *in vivo* use. *Ex vivo* biodistribution of ^{203}Pb-labelled A20 conjugates in naïve athymic nudes showed significant differences in renal uptake at one- and four-hours post-administration between peptides, both with and without pre-injections of selected modifiers to reduce renal uptake.

4. Conclusions

Affinity of A20 conjugates towards the integrin $\alpha_v\beta_6$ does not change significantly when introducing modifiers for reducing renal uptake or when changing chelator from DOTA to DOTAM. ^{203}Pb-labelled A20 conjugates show high stability up to 24 hours in human serum, which is slightly increased for DOTAM conjugates in line with the literature[1]. Using Pb-203 for these studies (instead of Pb-212) removed the effect of beta or alpha decay on stability. Significant differences in renal uptake between A20 analogues indicate that DOTA-RK2-A20 may be the best conjugate to use for therapy with Pb-212. These studies have thus far been carried out in naïve mice and a tumour-to-kidney ratio is required to make a better-informed decision. Further studies in tumour-bearing animals have been planned.

5. Involvement of the PRISMAP services

PRISMAP funding for the Pb-203 used in this project enabled us to carry out the extensive binding assay, stability and *in vivo* imaging studies needed to validate our approach, as the costs would have otherwise been too high. The flexible delivery schedule enabled us to able to plan between each batch based on our results and been able to get more valuable information out of the three batches. We have recommended Arronax as a high-quality Pb-203 supplier to other research groups, benefitting both those groups and Arronax.

6. Feedback to PRISMAP

We found the experience of using PRISMAP to be straightforward and have been happy with the level and clarity of communications. We appreciated the efforts to help us set up our contacts with Arronax and recommend PRISMAP program to anyone looking to work with research radionuclides they otherwise may struggle to source. We have developed a good relationship with Arronax and have ordered more Pb-203 to finish the work (outside of the PRISMAP funding scheme).

7. Publications and other dissemination activities

Paper disseminating these results is in preparation but pending further results.

A separate portion of the study has been accepted to be presented as a poster at 2nd Symposium on Molecular Radiotherapy dosimetry (Athens, November 2025) "^{212}Pb Human dosimetry estimates derived from ^{203}Pb SPECT imaging of an integrin-targeting peptide and the impact of ^{212}Bi disassociation on kidney dose" by Keryn Gresco, Perceptive Discovery.

1 L. L. Chappell, E. Dadachova, D. E. Milenic, K. Garmestani, C. Wu and M. W. Brechbiel, *Nucl. Med. Biol.*, 2000, **27**, 93–100.

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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