User project report

Title: Selective oncological theragnostic based on radioactively labeled exosomes (TheragnEso)

IP: Beatriz Salinas…

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

Dr. Beatriz Salinas, Instituto de investigación sanitaria Gregorio Marañón. Universidad Carlos III de Madrid

Dr. Maria Isabel González, Centro Nacional de Investigaciones Cardiovasculares

Dr. Manuel Desco, Instituto de investigación sanitaria Gregorio Marañón

Dr. Susanne Kossatz, Technische universität München

Dr. Wolfgang Weber, Technische universität München

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

Radiation therapy, in which ionizing radiation is administered locally to the tumor via an external beam or by surgical implantation of radionuclide-based seeds, is one of the gold standard treatments for cancer. Due to the non-selective nature of radiation, healthy tissue surrounding the cancerous region is often affected by the treatment. Therefore, new strategies are being studied to improve the selectivity of the treatment and minimize side effects. However, several challenges limit the current development of targeted radiotherapy, such as functionalization of the therapeutic agent (radioactive isotope) with vectors to enhance its accumulation in target tissue and control of release.

Nanoparticles offer unique opportunities as treatment delivery vehicles, since they have a large surface area that allows incorporation of a high amount of therapy (drug or isotope), improve cellular uptake of drugs and are easily functionalized with biomolecules for further accumulation in target processes. In the development of new nanotechnological tools, a new area of research has begun to emerge based on natural exosomes.

Exosomes are small, 30-140 nm, membrane defined particles of endosomal origin. Their natural origin provides them with greater biocompatibility and lower immuno-responsiveness, thus emerging as new nanotechnological tools, not only in diagnostics but also as platforms for the controlled release of drugs. In addition and due to their natural migration in tumor tissues and pre-metastasis niche, numerous current studies have begun to evaluate the role of exosomes as non-toxic and biocompatible nanoplatforms for drug delivery.

The main objective of this work is the development of new radiotheragnostic agents based on natural nanoparticles (exosomes) radioactively labeled with the novel therapeutic and diagnostic isotope Terbium 161 (161Tb).

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

The first step of the experimental setup was to determine the optimal conditions for passive labelling of exosomes with 161Tb. Ideally, syntheses with 161TbCl3 (commercial) was performed at 95 ºC, 30 min. We tested three different pH to evaluate the effect of the solvent; pH 5 with NaOAc, pH 6 with a solution of Na OAc and pH 7 with PBS. The radiolabelling performance was assessed by TLC (figure 1).TLC characterisation showed total isotope incorporation in the case of neutral conditions (PBS, pH 7) while in the case of pH 6 and 5 the chromatograms confirmed the presence of impurities from the free 161Tb

Once the optimal radiolabelling conditions were established, the second stage of the project was the evaluation of the in vitro stability of the new radiotracer RadioExo by iTLC. For this purpose, the sample (300uCi) was dissolved in 1 mL of PBS and kept at 37ºC with constant water mimicking physiological conditions. The iTLC plates showed outstanding stability, with degradation values below 5% even after 7 days of incubation (Figure 2)

The last step of the project was the in vitro evaluation of the radiotracer. In vitro toxicity studies revealed higher effectiveness in pulmonary cell lines (H2009) than in glioblastoma cell lines (U87). The longitudinal

assessment of the cytotoxicity at different times points and doses confirmed highest effect at 24h (Figure 3) with a higher therapeutic effect on lung cells than on brain cells.

4. Conclusions (800 characters max. including spaces)

i) We have successfully developed for the first time a direct tagging strategy with 161Tb of extracellular vesicles (exosomes) for subsequent teragnostic application.

ii) Stability studies demonstrated practically negligible release of the isotope from the vesicle structure.

iii) In vitro studies showed a higher therapeutic effectiveness at longer times (24h), as well as a more effective therapy in lung cells than in brain cells.

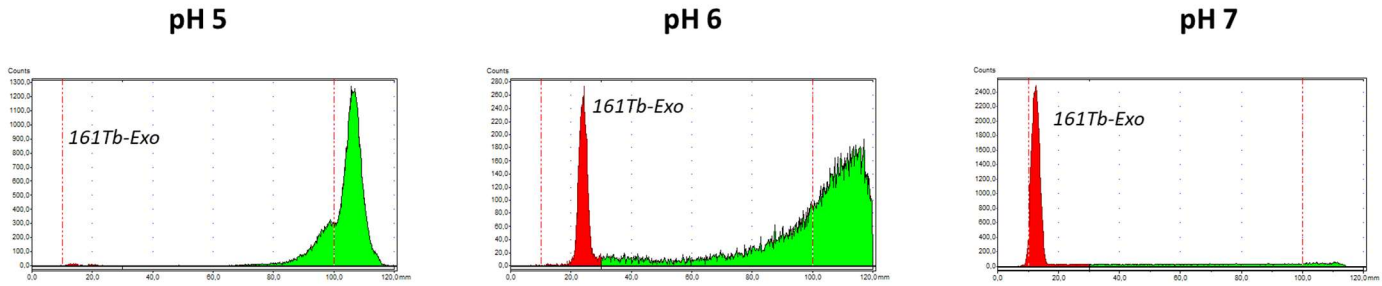

Figure 1. iTLC chromatogram of exosome labelling (RadioExo) at the different conditions

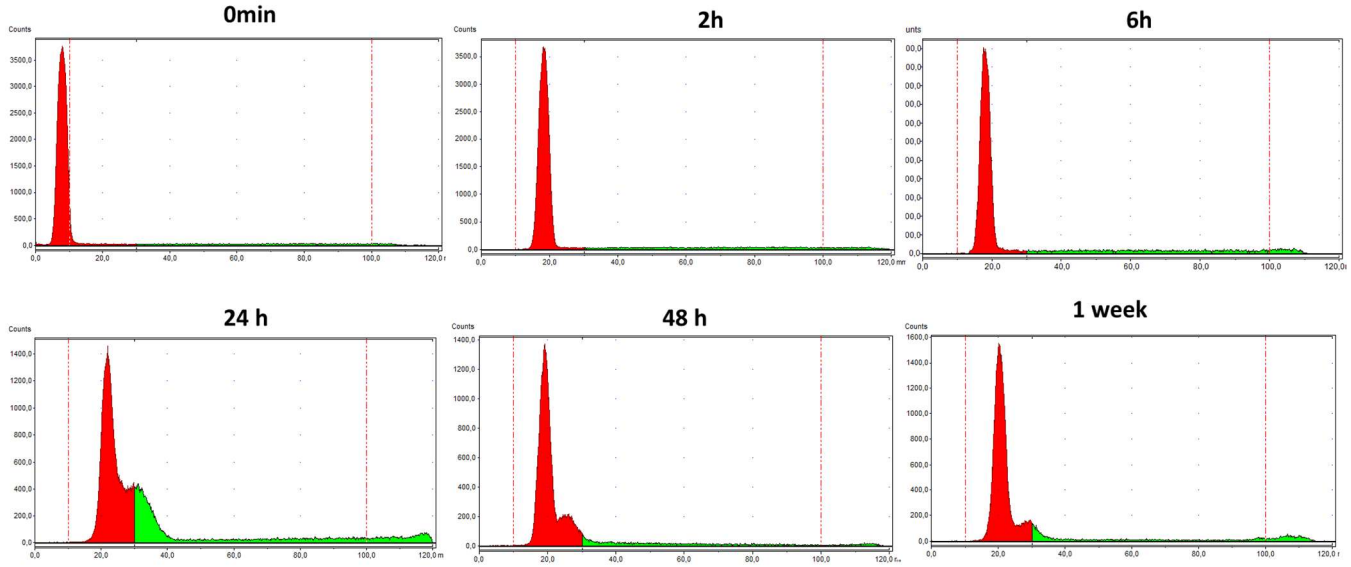

Figure 2. Stability assessment of RadioExo by TLC

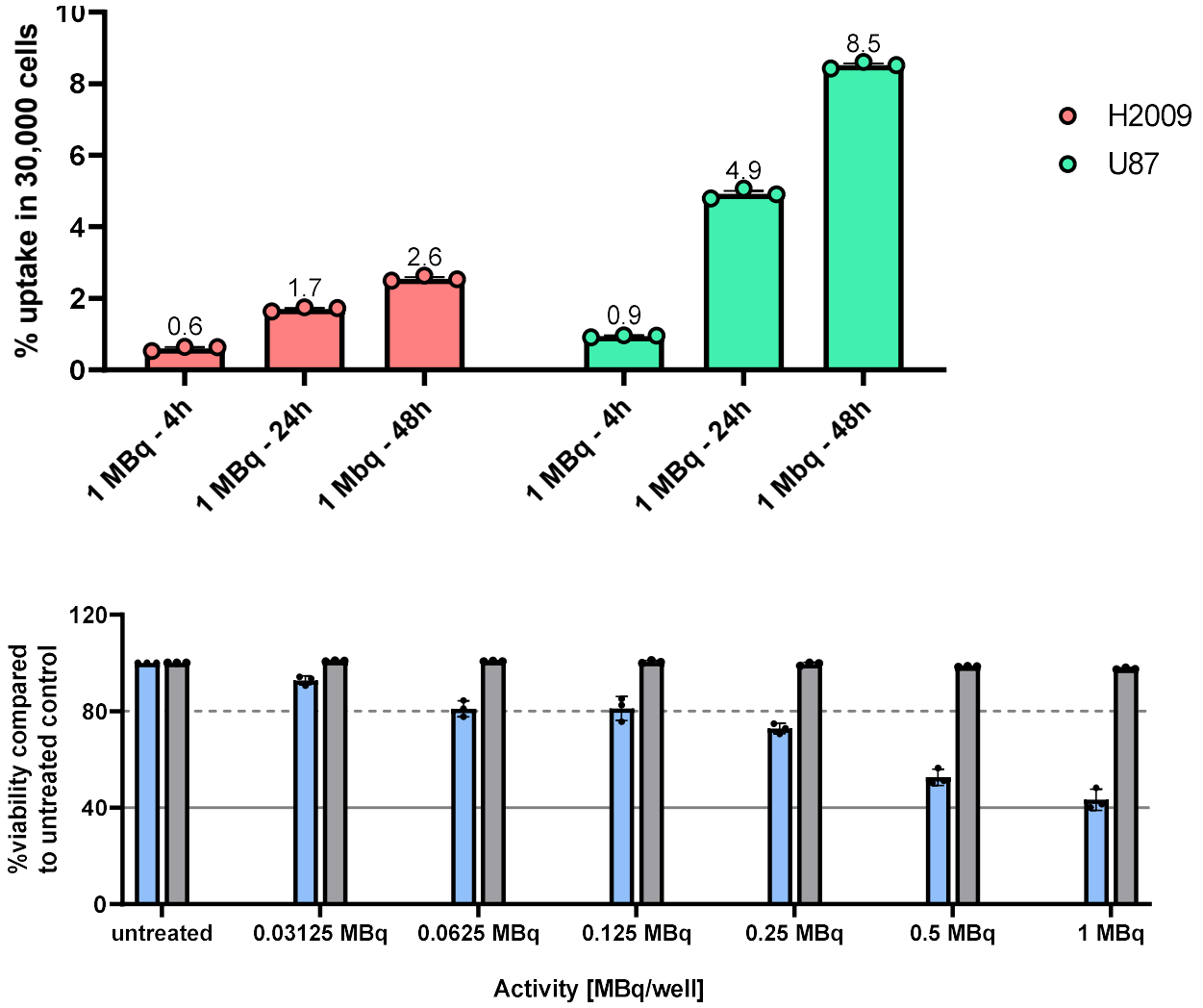

Figure 3. In vitro assessment of RadioExo in pulmonary and brain tumour cell lines at different timepoints (up) and different doses (bottom)

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

Thanks to the PRISMAP project, I have had access to the 161Tb radioisotope as this nuclide is not approved at my workplace. In this way I have been able to successfully carry out the synthesis studies of new teragnostic radiotracers and open a new line of research.

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

The project offers a great opportunity to establish scientific relationships with like-minded researchers and offers access to unique infrastructures. However, the development of the activities requires a high financial investment from the lead researcher, since on the one hand, it is necessary to pay for the transport of the isotopes and, on the other hand, for the accommodation and transport to the host centre.

In my case, the transport of the isotope was more than 1400€ and the accommodation and food in a city like Munich meant a daily investment of at least 200€. Although the original project proposed several stays to

carry out the different stages of the project, it has not been possible to carry out more than one due to the high economic cost involved (over 2500€ for a week), which has made it unfeasible to carry out the entire project.

For future editions, it would be advisable for PRISMAP to cover at least the researcher's accommodation and living expenses.

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

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)".