User project report

Terbium Chelation for Nuclear Medicine (TerbCheNuM)

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1. Authors

Partner	Name	First name	Position	Role & responsibilities in the project
Partner 1 ICMUB	MEYER	Michel	CNRS Researcher	Solution coordination chemistry
	BRANDES	Stéphane	CNRS Research Engineer	Organic/inorganic synthesis
	VACHEY	Lucas	Contractual Engineer	Solution coordination chemistry
Partner 2 IC-UNISTRA	CHAMBRON	Jean-Claude	CNRS Research Director	Organic/inorganic synthesis
	ZUJEW	Laurie	PhD student	Organic/inorganic synthesis, radiochemistry
	GALLER	Thibaut	Contractual Engineer	Organic/inorganic synthesis
Partner 3 IJCLab	SLADKOV	Vladimir	CNRS Researcher	Solution coordination chemistry
	LAM	Sabine	PhD student	Solution coordination chemistry

In the course of the project, a collaboration with the biotechnology company SYNDIVIA (Strasbourg, France) has been established. Prior to outsourcing the bioconjugation step, a Material Transfer Agreement (MTA) between the CNRS (partners 1 & 3), the University of Strasbourg (partner 2), and the SME has been signed.

PRISMAP partner: Dr David VIERTL, CHUV Lausanne

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

All current ^{161}Tb-based radiopharmaceuticals are DOTA-peptide bioconjugates tolerating the high temperatures used for inserting the metal into the cavity. As this is not the case of antibody (mAb) constructs, the aim of TerbCheNuM was to develop hitherto unavailable Tb-specific bifunctional chelators (BFC) that can be radiolabeled in mild conditions (20 °C), while keeping a stability and inertness comparable to those of the DOTA complexes. Owing to the slow binding kinetics of that macrocycle, we designed and evaluated mixed (O^-, N) BFC displaying either linear or branched topologies to ensure fast labeling. In a proof-of-concept approach, a novel enzyme-free and site specific bioconjugation technology targeting the mAb's hinge region was employed, that moreover allows a precise control of the drug-to-antibody ratio.

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

Four tetrapodal tetrapicolinate and one comb-like hydroxypyridonate chelators were synthesized. Crystallographic, solution equilibrium, and transchelation studies with DTPA as scavenger enabled to rank the corresponding Tb^{3+} complexes according to their stability and inertia. ^{161}Tb radiolabeling yields and *in vitro* stability tests in human serum performed at CHUV confirmed the ranking and allowed to select three compounds. Their bifunctional counterparts were prepared and bioconjugated to Trastuzumab (Tz), a mAb targeting the HER2 receptor overexpressed in breast, gastric, and ovarian cancers. After subjecting these constructs to the same *in vitro* tests, only two hits were retained, but only the ^{161}Tb-labeled tetrapicolinic TPACy–Tz conjugate could be evaluated *in vivo* so far. Biodistribution and µ-SPECT-CT scans of mice bearing a SK-OV-3 xenograft showed good tumor accumulation and inhibition of the tracer uptake when the animals were co-injected with a large excess of native Tz (Figure 1).

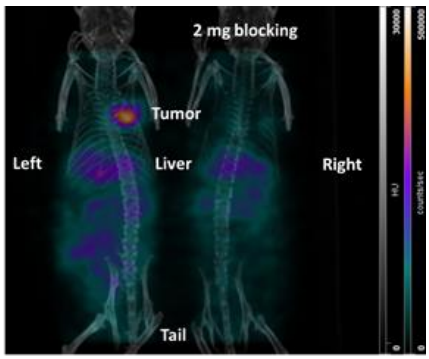

Figure 1. µ-SPECT-CT scans taken 4 days after injection of the [^{161}Tb]Tb(TPACy)–Tz bioconjugate to mice bearing a SK-OV-3 xenograft. The left picture shows significant tumor accumulation of the radiotracer, while uptake was completely inhibited in the control blocking experiment when a large excess of native Tz was co-administered to the animal on the right.

4. Conclusions (800 characters max. including spaces)

Within a year, the TerbCheNuM project allowed us to provide a first proof of concept, namely the ability to successfully radiolabel at room temperature and in less than 10 min with a yield higher than 99% a site-specific immunoconjugate with ^{161}Tb. The latter construct was stable in human plasma for 2 weeks, while µ-SPECT-CT images and biodistribution studies showed good tumor uptake, paving the way towards the development of new theranostic agents targeting PSMA to treat castration-resistant prostate cancer in the frame of the PRISMAP 2025 approved project TheraTerb.

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

Thanks to the support of PRISMAP, the French partners could initiate a most fruitful collaboration with Dr David Viertl (Centre Hospitalier Universitaire Vaudois, Lausanne). Our new Swiss partner has offered us an access to his accredited biomedical facilities (PRISMAP biomedical hub) in 2024, where 5 of us, including both PhD students involved in TerbCheNuM, could assist him in the experimental work (ten 3-day missions in total). The team could moreover benefit from 2 GBq of ^{161}Tb, delivered by the Paul Scherer Institute in April, May, June, and November 2024.

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

We are grateful to PRISMAP and to all our interlocutors for their kindness and dedication. Without this support, we could have never reached so rapidly the current level of maturity of our project.

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

So far, no scientific details about the design of the bioconjugates, nor on the results have been disclosed, as we are currently evaluating with the intellectual property advisors of our institutions the possibility to file in a patent application. Just an outline of the research activities of Partner 3, acknowledging PRISMAP and mentioning the TerbCheNuM project, has been presented during the annual meeting of a French network gathering CNRS research teams working in the field of nuclear medicine and cancer treatments:

Radiothérapie interne vectorisé à IJCLab. V. Sladkov, annual meeting of the French national network GdR Mi2B ("Outils et méthodes nucléaires pour la lutte contre le cancer"), Grenoble, 9–11 October 2024.

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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