# User project report

Towards 161Tb-PSMA cell targeting treatment of prostate cancer biochemical recurrence: Comparison with 177Lu-PSMA

Kirienko Margarita MD PhD

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

Kirienko Margarita[1], Anna Bogni[1], Elena Jachetti[2], Roberta Sulsenti[2], Claudio Pascali[1]

[1] Nuclear Medicine Department, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

[2] Molecular Immunology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

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

Prostate cancer (PC) represents the 5$^{th}$ cancer-related death worldwide. Prostate-specific membrane antigen (PSMA) is a specific target for both molecular imaging and radionuclide therapy in PC. PSMA is a class II transmembrane glycoprotein with enzymatic function. It is involved in folate and glutamate uptake, which are key to DNA synthesis and repair, amino acid and polyamine generation, and PI3K-Akt signaling. $^{177}$Lu-PSMA-targeted treatment is currently approved for clinical use in metastatic castration-resistant PC. A potentially more effective and less toxic therapeutic alternative to $^{177}$Lu is $^{161}$Tb. Using the commercially available PSMA I&T, our aim was to 1) Compare the efficacy of [$^{161}$Tb]Tb-PSMA I&T and [$^{177}$Lu]Lu-PSMA I&T in cell lines. 2) Imaging of PC patients to assess [$^{161}$Tb]Tb-PSMA I&T biodistribution and safety.

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

**Labelling tests**

Tests on the labelling conditions of PSMA I&T with $^{161}$Tb provided by PRISMAP are reported in Table 1.

Table 1.

| Exp | $^{161}$Tb MBq | $^{161}$Tb* µmol | PSMA I&T µmol | PSMA/$^{161}$Tb µmol/µmol | RCP % | AS MBq /µgPSMA |
|---|---|---|---|---|---|---|
| A | 126.5 | 0.0003 | 0.00267 | 10.33 | 100 | 31.2 |
| B | 126.5 | 0.0003 | 0.00134 | 5.17 | 100 | 62.4 |

*Estimated amount based on a specific activity of $^{161}$Tb = 3000MBq/µg

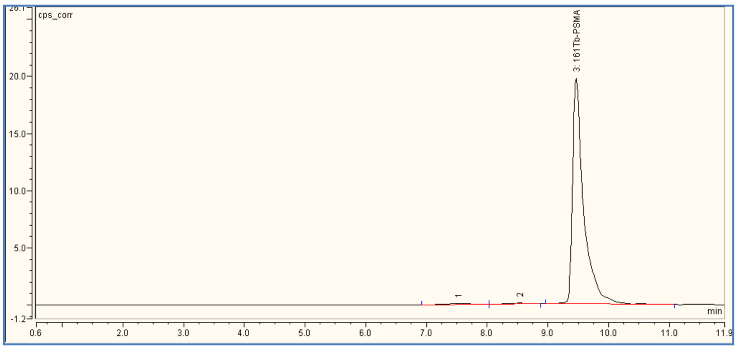
**Stability test on [$^{161}$Tb]Tb-PSMA I&T**

Purity of the products obtained in Exp. A and B were measured at different time points. The [$^{161}$Tb]Tb-PSMA I&T resulted stable (Table 2). Fig. 1 shows the HPLC radiochromatogram of sample A 7 days after labelling, confirming the results.

Table 2.

| Exp A | TLC (%) | HPLC (%) | Exp B | TLC (%) | HPLC (%) |
|---|---|---|---|---|---|
| t=0 | 100 | 100 | t=0 | 100 | 100 |
| t=18h | 100 | 100 | t=18h | 100 | 100 |
| t=7d | 100 | 98.7 | t=9d | 100 | 97.7 |

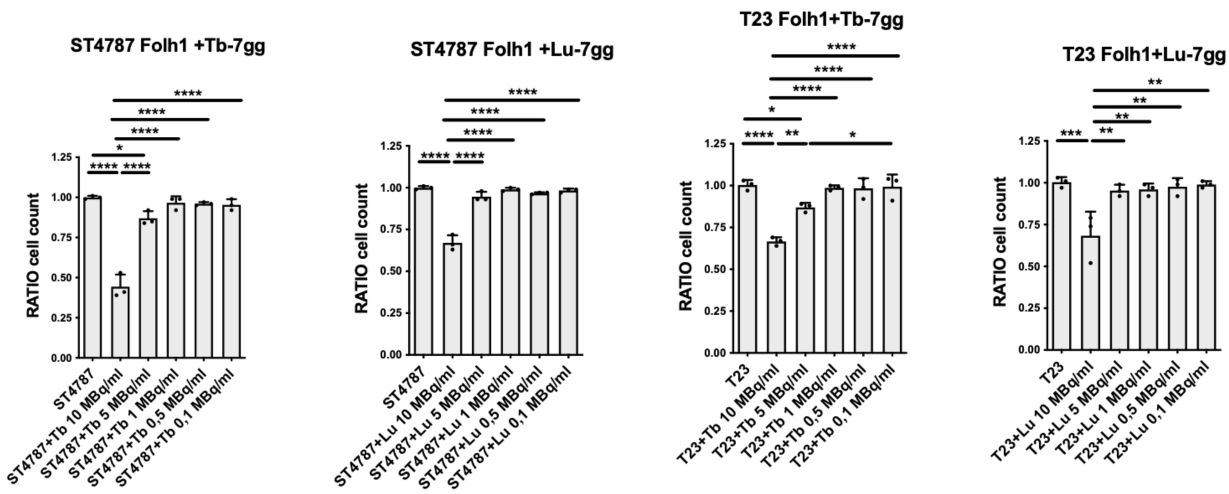
**Figure 1.** HPLC radiochromatogram of sample A after 7 days




**In vitro experiments in mouse cell lines**

We performed in vitro experiments using 161-Terbium provided by either PRISMAP and a private company (when deliveries from PRISMAP were not available) on both murine adenocarcinoma (T23) and neuroendocrine (ST4787) cell lines modified to express human PSMA. We aimed to compare the effect on cell proliferation of [$^{161}$Tb]Tb-PSMA I&T and [$^{177}$Lu]Lu-PSMA I&T in T23-*Folh1*-PURO and ST4787-*Folh1*-PURO. Both T23-Folh1-PURO and ST4787-Folh1-PURO, tested after infection and after at least two passages of puromycin selection, were positive for PSMA. In the first experiment, the reduction of viability of T23-Folh1-PURO and ST4787-Folh1-PURO tumor cells after exposure to [$^{161}$Tb]Tb-PSMA I&T and [$^{177}$Lu]Lu-PSMA I&T was observed not earlier than 7 days using 10MBq/mL. A viability reduction for T23-Folhl-PURO cells was observed also using 5 MBq/mL of [$^{161}$Tb]Tb-PSMA I&T, while [$^{177}$Lu]Lu-PSMA I&T did not provoke any change. In the second experiment we removed the radiopharmaceuticals after 4 hours and we observed that both cell lines resulted resistant to both radiopharmaceuticals at all the tested activities (0.5-10 MBq/ml).

**Figure 2. Cell proliferation in murine cell lines treated with [$^{161}$Tb]Tb-PSMA I&T and [$^{177}$Lu]Lu-PSMA I&T**



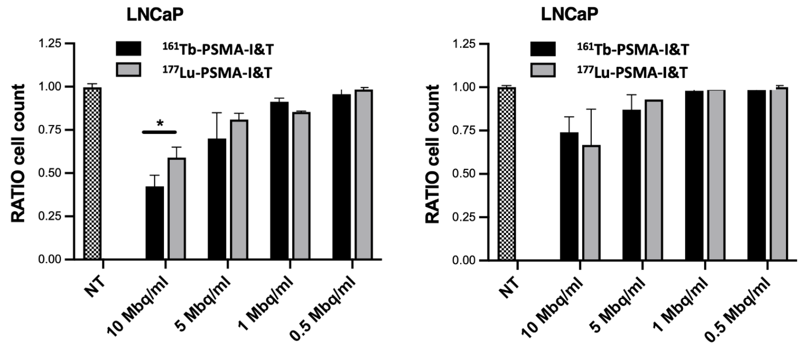

We concluded that the levels of PSMA were too low and thus the radiopharmaceuticals were not effective.

**In vitro experiments in human cell lines**

We used adenocarcinoma cell lines endogenously expressing PSMA (LNCaP) or not (PC3). PC3 cells have also been modified to express human PSMA (PC3- Folh1).

We aimed to compare the effect on cell proliferation of [$^{161}$Tb]Tb-PSMA I&T and [$^{177}$Lu]Lu-PSMA I&T in LNCaP and PC3 cell lines. We repeated the experiment twice. The reduction of viability of tumor cells after 4h exposure to [$^{161}$Tb]Tb-PSMA I&T and [$^{177}$Lu]Lu-PSMA I&T was observed after 7 days using 10MBq/mL. A viability reduction for tumor cells was observed also using 5 MBq/mL of [$^{161}$Tb]Tb-PSMA I&T in the first experiment. No significant difference was observed between the two radiopharmaceuticals at lower activities.

**Figure 3. Cell proliferation in human cell lines treated with [$^{161}$Tb]Tb-PSMA I&T and [$^{177}$Lu]Lu-PSMA I&T**




No effect against PSMA negative cells was observed.

## 4. Conclusions (800 characters max. including spaces)

Both radiopharmaceuticals - [$^{161}$Tb]Tb-PSMA I&T and [$^{177}$Lu]Lu-PSMA I&T resulted specific and promising in prostate cancer radioligand therapy. The $^{161}$Tb-labelled compound was found not inferior to the $^{177}$Lu-labelled counterpart. Additional tests are planned to confirm these preliminary results. We are preparing the Investigational Medicinal Product Dossier (IMPD) to be submitted to Italian pharma authorities to perform a biodistribution diagnostic study in prostate cancer patients.

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

PRISMAP facility in charge of our project was the Paul Scherrer Institute (PSI). PSI, Switzerland's largest research center for the natural sciences and engineering, in particular, in the field of matters and materials, energy, environment and human health.

PSI produced Terbium-161 and arranged the delivery to Milan. The access to Terbium-161 allowed us to perform the experiments that otherwise could not be performed. Thanks to PRISMAP and PSI, we acquired know-how on Terbium-161 labelling and handling. Additionally, we acquired knowledge on biological effects of [$^{161}$Tb]Tb-PSMA I&T on different models of prostate cancer cell lines.

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

We experienced nice collaboration with the staff from Paul Scherrer Institute, Dr. Zeynep Talip and Dr. Grundler Pascal Vincent. They supported us with information on the product any time we asked them. It might be useful for the users to receive a certificate, alongside the radionuclide, dealing with the batch specifications (activity per vial, specific activity, chemical purity, radionuclidic purity, radiochemical purity). After the problems rose during the first transport, the following run smoothly, with PSI staff taking care of documents' preparation for customs controls. The most challenging aspect in Terbium-161 transport is the timing. Customs operations made the delivery date shift in an unpredictable manner. This is not related to the PRISMAP or PSI. However, bureaucracy and logistics can constitute major barriers in promoting innovative radionuclide research and radiopharmaceuticals' development.

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

The manuscript is under preparation.

## 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)”.