User project report

Development and assessment of ^{161}Tb-targeted therapeutics in novel models of neuroendocrine cancer

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

Treatment development for neuroendocrine tumours (NETs) focuses on somatostatin receptor (SSTR) therapies using β-emitters like ^{177}Lu-DOTATATE. Terbium-161 (^{161}Tb) is a promising alternative due to its favourable decay properties and similar coordination chemistry to ^{177}Lu. Although ^{161}Tb complexes with DOTA, the high temperatures and long reaction times required limit its use. New chelators, L2and L3, may overcome these challenges. We are also developing 3D spheroid models from NET cell lines to better mimic tumour microenvironments and evaluate ^{161}Tb-based SSTR2-targeted (SSTR2T) radionuclide therapies, including agents like SSTR2T1, SSTR2T2 and SSTR2T3. They have shown potential in treating NETs but are untested with ^{161}Tb.

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

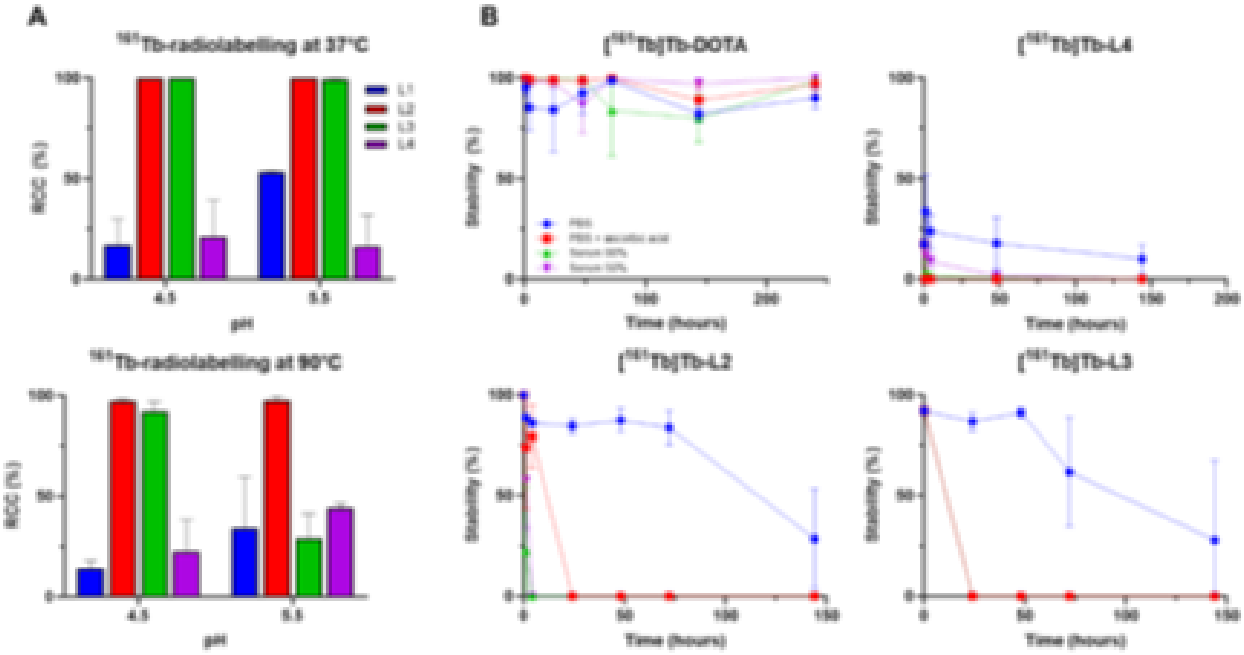
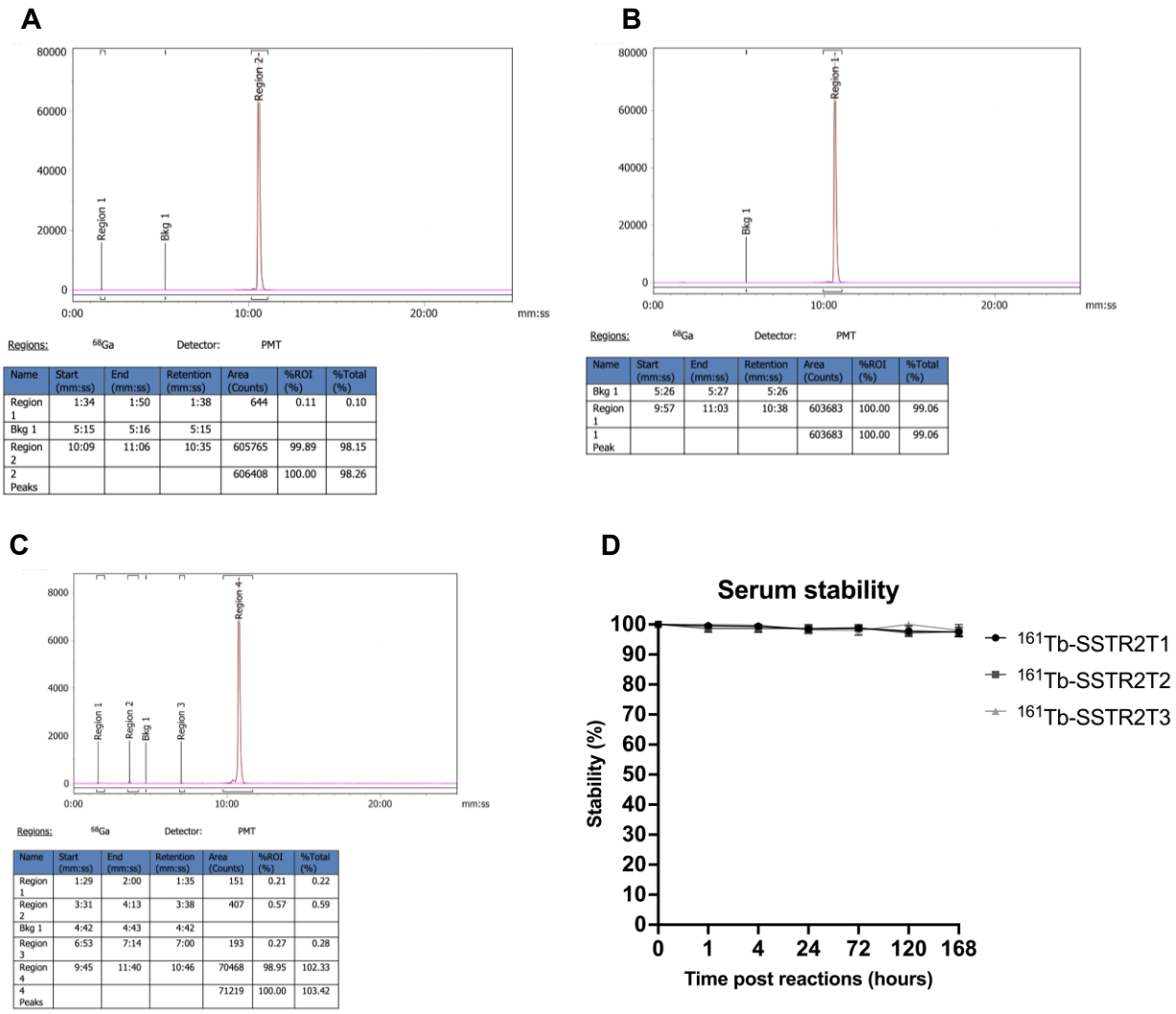


Figure 1. (A) Impact of pH and temperature on the radiochemical yield of ^{161}Tb-radiolabelling. 100 µM of L1, L2, L3 or L4 chelator, 37 or 90°C, 30 min, NaOAc buffer 0.1 M pH 4.5 or 5.5, 0.5 MBq. **(B) Stability study** at 37°C of [^{161}Tb]Tb-DOTA, [^{161}Tb]Tb-L4, [^{161}Tb]Tb-L2 and [^{161}Tb]Tb-L3, in PBS, PBS + 0.1% NaOAsc, serum 90% and serum 50%.

L2 showed the highest radiochemical yield (RCY >97%, Fig 1A). The RCY for L3 was also >97% except at 90°C, pH 5.5. ^{161}Tb did not effectively bind L1 and L4. Both ^{161}Tb-L2 and ^{161}Tb-L3 were stable with 87.5 ± 5.7% and 91.4 ± 2.2% remaining in PBS after 48 hours, respectively.



Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (Counts)	%ROI (%)	%Total (%)
Region 1	1:34	1:50	1:38	644	0.11	0.10
Bkg 1	5:15	5:16	5:15			
Region 2	10:09	11:06	10:35	605765	99.89	98.15
2 Peaks				606408	100.00	98.26

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (Counts)	%ROI (%)	%Total (%)
Bkg 1	5:26	5:27	5:26			
Region 1	9:57	11:03	10:38	603683	100.00	99.06
1 Peak				603683	100.00	99.06

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (Counts)	%ROI (%)	%Total (%)
Region 1	1:29	2:00	1:35	151	0.21	0.22
Region 2	3:31	4:13	3:38	407	0.57	0.59
Bkg 1	4:42	4:43	4:42			
Region 3	6:53	7:14	7:00	193	0.27	0.28
Region 4	9:45	11:40	10:46	70468	98.95	102.33
4 Peaks				71219	100.00	103.42

Figure 2 Successfully radiolabelled ^{161}Tb compounds show high stability in serum (A) Representative HPLC of (A) SSTR2T1, (B) SSTR2T2 and (C) SSTR2T3 demonstrating high radiochemical purity. (D) Serum stability of ^{161}Tb radiolabelled compounds. Stability was determined to be ≥96% by HPLC (n = 2).

SSTR2T1-3 showed high RCY and stability in serum at ≥96% after 7 days (Fig 2).

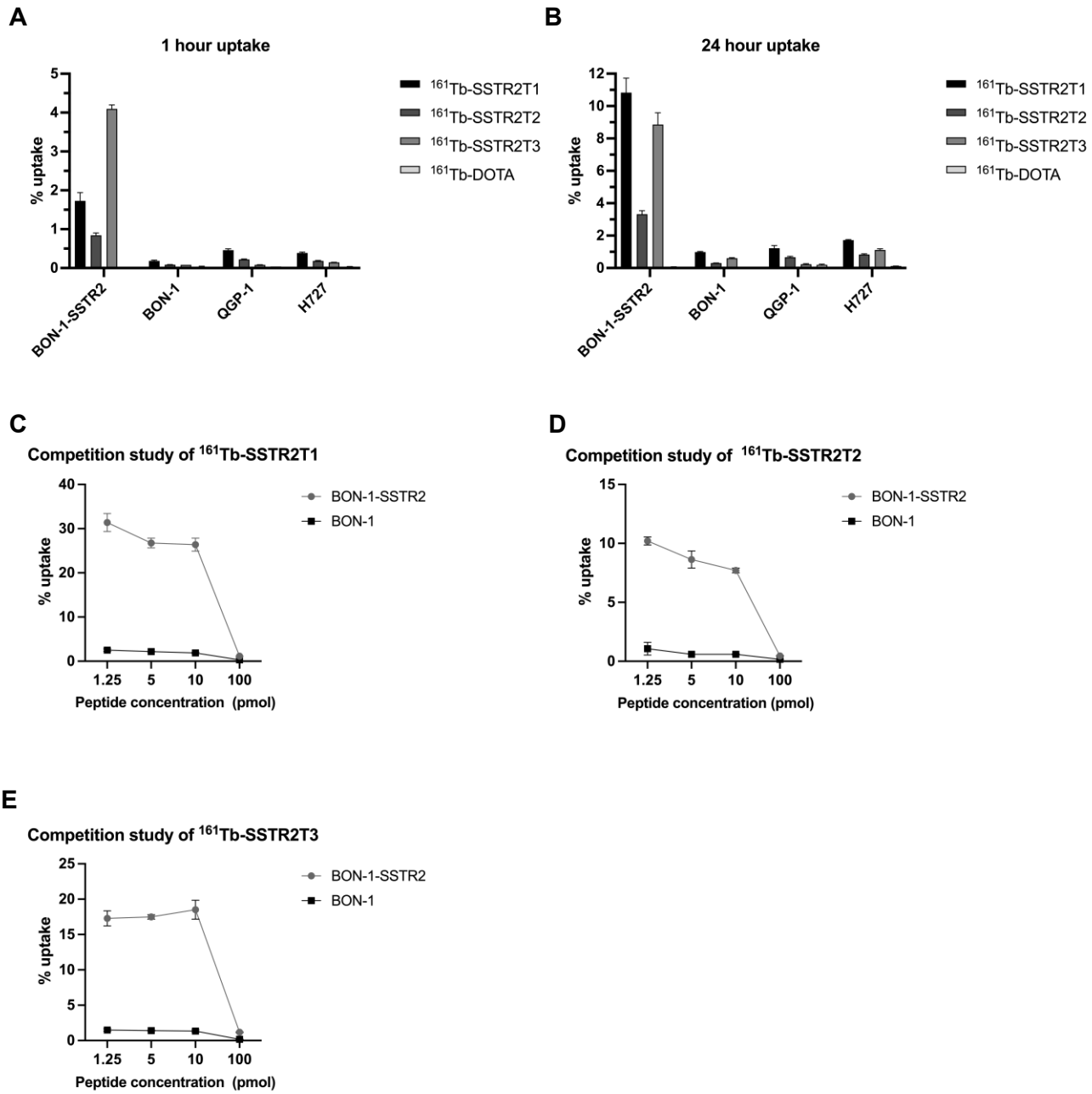


Figure 3 Uptake of ^{161}Tb-SSTR2T1, ^{161}Tb-SSTR2T2, ^{161}Tb-SSTR2T3 and ^{161}Tb-DOTA in neuroendocrine cell lines. Cellular uptake of 0.1 MBq/mL, 1.25 pmol, ^{161}Tb-DOTA, ^{161}Tb-SSTR2T1 ^{161}Tb-SSTR2T2 and ^{161}Tb- SSTR2T3 in neuroendocrine cell lines BON-1, BON-1-SSTR2, QGP-1 and H7272 at (A) 1 hour and (B) 24 hours. Results were calculated from independent biological replicates (n = 3) with ± standard deviation. Percentage uptake of (C) ^{161}Tb-SSTR2T1, (D) ^{161}Tb-SSTR2T2 and (E) ^{161}Tb- SSTR2T3 confirms SSTR2 specific uptake when adding increasing amounts of unconjugated peptide.

At 1 hr, uptake of ^{161}Tb-SSTR2T3 was 4.1 ± 0.18% in BON-1-SSTR2, significantly higher than ^{161}Tb-SSTR2T1 at 1.73 ± 0.37% (p = <0.0001) and ^{161}Tb- SSTR2T2 at 0.84 ± 0.11% (p = <0.0001, Fig 3A). At 24 hr, ^{161}Tb- SSTR2T1 had a value of 10.83 ± 1.55%, higher than ^{161}Tb-SSTR2T3 at 8.86 ± 1.26% (p = <0.001, Fig 3B). SSTR2 specific uptake was confirmed using competition studies (Fig 3C-E).

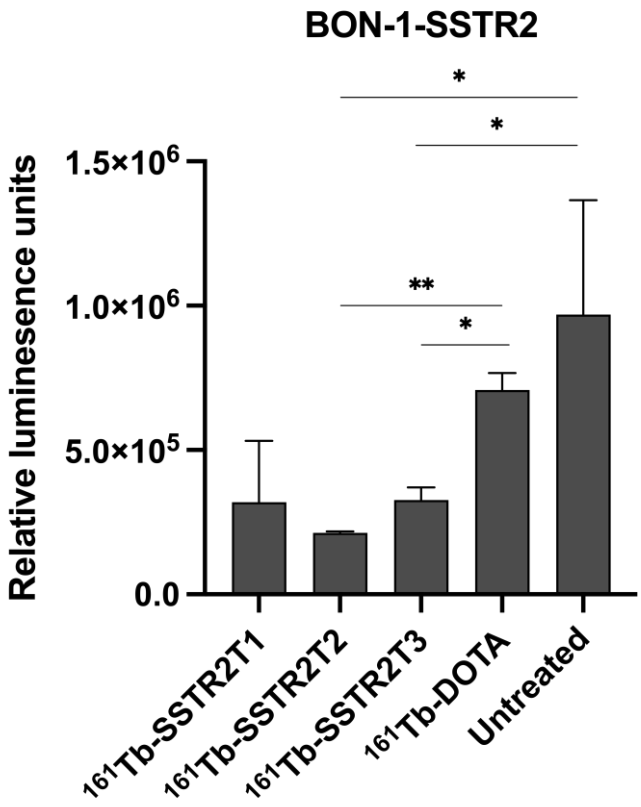


Figure 4 BON-1-SSTR2 spheroids show significantly decreased viability four days post treatment with ^{161}Tb-SSTR2T2 and ^{161}Tb-SSTR2T3. Compared with ^{161}Tb-DOTA and untreated controls, n = 3, ± standard deviation, * = $p < 0.05$, ** = $p < 0.01$.

At 4d post-treatment, spheroids treated with ^{161}Tb-SSTR2T2/3 showed significantly reduced viability compared to ^{161}Tb-DOTA or untreated controls, confirming treatment-induced cytotoxicity (Fig 4).

4. Conclusions (800 characters max. including spaces)

The L2 chelator showed the highest RCY (>97% for all conditions of pH and temperature tested). L3 also showed high RCY >97% except at 90°C, pH 5.5. In PBS, the stability of ^{161}Tb labelled compounds was good, but none showed stability in serum.

SSTR2T1, SSTR2T2 and SSTR2T3 were successfully labelled with ^{161}Tb (RCY >95%) and reactions showed ≥96% stability in serum over 7 days. Cellular uptake studies were performed, demonstrating uptake in a panel of NET cell lines, with SSTR2 receptor mediated binding was demonstrated for all radiotracers through competition study. At four days post treatment BON-1-SSTR2 spheroids showed a significant decrease in viability when treated with ^{161}Tb- SSTR2T3 and ^{161}Tb- SSTR2T2 but not ^{161}Tb- SSTR2T1 or ^{161}Tb-DOTA.

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

Access to PRISMAP's services enabled the procurement of ^{161}Tb which is currently not produced in the UK. Through the provision of ^{161}Tb, we were able to make connections with people at SCK CEN, Belgium.

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

The application process with PRISMAP was straightforward and well-supported, with clear communication throughout. Greater transparency regarding delivery associated costs would however be beneficial for future planning.

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

Currently not applicable.

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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