Improving the efficacy of pretargeted radioimmunotherapy in preclinical murine models by utilizing bioorthogonal click chemistry

Poster # 3051

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Background

Monoclonal antibodies (mAbs) are promising vectors for delivering therapeutic radiation to cancer cells, but the slow pharmacokinetics can lead to high radiation doses to healthy organs, which has hampered the translation of targeted radiommunotherapy (RIT) in the clinic. *Pretargeted* radioimmunotherapy (PRIT) has the potential to reduce the radiation burden to healthy tissue by harnessing the tumor-targeting properties of mAbs while avoiding their drawbacks by employing a process in which we are able to radioactively label mAbs after they have accumulated at their target site *in vivo*. Recently, an innovative approach has emerged based on the extraordinarily selective and rapid inverse electron demand Diels-Alder cycloaddition between a tetrazine (Tz) bearing radioligand and transcyclooctene (TCO) modified mAb.[1-3] Given the success of these pretargeted *imaging* modalities, the logical next step is to leverage this technology for the development of a safe and effective approach to PRIT.

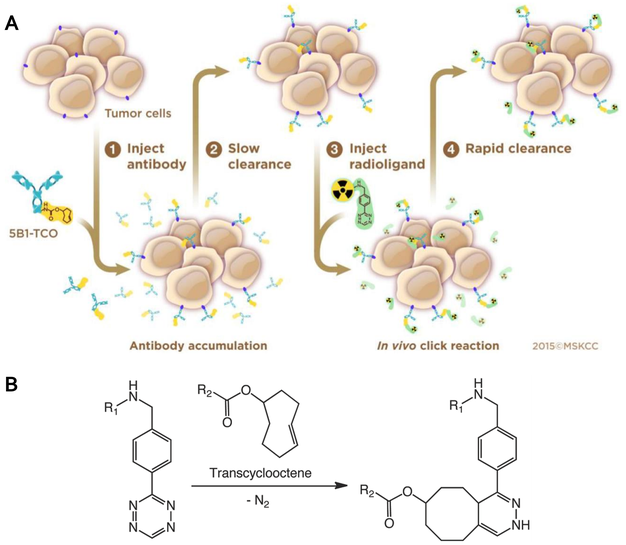


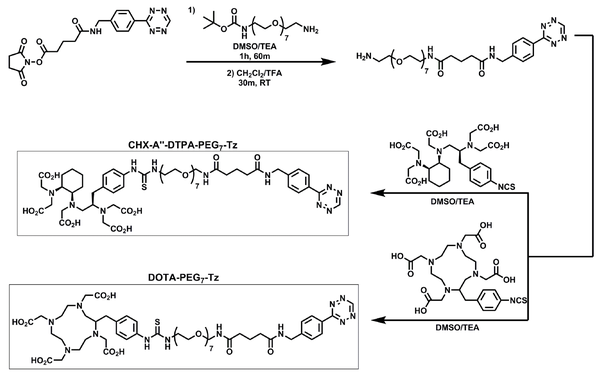
Figure 1. The pretargeting strategy utilized in these studies (**A**) and the rection between TCO and Tz (**B**) are depicted.

Methods

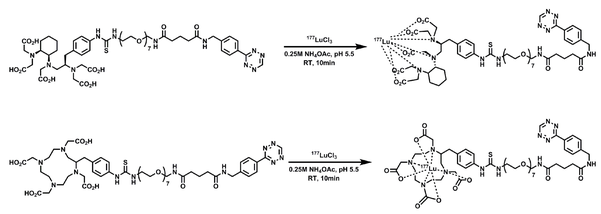
- TCO was conjugated to 5B1 – a fully human, anti CA19.9 antibody – as previously reported[3]
- Athymic nude mice with subcutaneous BxPC3 xenografts were used for all biodistribution and therapy studies
- Two novel bifunctional (chelator + tetrazine) ligands were synthesized and characterized
- Both ligands were radiolabeled with ^{177}Lu
- Biodistribution studies were performed to compare the two novel ligands and choose a lead candidate
- ^{177}Lu-DOTA-PEG$_7$-Tz was further characterized via biodistribution studies
- Therapy studies were performed using constant amounts of 5B1-TCO and varying amounts of activity to establish dose-dependent response in a murine model of PDAC

Results

Synthesis and radiolabeling



Scheme 1. Synthetic strategy use to produce CHX-A″-DTPA-PEG$_7$-Tz and DOTA-PEG$_7$-Tz precursor.



Scheme 2. Radiolabeling conditions used to produce ^{177}Lu-CHX-A″-DTPA-PEG$_7$-Tz (**top**) and ^{177}Lu-DOTA-PEG$_7$-Tz (**bottom**) for in vitro and in vivo studies.

In vitro and in vivo comparison of novel radioligands

	^{177}Lu-DOTA-PEG$_7$-Tz		^{177}Lu-CHX-A″-DTPA-PEG$_7$-Tz	
Time (h)	PBS	Serum	PBS	Serum
1	94.1 ± 0.65	97.7 ± 0.36	94.9 ± 0.80	97.5 ± 0.78
4	91.8 ± 3.0	96.2 ± 0.80	92.9 ± 3.9	93.9 ± 3.4
24	89.1 ± 5.9	95.2 ± 0.80	86.6 ± 9.7	90.4 ± 1.9
48	80.2 ± 8.0	93.6 ± 0.93	81.8 ± 5.4	85.5 ± 3.0

Table 1. Percent of ^{177}Lu-Tz-PEG$_7$-DOTA and ^{177}Lu-Tz-PEG$_7$-CHX-A″-DTPA intact after incubation in PBS (pH 7.4) or human serum at 37 °C as determined by HPLC analysis. Data represent an average of three experiments.

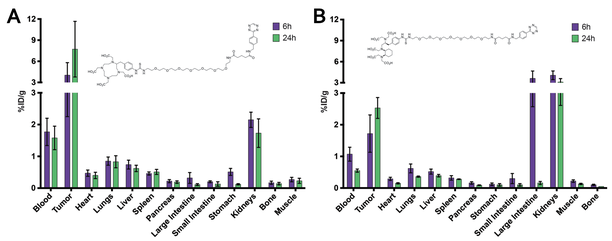


Figure 2. Results from biodistribution studies of the 5B1-TCO and either ^{177}Lu-DOTA-PEG$_7$-Tz (**A**) or ^{177}Lu-CHX-A″-DTPA-PEG$_7$-Tz (**B**) in mice (n = 5 per construct per time point) bearing BxPC3 subcutaneous xenografts, indicating that the DOTA construct demonstrated superior uptake in the tumor at both 4h and 24h post injection of radioligand.

Results

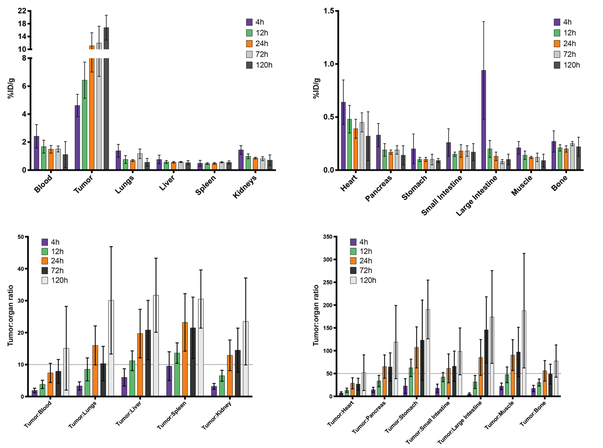
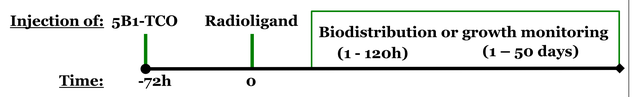


Figure 3. Biodistribution results from mice (n = 5 per itme point) injected with 5B1-TCO and ^{177}Lu-Tz-PEG$_7$-DOTA 72h after. Top panels report the injected dose per gram of tissue (%ID/g) whereas the bottom panels report the tumor to organ ratios.

Design of in vivo studies based on previous results[3]



Scheme 3. Experimental approach to pretargeted biodistribution and therapy studies.

In vivo therapy

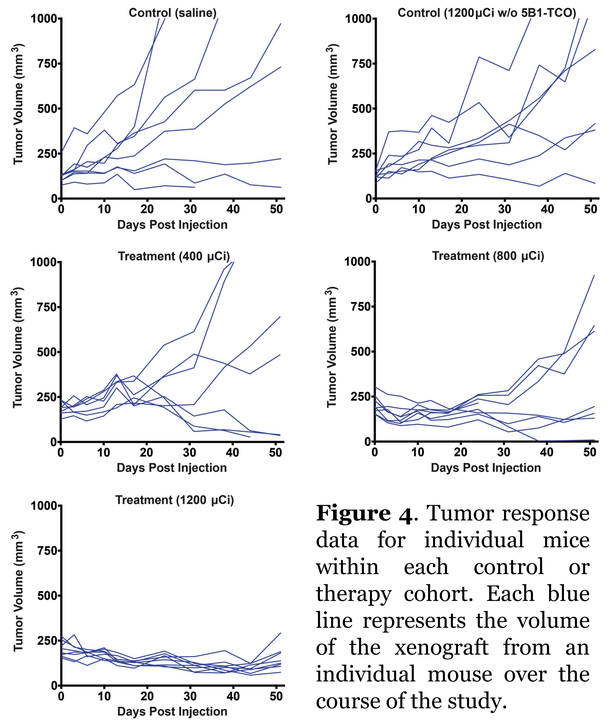


Figure 4. Tumor response data for individual mice within each control or therapy cohort. Each blue line represents the volume of the xenograft from an individual mouse over the course of the study.

Results

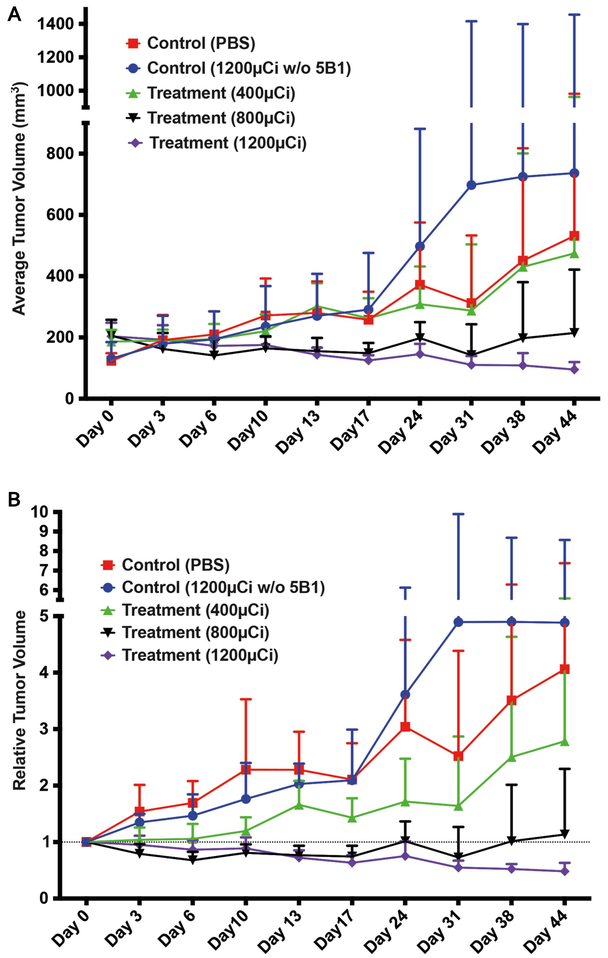


Figure 5. Tumor dose response data showing average tumor size (**A**) and relative tumor volume (**B**) over the course of the pretargeted radioimmunotherapy study, indicating a distinct dose-specific response in a murine model of pancreatic cancer.

Conclusions

- Two novel radioligands (DOTA-PEG$_7$-Tz and CHX-A″-DTPA-PEG$_7$-Tz) were synthesized and characterized
- Pilot studies of the radiolabeled constructs indicated that ^{177}Lu-DOTA-PEG$_7$-Tz was the better ligand for therapy
- In vivo biodistribution and PRIT showed that this systems has significant potential for clinical applications
- Studies to support clinical translation are underway

References and Acknowledgements

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2) Zeglis, BM et al. (**2013**) *J Nucl Med.* 54, 1389-96.
3) Houghton, JL et al. (**2015**) *J Nucl Med.* 57, 453-9.