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Towards the Optimization of Click-Mediated Pretargeted Radioimmunotherapy

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Abstract

Pretargeted radioimmunotherapy (PRIT) based on the inverse electron demand Diels-Alder reaction has shown promise in murine models of disease, yet the radiation dosimetry of this approach must be optimized to make it a viable clinical option. To this end, we have leveraged two recent developments in pretargeted imaging — dendritic scaffolds and masking agents — to improve the dosimetric profile of a proof-of-concept PRIT system that is based on the huA33 antibody, a ¹⁷⁷Lu-labeled tetrazine radioligand ([¹⁷⁷Lu]Lu-DOTA-PEG₇-Tz), and a mouse model of A33 antigen-expressing colorectal carcinoma. Pretargeting using an huA33 immunoconjugate bearing a *trans*-cyclooctene-decorated dendritic scaffold (⁸⁸huA33-DEN-TCO) produced significantly higher tumoral activity concentrations at 120 h post-injection (23.0 ± 2.2 %ID/g) than those achieved with an analogous, dendrimer-lacking immunoconjugate (12.7 ± 2.6 %ID/g). However, pretargeting using ⁸⁸huA33-DEN-TCO also resulted in increased activity concentrations in the blood at the same time point (1.9 ± 0.4 %ID/g) compared to the dendrimer-lacking construct (0.7 ± 0.2 %ID/g), thereby curtailing improvements to the tumor-to-blood therapeutic ratio of the system. In order to circumvent this issue, a tetrazine-labeled, dextran-based masking agent (Tz-DP) was injected prior to the radioligand in order to prevent the ligation between [¹⁷⁷Lu]Lu-

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Supporting Information

Reagents and general procedures; detailed experimental methods for chemical syntheses, radiosyntheses, *in vivo* experiments; dosimetry protocols; and tables of biodistribution data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

All animals were treated according to the guidelines approved by the Research Animal Resource Center and Institutional Animal Care and Use Committee at Memorial Sloan Kettering Cancer Center.

DOTA-PEG₇-Tz and circulating immunoconjugate. This approach dramatically decreased the absorbed dose to the blood but also attenuated the absorbed dose to the tumor and increased the absorbed dose to the lungs. Ultimately, these data suggest that dendritic scaffolds and masking agents could be used to improve the dosimetry of PRIT, but the combination of these technologies will require extensive optimization.

Graphical abstract

Keywords

radioimmunotherapy; click chemistry; pretargeting; pretargeted radioimmunotherapy; Lu-177; colorectal cancer; inverse electron demand Diels-Alder reaction; tetrazine; *trans*-cyclooctene; dendrimer; masking agent

Radioimmunotherapy has become established as an effective way to selectively and specifically localize therapeutic radioactivity to sites of disease.^{1, 2} Yet despite preclinical and clinical successes, the high radiation doses delivered by radioimmunotherapeutics to healthy non-target organs, most notably red marrow and osteogenic cells, have long been a major limiting factor for the modality.^{1, 3} Not surprisingly, a great deal of effort has been dedicated to developing strategies that circumvent this issue. One promising — yet undeniably mercurial — approach that has been explored for nearly three decades is *in vivo* pretargeting, a strategy founded on decoupling the antibody and radioactivity, injecting them separately, and allowing them to combine at the target tissue.⁴ Over the last decade, an approach to *in vivo* pretargeting based on the rapid and bioorthogonal inverse electron demand Diels-Alder reaction between *trans*-cyclooctene (TCO) and 1,2,4,5-tetrazine (Tz) has emerged, with several groups reporting exciting results in the context of PET, SPECT, radioimmunotherapy, and even chemotherapy.^{5, 67–11} Generally speaking, this approach is predicated on (i) the systemic administration of a TCO-bearing immunoconjugate, (ii) a multi-day interval period during which the antibody accumulates in the target tissue and clears from circulation, (iii) the systemic administration of the radiolabeled tetrazine, and (iv) the *in vivo* click ligation between the two components followed by the rapid clearance of any unreacted radioligand (Figure 1).

Over the last two years, our groups have published several studies demonstrating the potential of pretargeted radioimmunotherapy (PRIT) and comparing this approach to traditional radioimmunotherapy.^{9, 12, 13} In 2018, for example, we clearly illustrated the efficacy of ¹⁷⁷Lu-based PRIT in a murine model of colorectal carcinoma. While many of the tumor-to-background activity concentration ratios of ¹⁷⁷Lu-PRIT were undoubtedly higher than the analogous values produced via traditional radioimmunotherapy, the improvements to the dosimetry were less pronounced than in pretargeted PET studies in which we were

able to use short-lived radionuclides that are incompatible with traditional immunoPET.^{14, 15} Short-lived radionuclides with therapeutic emissions are, of course, much less common. In light of this, the investigation at hand is dedicated to leveraging two recent developments in pretargeted imaging — dendritic scaffolds and masking agents — to improve the dosimetric profile of a proof-of-concept PRIT system based on the huA33 antibody, a ¹⁷⁷Lu-labeled tetrazine radioligand ([¹⁷⁷Lu]Lu-DOTA-PEG₇-Tz), and a mouse model of A33 antigen-expressing colorectal carcinoma.^{16, 17} The huA33 antibody targets the A33 antigen, a surface-persistent glycoprotein overexpressed in >95% of colorectal carcinomas.¹⁸ Our approach to optimization is predicated on the fact that there are two ways to improve therapeutic ratios: *increasing activity concentrations in the tumor* and *decreasing activity concentrations in the blood*.

In this work, we first seek to address the former. Rossin *et al.* have clearly shown that the *in vivo* isomerization of reactive *trans*-cyclooctene to inert *cis*-cyclooctene has a deleterious effect on *in vivo* pretargeting.^{5, 10} It stands to reason that compensating for this isomerization will improve the performance of *in vivo* click ligations. To this end, our laboratory has previously developed a TCO-bearing poly(amidoamine) dendrimer (DEN-TCO).¹⁶ This scaffold can be attached to the heavy chain glycans of an antibody via a chemoenzymatic bioconjugation strategy in order to increase the number of TCO moieties appended to the immunoglobulin while simultaneously avoiding any adverse influence on the ability of the antibody to bind its target. We have previously shown that the site-specific modification of the huA33 antibody with these dendrimer scaffolds produces an immunoconjugate — ^{ss}huA33-DEN-TCO — with a degree of labeling of 8 TCOs/mAb, a marked increase over previously reported mAb-TCO conjugates. Even more importantly, PET imaging studies with ^{ss}huA33-DEN-TCO and a ⁶⁴Cu-labeled tetrazine radioligand ([⁶⁴Cu]Cu-SarAr-Tz) in murine models of colorectal cancer revealed that pretargeting with the dendrimer-bearing antibody produces nearly double the tumoral activity concentrations relative to pretargeting with a more traditional, dendrimer-lacking analogue. While our data suggest that the root of this increase lies in the higher number of TCOs/mAb on ^{ss}huA33-DEN-TCO, the longer serum half-life of the dendrimer-bearing immunoconjugate analogue likely contributes as well.

For this investigation, DEN-TCO, ^{ss}huA33-DEN-TCO, and a non-site-specifically modified, dendrimer-lacking immunoconjugate (huA33-TCO) were synthesized and characterized according to previously published protocols.^{12, 16} The ^{ss}huA33-DEN-TCO and huA33-TCO were found to have degrees of labeling of approximately 8.0 and 3.0 TCO/mAb respectively. In order to adapt this work for PRIT, a tetrazine-bearing radioligand labeled with the β-emitting radiometal ¹⁷⁷Lu — [¹⁷⁷Lu]Lu-DOTA-PEG₇-Tz — was likewise synthesized and characterized according to published procedures.⁹ In order to explore the *in vivo* behavior of PRIT with ^{ss}huA33-DEN-TCO and [¹⁷⁷Lu]Lu-DOTA-PEG₇-Tz, biodistribution experiments were performed in athymic nude mice bearing subcutaneous A33 antigen-expressing SW1222 colorectal carcinoma xenografts. To this end, ^{ss}huA33-DEN-TCO or huA33-TCO (100 μg, 0.67 nmol) were injected either 72 or 120 h prior to the administration of [¹⁷⁷Lu]Lu-DOTA-PEG₇-Tz (9.14 MBq, 0.74 nmol). Subsequently, at various times post-injection of radioligand, the animals were euthanized and dissected, and their organs were assayed for radioactivity using a γ-counter. As shown in Figure 2A, the data clearly show

that pretargeting with $^{88}\text{huA33-DEN-TCO}$ produces dramatically higher tumoral activity concentrations than pretargeting with huA33-TCO . To wit, using a 72 h interval between the administration of the two components, the tumoral activity concentration 120 h after the administration of the radioligand was $23.0 \pm 2.2\% \text{ID/g}$ with $^{88}\text{huA33-DEN-TCO}$ and $12.7 \pm 2.6\% \text{ID/g}$ with huA33-TCO . A similar trend can be seen with a 120 h injection interval, though the absolute activity concentration values are lower (Figure 3A). From a dosimetric perspective, this increase in tumoral activity concentrations translates into greater radiation doses absorbed by the tumor (Figures 2B and 3B). With a 72 h injection interval, for example, the absorbed dose to the tumor is 833.4 cGy/MBq with $^{88}\text{huA33-DEN-TCO}$ and 448.5 cGy/MBq with huA33-TCO .

Unfortunately, however, a concomitant — yet much less desirable — trend negates any net improvements to the overall dosimetric profile of this approach: pretargeting with $^{88}\text{huA33-DEN-TCO}$ produces higher activity concentrations in the blood than pretargeting with huA33-TCO . This trend is likely a result of the increased serum half-life of $^{88}\text{huA33-DEN-TCO}$ as well as the higher efficiency of click ligations between the radioligand and any dendrimer-bearing immunoconjugate remaining in the bloodstream (Figures 2 and 3). This increase in activity concentrations naturally increases the absorbed dose to the blood. Using a 72 h injection interval, for example, the absorbed dose to the blood is 80.5 cGy/MBq with $^{88}\text{huA33-DEN-TCO}$ and 40.5 cGy/MBq with huA33-TCO . As a result, the tumor-to-blood therapeutic indices for pretargeting with the two immunoconjugates are very similar: 11.1 (huA33-TCO) and 10.4 ($^{88}\text{huA33-DEN-TCO}$). Similar therapeutic indices are seen with a 120 h injection interval: 17.7 (huA33-TCO) and 15.1 ($^{88}\text{huA33-DEN-TCO}$).

The data make it clear that the use of the dendrimer-bearing immunoconjugate is — regrettably — a double-edged sword. While it increases activity concentrations in the tumor, it simultaneously boosts activity concentrations in the blood. The next phase of this investigation hinged on the question of whether it is possible to harness the increased tumor dose associated with pretargeting with $^{88}\text{huA33-DEN-TCO}$ while skirting the boosted radiation dose to the blood. To this end we sought to pair $^{88}\text{huA33-DEN-TCO}$ with a Tz-bearing masking agent.^{17, 19, 20} In our pretargeting schema, the masking agent is injected *after* the TCO-bearing immunoconjugate yet *before* the Tz-containing radioligand (Figure 1). The goal of a masking agent is to prevent ligations between radioligands and immunoconjugates in the blood by *inactivating* the TCO groups of any immunoconjugates still in circulation. The masking agent for this study — Tz-DP — is a dextran-based macromolecule that has been functionalized with an average of 304 tetrazine moieties per dextran.¹⁷ The large size of the masking agent (~2 MDa) theoretically prevents its extravasation to tumor tissue.^{21–23} In this manner, the masking agent can inactivate the TCO moieties of circulating immunoconjugates without interfering with the tumor-bound immunoconjugates. In previously published pretargeted PET imaging studies, the use of a variant of this dextran-based masking agent significantly increased tumor-to-blood activity concentration ratios in a murine model of pancreatic and colorectal cancer.¹⁷ Yet despite these positive results, it is nonetheless important to note that masking (or clearing) agents come with their own set of drawbacks: to wit, their very introduction inevitably adds complexity to an already complex system.

For this investigation, Tz-DP was prepared according to published protocols.¹⁷ For *in vivo* biodistribution studies, ⁸⁸huA33-DEN-TCO (100 µg; 0.67 nmol) was administered via the tail vein to athymic nude mice bearing subcutaneous SW1222 xenografts 72 h prior to the i.v. injection of 55 nmol of Tz-DP. Then, after a short interval of 30 minutes — the timing used in the original report on Tz-DP — [¹⁷⁷Lu]Lu-DOTA-PEG₇-Tz (11.1 MBq, 0.74 nmol) was administered via the tail vein. As can be seen in Figure 4, the use of Tz-DP in the pretargeting scheme dramatically reduces the activity concentrations in the blood at all time points. At 24 h after injection, for example, the activity concentration in the blood is 0.74 ± 0.16 %ID/g with Tz-DP compared to 3.4 ± 1.1 %ID/g without Tz-DP. Unfortunately, however, the activity concentrations in the tumor are also significantly reduced. Again, at 24 h after injection, the activity concentration in the tumor is 3.9 ± 0.4 %ID/g with Tz-DP compared to 11.0 ± 2.9 %ID/g without Tz-DP. We believe that this reduction has its roots in two phenomena. First, the use of the masking agent prevents the ligation of [¹⁷⁷Lu]Lu-DOTA-PEG₇-Tz and ⁸⁸huA33-DEN-TCO in the blood and thereby prevents the formation of ¹⁷⁷Lu-labeled immunoconjugate that can subsequently travel to the tumor. Second, the masking agent may also be penetrating the tumor and thereby preventing ligations between [¹⁷⁷Lu]Lu-DOTA-PEG₇-Tz and tumor-localized ⁸⁸huA33-DEN-TCO. As a result of these changes in the biodistribution, the absorbed dose to both the tumor (256 cGy/MBq) and the blood (15.6 cGy/MBq) are significantly lower than those produced upon pretargeting *without* the masking agent (833 cGy/MBq and 80.5 cGy/MBq, respectively). The tumor-to-blood therapeutic index of the masking agent-based pretargeting scheme — 16.4 — is slightly higher than that produced by pretargeting without Tz-DP — 10.4.

Easily the most surprising facet of the biodistribution experiments with ⁸⁸huA33-DEN-TCO, [¹⁷⁷Lu]Lu-DOTA-PEG₇-Tz, and Tz-DP is the accumulation of radioactivity in the lungs. Indeed, by 120 h after the injection of the radioligand, the activity concentration in the lungs is 16.3 ± 12.0 %ID/g, nearly 14 times the activity concentration in the lungs at the same time point in the biodistribution *without* the masking agent (1.2 ± 0.2 %ID/g). While extrapolating dosimetry data from mice to humans can be problematic, the radiation dose rate to the lungs created by this approach (531 cGy/MBq) is concerning high even though the red marrow is typically the dose-limiting organ in the context of radioimmunotherapy. We are currently investigating the root of this phenomenon. This elevated lung uptake is not observed in biodistribution experiments featuring [¹⁷⁷Lu]Lu-DOTA-PEG₇-Tz, Tz-DP, and the dendrimer-*lacking* huA33-TCO, though these data likewise reveal masking agent-associated reductions in the activity concentrations of both the blood and tumor (Supplemental Figure S1). Furthermore, the data plainly show that this lung uptake is absent upon the administration of [¹⁷⁷Lu]Lu-DOTA-PEG₇-Tz alone, upon pretargeting with ⁸⁸huA33-DEN-TCO and [¹⁷⁷Lu]Lu-DOTA-PEG₇-Tz, and upon the injection of Tz-DP followed by a ⁸⁹Zr-labeled mAb-TCO immunoconjugate.¹⁷ We have also demonstrated that [¹⁷⁷Lu]Lu-DOTA-PEG₇-Tz does not bind non-specifically to Tz-DP (Supplemental Figures S2 b S3). Thus, the uptake in the lungs clearly requires the presence of all three unique components. We hypothesize that it is the result of (a) the formation of cross-bridged Tz-DP/⁸⁸huA33-DEN-TCO/[¹⁷⁷Lu]Lu-DOTA-PEG₇-Tz aggregates in the blood that accumulate in the lungs or (b) the *in vivo* reaction of ⁸⁸huA33-DEN-TCO and [¹⁷⁷Lu]Lu-DOTA-PEG₇-Tz with Tz-DP aggregates that have accumulated in the lungs. In both cases, the presence of

several TCO moieties within the DEN-TCO scaffolds could facilitate the reaction of immunoconjugates with *both* Tz-DP and [^{177}Lu]Lu-DOTA-PEG₇-Tz. These working models are supported by literature reports on the accumulation of aggregated macromolecules and nanoparticles in the lungs.^{24, 25}

In conclusion, we have clearly demonstrated that *in vivo* pretargeting with an immunoconjugate bearing a dendritic scaffold boosts tumoral activity concentrations and absorbed radiation dose to the tumor. However, this approach does not improve the tumor-to-blood therapeutic ratio due to a concomitant increase in the absorbed radiation dose to the blood. The introduction of a masking agent — Tz-DP — to the ⁸⁹SuA33-DEN-TCO/[^{177}Lu]Lu-DOTA-PEG₇-TZ pretargeting strategy reduces the activity concentrations in the blood and slightly improves the tumor-to-blood therapeutic ratio, yet simultaneously attenuates the absorbed dose to the tumor dramatically and introduces a new uptake phenomenon in the lungs. In the end, we remain hopeful that dendritic scaffolds and masking agents can yet be combined to improve the dosimetry of pretargeted radioimmunotherapy, but we nonetheless concede that much more extensive and systematic studies must be conducted in order to optimize this approach.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

RIT	radioimmunotherapy
PRIT	pretargeted radioimmunotherapy
IEDDA	inverse electron demand Diels-Alder
Tz	tetrazine
TCO	<i>trans</i> -cyclooctene
iTLC	instant thin layer chromatography
%ID/g	percent injected dose per gram
p.i	post-injection
NHS	<i>N</i> -hydroxysuccinimide

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Figure 1.

Cartoon depiction of pretargeted radioimmunotherapy. This strategy proceeds via (1) the injection of a TCO modified antibody (either huA33-TCO or $^{ss}\text{huA33-DEN-TCO}$), (2) the optional administration of a tetrazine-functionalized masking agent (Tz-DP), (3) the injection of a tetrazine-bearing radioligand ($[^{177}\text{Lu}]\text{Lu-DOTA-PEG}_7\text{-Tz}$), and (4) the irradiation of the tumor site.

Figure 2.

Biodistribution (A) and dosimetry (B) data obtained following pretargeting with a 72 h injection interval. Athymic nude mice bearing subcutaneous A33 antigen-expressing SW1222 xenografts (n = 4) were administered 100 µg (0.67 nmol) of either huA33-TCO (gray) or ^{ss}huA33-DEN-TCO (red) via the tail vein, followed 72 h later by the injection of 9.14 MBq (0.74 nmol) of [¹⁷⁷Lu]Lu-DOTA-PEG₇-Tz.

Figure 3.

Biodistribution (A) and dosimetry (B) data obtained following pretargeting with a 120 h injection interval. Athymic nude mice bearing subcutaneous A33 antigen-expressing SW1222 xenografts (n = 4) were administered 100 µg (0.67 nmol) of either huA33-TCO (gray) or ^{ss}huA33-DEN-TCO (red) via the tail vein, followed 120 h later by the injection of 9.4 MBq (0.74 nmol) of [¹⁷⁷Lu]Lu-DOTA-PEG₇-Tz.

Figure 4.

Biodistribution (A) and dosimetry (b) data obtained via pretargeting with a Tz-bearing masking agent (Tz-DP). Athymic nude mice bearing A33 antigen-expressing SW1222 xenografts were injected *i.v.* with ^{ss}huA33-DEN-TCO (100 µg; 0.67 nmol) 72 h prior to the *i.v.* administration of [¹⁷⁷Lu]Lu-DOTA-PEG₇-Tz (11.1 MBq; 0.74 nmol). Cohorts of mice that received a dose of the Tz-DP masking agent (55 nmol) 30 minutes prior to the administration of the radioligand are shown in green, while those that did not are shown in red.