18F-Based Pretargeted PET Imaging Based on Bioorthogonal Diels–Alder Click Chemistry

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Alder Click Chemistry

ABSTRACT: A first-of-its-kind 18F pretargeted PET imaging approach based on the bioorthogonal inverse electron demand Diels–Alder (IEDDA) reaction between tetrazine (Tz) and trans-cyclooctene (TCO) is presented. As proof-of-principle, a TCO-bearing immunoconjugate of the anti-CA19.9 antibody SB1 and an Al[18F]NOTA-labeled tetrazine radioligand were harnessed for the visualization of CA19.9-expressing BxPC3 pancreatic cancer xenografts. Biodistribution and 18F-PET imaging data clearly demonstrate that this methodology effectively delineates tumor mass with activity concentrations up to 6.4 %ID/g at 4 h after injection of the radioligand.

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order to be able to use readily available TCO-modified SBI for in vivo pretargeting of CA19.9. Herein, we report the development of a novel Tz/TCO-based pretargeting strategy using an Al\(^{[18F]}\)-NOTA-labeled tetrazine radioligand. For our proof-of-concept system, we selected the SBI antibody, a fully human IgG that targets a promising biomarker for pancreatic ductal adenocarcinoma: carbohydrate antigen 19.9 (CA19.9).\(^{23,24}\) In order to arm the promising biomarker for pancreatic ductal adenocarcinoma: selected the 5B1 antibody, a fully human IgG that targets a
decaethylene glycol (NH\(_2\)-PEG\(_{11}\)-NHBoc), and NHS), commercially available building blocks: 2,5-dioxo-1-pyrrolidinyl (TCO-NHS, 35 equiv.) at room temperature for 1 h. The immunoconjugate was subsequently purified by gel-filtration chromatography. The precursor to the radioligand, Tz-PEG\(_{11}\)-NOTA (1, Scheme 1), was synthesized from three

Scheme 1. Radiochemical Synthesis of the Radioligand Tz-PEG\(_{11}\)-Al\(^{[18F]}\)-NOTA (\([18F]\mathbf{2})\)

\[ \text{AlCl}_3^{[18F]}\text{fluoride} \rightarrow \text{MeCN/H}_2\text{O, 90 °C, 15 min} \]

\[ 54-65 \% \text{RCY (d.c.)} \]

\[ ^{[18F]}\mathbf{2} \] was obtained in 54−56% RCY (d.c.) and high SAs (21.4−26.7 GBq/\(\mu\)mol) after a total synthesis time of 108 min. Purification of the crude reaction mixture using a C18-cartridge gave \([18F]2\) in purities >96%.

commercially available building blocks: 2,5-dioxo-1-pyrrolidinyl S-[4-(1,2,4,5-tetrazin-3-yl)benzylationio]-5-oxopentanoate (Tz-NHS), O-(2-aminoethyl)-O’-[2-(boc-amino)ethyl]-decaethylene glycol (NH\(_2\)-PEG\(_{11}\)-NHBoc), and S-2-(4-isothiocyanatobenzyl)-1,4,7-triazacyclononane-1,4,7-triacetic acid (p-SCN-Bn-NOTA). After the peptide coupling between Tz-NHS and NH\(_2\)-PEG\(_{11}\)-NHBoc and the subsequent deprotection of the terminal tert-butyloxycarbonyl protecting group, the resulting Tz-PEG\(_{11}\)-NH\(_2\) moiety was reacted with the bifunctional p-SCN-Bn-NOTA chelator. Ultimately, the precursor was prepared in very high purity (>98%) and with an overall yield of 15% (\(n = 3\)).

The \(^{18F}\)-labeled radioligand Tz-PEG\(_{11}\)-Al\(^{[18F]}\)-NOTA (\([18F]\mathbf{2})\) was obtained in 54−65% radiochemical yield [decay-corrected (d.c.) to the start of synthesis] in high purity (>96%) and a specific activity between 21.4 and 26.7 GBq/\(\mu\)mol (for more detailed experimental data, see Supporting Information). The use of metal-free solvents, the pH of the Al\(^{[18F]}\)-NOTA complexation reaction (pH = 4), and the ratio of reaction solvents (at least 3:1 MeCN/H\(_2\)O) all proved to be crucial factors in obtaining high radiochemical yields. The in vitro stability of \([18F]2\) was assayed by incubation in phosphate buffered saline (PBS, pH 7.4) or human serum at 37 °C, followed by analysis via radio-HPLC. In PBS, negligible decomposition could be observed after 4 h (92 ± 2.3% intact), and 79 ± 4.4% (\(n = 4\)) of the radioligand remained intact in human serum at the same time point. The in vivo stability was determined by injecting \([18F]\mathbf{2}\) (150 \(\mu\)Ci in 150 \(\mu\)L 0.9% sterile saline) into healthy athymic nude mice. Blood was subsequently collected via cardiac puncture and 63 ± 8.9% (\(n = 3\)) of the radioligand was found intact 4 h after injection. Given the fast reaction kinetics of the IEDDA ligation as well as the relatively short half-life of \(^{18F}\), the observed degradation rate is not considered a detriment to the system, as shown for other Tz/TCO approaches.\(^{3,19}\)

The bioorthogonal click reaction between \([18F]\mathbf{2}\) and the TCO moiety on the antibody was demonstrated by incubation of equimolar amounts (1.33 nmol) of the purified radioligand with SBI-TCO at room temperature. Analysis of the reaction via radio-TLC (mobile phase: 90% MeCN in H\(_2\)O) revealed a >94% yield for the reaction measured by the consumption of \([18F]\mathbf{2}\), with the \(^{18F}\)-labeled click reaction product situated at the origin, while the free radioligand can be detected at the solvent front (see Supporting Information). In all experiments throughout this study, the equimolar amount of tetrazine is calculated relative to the antibody SBI (and not the TCO).

Ex vivo biodistribution data for Tz-PEG\(_{11}\)-Al\(^{[18F]}\)-NOTA were first obtained in healthy mice by injecting \([18F]\mathbf{2}\) alone (1.8−2.0 MBq) via the tail vein (Figure 1). The data shows accumulation and retention of the radiotracer in the large intestines and feces with 30.2 ± 0.87% injected dose per gram (%ID/g) at 1 h after injection to 1.73 ± 0.45 %ID/g at 4 h. The uptake and retention of \([18F]\mathbf{2}\) could also be observed in the kidneys (2.12 ± 0.23 %ID/g at 1 h to 1.17 ± 0.12% ID/g at 4 h), indicating dual renal and fecal elimination pathways for the radioligand. The amount of activity in the blood decreases over time, from 1.94 ± 0.23 %ID/g at 1 h to 0.78 ± 0.08 %ID/g at 4 h after injection, while the uptake in all other healthy tissues remained <1 %ID/g. Critically, the activity concentrations in the bone were particularly low (≤0.2 %ID/g), illustrating the high in vivo stability of the Al\(^{[18F]}\)-NOTA complex. In accompanying experiments, the blood half-life of the radioligand was calculated to be 71.2 ± 5.4 min.

In subsequent pretargeted biodistribution experiments, nude, athymic mice bearing subcutaneous CA19.9-expressing BxPC3 xenografts were injected with SBI-TCO (1.33 nmol of SBI) 72 h prior to the administration of \([18F]\mathbf{2}\) (1.33 nmol, 1.8−2.0 MBq) (Figure 2).
PET images of Tz-PEG$_{11}$-Al[$^{18}$F]-NOTA/SB1-TCO pretargeting strategy. Subcutaneous BxPC3 xenograft bearing mice were administered SB1-TCO (1.33 nmol) 72 h prior to the injection of the $^{18}$F-labeled tracer (1.33 nmol, 18–20 MBq) via the tail vein. Transverse (top) and coronal (middle) planar images intersect the center of the tumors. The maximum intensity projections (MIPs, bottom) clearly illustrate tumor uptake after 1 h with increasing tumor-to-background ratios over the course of the experiment.

In light of these results, second generation tetrazine-bearing radioligands are currently in development in our laboratory in an effort to determine whether structural alterations can increase the fraction of the radioligand that is excreted via the renal system and thus create higher tumor-to-background ratios at earlier time points. Finally, using the biodistribution data, we performed a dosimetric analysis of the pretargeting strategy that confirms that pretargeted PET imaging with Tz-PEG$_{11}$-Al[$^{18}$F]-NOTA and SB1-TCO confers a significant dosimetric advantage over the use of antibodies directly labeled with long-lived radioisotopes (in this case $^{89}$Zr-DFO-SB1). The effective dose of the presented $^{18}$F-based pretargeting system (0.03 rem/mCi) is more than 60 times lower than directly labeled $^{89}$Zr-DFO-SB1 (2.02 rem/mCi; see Supporting Information).

In sum, this novel $^{18}$F-based pretargeted PET imaging system shows highly promising biodistribution results and produced tumoral activity concentrations of up to 6.4 %ID/g at 4 h after injection of the radiotracer.

### ASSOCIATED CONTENT

#### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.bioconjchem.5b00504.

Synthesis of the precursor 1, dosimetry calculations, and experimental details (PDF)

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