Supporting Information

Synthesis and Comparative In Vivo Evaluation of Site-Specifically Labeled Radioimmunoconjugates for DLL3-Targeted ImmunoPET

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MATERIALS AND METHODS

Reagents

All chemicals, unless otherwise noted, were acquired from Sigma-Aldrich and Fisher Scientific and were used as received without further purification. All water used was ultra-pure (>18.2 MΩcm⁻¹), and dimethylsulfoxide was of molecular biology grade (>99.9%). PODS-DFO-Fe was prepared as previously described, and Mal-DFO was purchased from Macroyclics, Inc. (1) SC16-MB1 is an engineered variant of the fully humanized DLL3-targeting IgG1 antibody, whose cysteine at position 220 in the upper hinge of the heavy chain has been mutated to serine (C220S). This modification yields an unpaired cysteine within the light chain at position 214. (2) A similar non-specific isotype control variant of this antibody — hIgG1-MB1 — was also produced. Both antibodies were obtained from Abbvie-Stemcentrx, USA.

Synthesis of Immunoconjugates

The bioconjugation of Mal-DFO (B-772, Macroyclics) to the antibodies was performed using two methods. The first method used tris-(2-carboxyethyl) phosphine (TCEP; 20490; Thermo Scientific) alone as the reducing agent. To this end, 2 mg of the SC16-MB1 antibody (2 mg/mL in Chelex-treated PBS, pH 7.2) were reduced by incubation with 4 molar equivalents versus 10 molar equivalents of TCEP (10 mM solution in water) at 25 °C for 2 hours. As explained in the text, two stoichiometric ratios of TCEP:mAb were employed to achieve partial versus complete reduction of the interchain disulfide bonds within SC16-MB1 and facilitate the evaluation of any differences created by these two approaches. Thereafter, the TCEP-reduced SC16-MB1 antibodies were used directly for conjugation with Mal-DFO by adding 4- and 10-molar equivalents of Mal-DFO, respectively, from a 14 mM stock solution in DMSO whilst keeping the volume of DMSO in the reaction mixture <2% (v/v). The conjugation mixture was incubated with gentle agitation (100 rpm) at 25 °C for 1.5 hours. Finally, two immunoconjugates were obtained: DFO_{Mal-TCEP}SC16-MB1 and DFO_{Mal-TCEP}SC16-MB1. The immunoconjugates were purified from the reaction mixture via size exclusion chromatography using PD-10 desalting columns (17085101, Cytiva Life Sciences) equilibrated with Chelex-treated PBS pH 7.2 (dead volume of 2.5 mL, elution volume of 2mL) and subsequent concentrated to 4-5 mg/mL using Ultra-2 Amicon 30 kDa molecular weight cutoff (MWCO) filtration spin columns (UFC203024, Millipore).
The second method employed a reduction buffer containing 7 mM reduced L-glutathione (G4251, Sigma Aldrich), 1 M arginine (A5006, A5131, Sigma-Aldrich), and 5 mM ethylenediaminetetraacetic acid (EDTA, E6758, Sigma Aldrich). A 4× dilution of the reducing buffer was prepared in milli-Q water and added to 2 mg (4 mg/mL in Chelex-treated PBS, pH 7.2) of the antibodies — either SC16-MB1 or hIgG-MB1 — and incubated at 25 °C for 2 h. The reduced antibodies were buffer exchanged via 50 kDa MWCO Amicon Ultra-15 centrifugal filtration columns (UFC905024, Millipore) using at least 12 diafiltration volumes of a conjugation buffer containing predetermined concentrations of Tris-EDTA [Tris Base, 93362, Sigma-Aldrich; Tris HCl, AAJ22638K2, Thermo Scientific] and EDTA (E6758, Sigma Aldrich). The buffer-exchanged antibody solutions were concentrated to 2 mg/mL using Ultra-2 Amicon 30 kDa molecular weight cutoff (MWCO) filtration spin columns (UFC203024, Millipore). Next, 4 molar equivalents of Mal-DFO from a 14 mM stock solution in DMSO was added to the antibodies whilst keeping the volume of DMSO in the reaction mixture <2% (v/v). The conjugation mixture was incubated with gentle agitation (100 rpm) at 25 °C for 1.5 hours. At the end of the incubation period, 1.2 molar excess of N-acetyl cysteine (A8199, Sigma Aldrich) was added from a freshly prepared 10 mM stock solution to each of the conjugation mixtures to quench the reaction for 20 min. (2)

All of the steps described above were followed for the synthesis of PODS-based conjugates of SC16-MB1 and hIgG-MB1. However, as previously described by Adumeau et al.,(1) a 2 mM solution of PODS-DFO-Fe was used to add 4 molar equivalents of PODS-DFO-Fe to the conjugation reaction. In order to remove the Fe, at the end of the 1.5 h conjugation reaction, 100 µL of a 25 g/L solution of EDTA was added, and the pH of the solution was quickly adjusted to 4.5 by adding 30 µL of 0.25 M H₂SO₄. This solution was then allowed to stir gently for 30 min. Finally, the reaction mixtures of all of the maleimide- and PODS-based immunoconjugates were purified via size exclusion chromatography using PD-10 desalting columns and subsequently concentrated to 8-12 mg/mL as described above.

**Characterization of Immunoconjugates**

The immunoconjugates synthesized using the methods described above were characterized at an intermediate stage in the process to verify the presence of free thiols using Ellman’s reagent. In addition, all of the final immunoconjugates were analyzed using mass spectrometry.
Ellman’s assay was carried out using a Biospec-nano micro-volume UV-Vis spectrometer (Shimadzu). Briefly, a solution of Ellman’s reagent was prepared by dissolving 4 mg of dithio-bis-(2-nitrobenzoic acid) (22582, Thermo Scientific) in 1 mL of 0.1M sodium phosphate, pH 8.0 containing 1 mM EDTA. The spectrometer was blanked using Chelex-treated PBS pH 7.2, and the absorbance of the reduced antibody solutions was measured in triplicates at 280 nm and 340 nm. Thereafter, 10 µL of each reduced antibody was aliquoted into a separate 100 µL microcentrifuge tube (in triplicates). Ellman’s blank solution was prepared by adding 10 µL of Chelex-treated PBS containing 50 mM EDTA. 2 µL of Ellman’s reagent was added to each of the tubes containing reduced antibody solutions and the blank solution. The tubes were vortexed briefly and allowed to incubate by standing at 25 °C for 10 min. The spectrometer was first blanked using the Ellman’s blank solution. Subsequently, the absorbance of the mixture of the reduced antibody and the Ellman’s reagent was measured at 412 nm and 750 nm. The free thiols in each sample were calculated using the following steps. First, the antibody concentration was determined by subtracting the absorbance values at 280 nm and 340 nm (A\textsubscript{280} - A\textsubscript{340}) and then dividing the result by the molar extinction coefficient for antibody (210,000). Next, the concentration of free thiols was determined by subtracting the absorbance values at 412 nm and 750 nm (A\textsubscript{412} - A\textsubscript{750}) and then dividing the result by a constant (14,150). Finally, the ratio of free thiols per antibody was determined by dividing the concentration of free thiols by the concentration of antibody.

The final immunoconjugates were characterized by mass spectrometry using a Thermo U3000 LC system connected with a Bruker maXis II UHR QTOF-MS system that enables native LC-MS analysis of intact full-length antibody molecules. Briefly, the antibody and immunoconjugate samples were diluted to 2 mg/mL and injected directly onto a pseudo-SEC column under native conditions using 0.2 M ammonium acetate buffer under isocratic conditions. The MS data was analysed within the Bruker Biopharma Compass software using the maximum entropy deconvolution algorithm. Further characterization of the light and heavy chains of the antibodies and immunoconjugates was performed after the reduction of the disulfides using 50 mM DTT and analysis under denatured ESI-MS conditions using the same LC-MS system as above. The deconvolution of the mass spectra from the reduced ESI-MS yielded monoisotopic masses which facilitated the further analysis of the integrity of the conjugation reaction and confirmed whether modifications had been made to the heavy or light chains of each antibody. The average DAR (DFO-to-antibody ratio) of each immunoconjugate was calculated by using the weighted average
of the deconvoluted peak area of each species multiplied by the DAR and divided by the total area of all DAR species.

**Radiopharmaceutical Chemistry**

High specific activity zirconium-89 (\(^{89}\)Zr-oxalate) was procured from 3D Imaging (Little Rock, AR, USA). The \(^{89}\)Zr-labeled radioimmunoconjugates were prepared by mixing pH-adjusted \(^{89}\)Zr (44.4 MBq; 1.2 mCi) with 236 ± 1.6 µg (~1.6 nmol) of each DFO-modified antibody suspended in Chelex-treated PBS pH 7.2. The mixture was incubated with gentle stirring for 1 h at 25 °C in a reaction volume of 300 µL whilst achieving an activity concentration of 4 µCi/µL. The reaction progress was assayed via radio-thin layer chromatography (radio-TLC) on silica-impregnated paper using an eluent of 50 mM EDTA, pH 5. After 1 h, the reaction was quenched by adding 1/10 (v/v) 10 mM EDTA. Finally, the radioimmunoconjugates were purified using a PD-10 desalting column for size-exclusion chromatography equilibrated with Chelex-treated PBS (dead volume 2.5 mL and elution volume 2 mL). The purity of each radioimmunoconjugate preparation was assayed by radio-TLC. The radioimmunoconjugates were tested for antibody stability and the demetallation of \(^{89}\)Zr\(^{4+}\) by incubating them in human AB-type serum for 6 days at 37 °C, during which the radiochemical purity was assayed daily via radio-TLC.

**Animal Model of Small Cell Lung Cancer**

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, NY. H82 xenografts expressing relatively high amounts of DLL3 were generated by subcutaneously injecting a 100 µL 1:1 (v/v) mixture of H82 cells in media and matrigel (BD Biosciences) on the right shoulder of female athymic nude mice (01B74-Athymic NCr-nu/nu; Charles River Laboratories, NY, USA). The xenograft-bearing mice were used for in vivo studies when the tumor volumes reached ~150-250 mm\(^3\).

**PET Imaging**

PET imaging was conducted on an Inveon PET/CT scanner (Siemens). Each xenograft-bearing mice (n = 3 per radioimmunoconjugate) was injected with ~143 µCi of activity (4.5 mCi/mg; 32 µg of radioimmunoconjugate; 1.3 mg/kg) in 200 µL Chelex-treated PBS via the lateral tail vein.
Static PET scans were acquired between 120 h after the administration of the radioimmunoconjugate. 120 h p.i. was chosen as the time point for PET imaging to allow for clearance of the radioimmunoconjugate from systemic circulation whilst achieving high accretion in the DLL3-expressing H82 tumors. In a separate longitudinal PET imaging study, H82 xenograft mice (n = 3 mice per group) were injected with ~230 μCi of activity (5.4 mCi/mg; 42 μg of radioimmunoconjugate; 1.7 mg/kg per mouse) in 200 μL Chelex-treated PBS via the lateral tail vein. Serial static PET scans were acquired at 24, 72, and 120 h after the injection of the radioimmunoconjugate. A minimum of 20 million coincident events was recorded for each scan, which lasted between 10-20 min. An energy window of 350-700 keV and a coincidence-timing window of 6 ns were used. The counting rates in the reconstructed images were converted to activity concentrations (percentage injected dose [%ID] per gram of tissue) using a system calibration factor derived from the imaging of a mouse-sized water-equivalent phantom containing $^{89}$Zr. Data were sorted into 2-dimensional histograms by Fourier re-binning, and transverse images were reconstructed by filtered back-projection (FBP) into a $128 \times 128 \times 63$ ($0.72 \times 0.72 \times 1.3$ mm) matrix. The image data were normalized to correct for non-uniformity of response of the PET, dead-time count losses, positron branching ratio, and physical decay to the time of injection, but no attenuation, scatter, or partial-volume averaging correction was applied. PET images were analyzed using ASIPro VM™ software (Concorde Microsystems).

**Biodistribution Analysis**

*Ex vivo* biodistribution analysis of the various $^{89}$Zr-radioimmunoconjugates was performed in mice bearing subcutaneous H82 xenografts. The mice (n = 5 per radioimmunoconjugate) were administered 27 μCi (4.5 mCi/mg; 6 μg per mouse; 0.2 mg/kg) in 200 μL of Chelex-treated PBS via lateral tail vein injection. The mice were euthanized by CO2 asphyxiation and vital tissues including the blood, heart, lungs, liver, spleen, stomach, pancreas, large intestine, small intestine, pancreas, reproductive tract, kidneys, muscle, bone, tail and tumor were harvested, weighed, and assayed for radioactivity on a gamma counter calibrated for $^{89}$Zr. Counts were converted into activity using a calibration curve generated from known standards. Count data was background and decay corrected to the time of injection, and the percent injected dose per gram (%ID/g) for each tissue sample was calculated by normalization to the total activity injected per mouse.
**Statistical Analysis**

All data presented are expressed as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism version 8.0. Statistical comparisons of the radioactivity concentrations in a given organ across different groups in the *ex vivo* biodistribution studies were done using unpaired t tests (one per row) with the Welch correction and applying the Holm-Sidak method with alpha = 0.05. Each row was analyzed individually, without assuming a consistent SD.
**SUPPLEMENTARY FIGURES**

**Figure S1.** Deconvoluted mass spectra from intact native ESI-MS and reduced denatured mass spectra for the light (LC) and heavy (HC) chains of unconjugated SC16-MB1 and DFO\textsubscript{Mal-TCEP\textsubscript{low}}SC16-MB1.
Figure S2. Deconvoluted mass spectra from intact native ESI-MS and reduced denatured mass spectra for the light (LC) and heavy (HC) chains of unconjugated SC16-MB1, DFOMal-\textsuperscript{TCEPh}SC16-MB1, and DFOMal-\textsuperscript{DAR2}SC16-MB1.
Figure S3. Deconvoluted mass spectra from intact native ESI-MS and reduced denatured mass spectra for the light (LC) and heavy (HC) chains of unconjugated SC16-MB1 and DFO_{PODS-DAR2SC16-MB1}. 
Figure S4. Deconvoluted mass spectra from intact native ESI-MS and reduced denatured mass spectra for the light (LC) and heavy (HC) chains of unconjugated hlgG-MB1, DFO_{Mal}^{DAR2}hlgG1-MB1, and DFO_{PODS}^{DAR2}hlgG1-MB1.
**Figure S5.** Instant thin layer chromatography assay to analyze the stability of the radioimmunoconjugates in serum over a period of 6 days. All of the radioimmunoconjugates showed signs of minor demetallation and/or the migration of activity from the baseline between days 3 and 6. This effect was most pronounced by day 6 for $[^{89}\text{Zr}]{\text{Zr}}\text{DFO}_{\text{Mal}}^{\text{TCEPlow}}\text{SC16-MB1}$. 
**SUPPLEMENTARY TABLES**

**Table S1.** *Ex vivo* biodistribution data (%ID/g ± S.D.) for the sextet of $^{89}$Zr-labeled radioimmunoconjugates in immunodeficient mice bearing subcutaneous H82 xenografts at a dose of 0.2 mg/kg. The stomach, small intestine, and large intestine values contain the contents.

| Tissue     | $[^{89}\text{Zr}]\text{Zr-DFO}_{\text{TCEP-low}}$ | $[^{89}\text{Zr}]\text{Zr-DFO}_{\text{TCEP-high}}$ | $[^{89}\text{Zr}]\text{Zr-DFO}_{\text{PODS-low}}$ | $[^{89}\text{Zr}]\text{Zr-DFO}_{\text{PODS-high}}$ | $[^{89}\text{Zr}]\text{Zr-DFO}_{\text{hIgG-low}}$ | $[^{89}\text{Zr}]\text{Zr-DFO}_{\text{hIgG-high}}$ |
|------------|--------------------------------|----------------|----------------|----------------|----------------|----------------|
| Blood      | 8.6 ± 1.4                      | 8.6 ± 1.0       | 6.8 ± 0.8       | 8.6 ± 1.2       | 9.9 ± 0.8       | 9.9 ± 0.8       |
| Heart      | 2.6 ± 0.4                      | 2.8 ± 0.5       | 2.3 ± 0.3       | 2.7 ± 0.7       | 3.1 ± 0.3       | 3.0 ± 0.3       |
| Lungs      | 3.3 ± 0.5                      | 3.2 ± 0.6       | 2.6 ± 0.4       | 3.5 ± 1.4       | 3.5 ± 0.7       | 4.4 ± 0.7       |
| Liver      | 2.9 ± 0.1                      | 2.4 ± 0.3       | 2.7 ± 0.4       | 3.1 ± 0.3       | 3.6 ± 0.1       | 3.4 ± 0.1       |
| Spleen     | 3.1 ± 0.3                      | 2.8 ± 0.2       | 3.1 ± 1.0       | 2.7 ± 0.2       | 2.9 ± 0.4       | 2.5 ± 0.4       |
| Stomach    | 0.9 ± 0.2                      | 0.5 ± 0.1       | 0.9 ± 0.1       | 1.0 ± 0.1       | 1.0 ± 0.4       | 1.2 ± 0.4       |
| Pancreas   | 0.8 ± 0.3                      | 1.0 ± 0.1       | 0.8 ± 0.2       | 1.0 ± 0.3       | 1.2 ± 0.3       | 1.0 ± 0.3       |
| L. Intestine | 0.5 ± 0.1                     | 0.5 ± 0.1       | 0.5 ± 0.0       | 0.9 ± 0.1       | 0.7 ± 0.1       | 0.9 ± 0.1       |
| S. Intestine | 0.8 ± 0.2                     | 0.8 ± 0.1       | 0.8 ± 0.1       | 0.9 ± 0.1       | 1.1 ± 0.1       | 1.0 ± 0.1       |
| Uterus     | 2.0 ± 0.9                      | 2.7 ± 1.1       | 1.8 ± 0.8       | 2.0 ± 0.8       | 2.8 ± 1.0       | 2.6 ± 1.0       |
| Kidneys    | 5.8 ± 0.6                      | 6.3 ± 1.5       | 4.7 ± 0.5       | 3.3 ± 0.5       | 6.7 ± 1.1       | 4.0 ± 1.1       |
| Muscle     | 0.6 ± 0.1                      | 0.8 ± 0.1       | 0.6 ± 0.1       | 0.7 ± 0.1       | 0.7 ± 0.1       | 0.7 ± 0.1       |
| Bone       | 7.1 ± 2.5                      | 4.9 ± 1.5       | 7.5 ± 0.9       | 8.0 ± 2.9       | 5.1 ± 3.3       | 5.6 ± 3.3       |
| Tail       | 1.5 ± 0.1                      | 1.7 ± 0.6       | 1.5 ± 0.3       | 1.6 ± 0.1       | 1.8 ± 0.1       | 1.6 ± 0.1       |
| Tumor      | 20.7 ± 3.9                     | 23.0 ± 2.1      | 19.2 ± 3.5      | 23.3 ± 4.8      | 4.5 ± 1.0       | 4.0 ± 1.0       |
Table S2. Tissue-to-blood activity concentration ratios (ratio ± S.D.) derived from the biodistribution data for the six 89Zr-labeled radioimmunoconjugates in immunodeficient mice bearing subcutaneous H82 xenografts at a dose of 0.2 mg/kg.

| Tissue-to- | [89Zr]Zr-DFOMal-SC16-MB1 | [89Zr]Zr-DFOMal-SC16-MB1 | [89Zr]Zr-DFOMal-DAR2SC16-MB1 | [89Zr]Zr-DFOPODS-DAR2hIgG-MB1 | [89Zr]Zr-DFOPODS-DAR2hIgG-MB1 |
|------------|--------------------------|--------------------------|-------------------------------|-------------------------------|-------------------------------|
| Blood      | 1.0 ± 0.0^0              | 1.0 ± 0.0^0              | 1.0 ± 0.0^0                   | 1.0 ± 0.0^0                   | 1.0 ± 0.0^0                   |
| Heart      | 0.3 ± 0.0^0              | 0.3 ± 0.0^0              | 0.3 ± 0.0^4                   | 0.3 ± 0.1                     | 0.3 ± 0.1                     |
| Lungs      | 0.4 ± 0.0^1              | 0.4 ± 0.0^1              | 0.4 ± 0.0^4                   | 0.4 ± 0.2                     | 0.4 ± 0.1                     |
| Liver      | 0.3 ± 0.1                | 0.3 ± 0.1                | 0.4 ± 0.1                     | 0.4 ± 0.1                     | 0.3 ± 0.1                     |
| Spleen     | 0.4 ± 0.0^4              | 0.3 ± 0.0^4              | 0.5 ± 0.2                     | 0.3 ± 0.1                     | 0.3 ± 0.1                     |
| Stomach    | 0.1 ± 0.0^5              | 0.1 ± 0.0^1              | 0.1 ± 0.0^3                   | 0.1 ± 0.0^3                   | 0.1 ± 0.0^3                   |
| Pancreas   | 0.1 ± 0.0^0              | 0.1 ± 0.0^0              | 0.1 ± 0.0^3                   | 0.1 ± 0.0^2                   | 0.1 ± 0.0^3                   |
| L. Intestine| 0.1 ± 0.0^2              | 0.1 ± 0.0^1              | 0.1 ± 0.0^1                   | 0.1 ± 0.0^1                   | 0.1 ± 0.0^2                   |
| S. Intestine| 0.1 ± 0.0^3              | 0.1 ± 0.0^1              | 0.1 ± 0.0^1                   | 0.1 ± 0.0^1                   | 0.1 ± 0.0^3                   |
| Uterus     | 0.2 ± 0.1                | 0.3 ± 0.1^4              | 0.3 ± 0.1                     | 0.2 ± 0.1                     | 0.3 ± 0.1                     |
| Kidneys    | 0.7 ± 0.2                | 0.7 ± 0.1^2              | 0.8 ± 0.2                     | 0.4 ± 0.1                     | 0.7 ± 0.1                     |
| Muscle     | 0.1 ± 0.0^1              | 0.1 ± 0.0^0              | 0.1 ± 0.0^2                   | 0.1 ± 0.0^1                   | 0.1 ± 0.0^1                   |
| Bone       | 0.9 ± 0.5                | 0.6 ± 0.1^2              | 1.1 ± 0.2                     | 0.9 ± 0.3                     | 0.5 ± 0.3                     |
| Tail       | 0.2 ± 0.0^4              | 0.2 ± 0.1                | 0.2 ± 0.1                     | 0.2 ± 0.0^2                   | 0.2 ± 0.1                     |
| Tumor      | 2.5 ± 0.7                | 2.3 ± 0.6                | 2.8 ± 0.4                     | 2.8 ± 0.7                     | 0.5 ± 0.1                     |

^0^ denotes significance level.
Table S3. *Ex vivo* biodistribution data (%ID/g ± S.D.) for $[^{89}\text{Zr}]\text{Zr-DFO}_{\text{Mal-^DAR2SC16-MB1}}$ and $[^{89}\text{Zr}]\text{Zr-DFO}_{\text{PODS-DAR2SC16-MB1}}$ in immunodeficient mice bearing subcutaneous H82 xenografts at a dose of 1.7 mg/kg.

| Tissue   | $[^{89}\text{Zr}]\text{Zr-DFO}_{\text{Mal-^DAR2SC16-MB1}}$ | $[^{89}\text{Zr}]\text{Zr-DFO}_{\text{PODS-DAR2SC16-MB1}}$ |
|----------|-------------------------------------------------|-------------------------------------------------|
| Blood    | 12.4 ± 3.6                                      | 9.8 ± 0.7                                       |
| Lungs    | 5.3 ± 1.0                                       | 3.5 ± 0.1                                       |
| Liver    | 4.1 ± 1.1                                       | 3.2 ± 0.4                                       |
| Spleen   | 6.7 ± 2.5                                       | 4.2 ± 0.5                                       |
| Kidneys  | 9.2 ± 0.7                                       | 6.2 ± 0.2                                       |
| Muscle   | 0.9 ± 0.1                                       | 0.6 ± 0.2                                       |
| Bone     | 7.5 ± 1.9                                       | 5.9 ± 0.1                                       |
| Tumor    | 30.2 ± 4.0                                      | 19.9 ± 4.5                                      |
Table S4. Tissue-to-blood activity concentration ratios (ratio ± S.D.) for $[^{89}\text{Zr}]\text{Zr-DFO}_{\text{Mal-DAR}^2}\text{SC16-MB1}$ and $[^{89}\text{Zr}]\text{Zr-DFO}_{\text{PODS-DAR}^2}\text{SC16-MB1}$ in subcutaneous H82 xenografts at a dose of 1.7 mg/kg.

| Tissue-to- | $[^{89}\text{Zr}]\text{Zr-DFO}_{\text{Mal-DAR}^2}\text{SC16-MB1}$ | $[^{89}\text{Zr}]\text{Zr-DFO}_{\text{PODS-DAR}^2}\text{SC16-MB1}$ |
|------------|-------------------------------------------------|-------------------------------------------------|
| Blood      | 1.0 ± 0.0$^p$                                  | 1.0 ± 0.0$^p$                                  |
| Lungs      | 0.4 ± 0.1                                       | 0.4 ± 0.0$^t$                                  |
| Liver      | 0.3 ± 0.1                                       | 0.4 ± 0.0$^t$                                  |
| Spleen     | 0.6 ± 0.2                                       | 0.5 ± 0.0$^p$                                  |
| Kidneys    | 0.8 ± 0.2                                       | 0.6 ± 0.0$^t$                                  |
| Muscle     | 0.1 ± 0.0$^t$                                   | 0.1 ± 0.0$^t$                                  |
| Bone       | 0.6 ± 0.3                                       | 0.6 ± 0.1                                     |
| Tumor      | 2.5 ± 0.4                                       | 2.0 ± 0.6                                     |
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