SUPPLEMENTARY INFORMATION

METHODS

Cell Culture

GFP-Luciferase positive (Gfp/Luc) MM.1S MM cells were provided by Dr. Irene Ghobrial (Dana-Farber Cancer Institute, Boston, MA), and U266 Luc+ cells were provided by Dr. Steven Grant (Commonwealth University, Richmond, Virginia). MM.1S and U266 parental cell lines were purchased from the American Tissue Culture Collection (U.S.A.). MM cells were grown in RPMI-1640 medium. All media were supplemented with 10% fetal bovine serum. Cells were maintained in a humidified incubator at 5% CO_2 and 37°C.

Flow Cytometry

Flow cytometry was used to verify the levels of CD38 expression in Gfp/Luc MM.1S and U266 Luc cells and parental cell lines using PE Mouse Anti-human CD38 (clone IB6 cat# 130-092-260, Miltenyi Biotec). A proper isotype control (mouse IgG2a PE cat# 130-098-849, Miltenyl Biotec.) was used. Staining and flow cytometry analysis followed standard protocols. Analysis was performed using the BDLSR II Becton Dickinson and Kaluza analysis 1.3.

Preparation of Radiolabeled Dara

A 20 ml vial of Dara (NDC57894-502-20 Lot DPF1700) was purchased from the City of Hope (COH) pharmacy (400 mg/vial) and conjugated with DOTA in the COH cGMP manufacturing facility. The antibody was buffer exchanged into sodium bicarbonate buffer and subsequently conjugated to the metal chelator NHS-DOTA (1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid mono-N-hydroxysuccinimide ester) (Macrocyclics™, Plano Tx), buffer exchanged into 0.25 M ammonium acetate (pH7), then aseptically filtered and vialed. The Dara pre-conjugation, DOTA-Dara post-conjugation and DOTA-Dara mAb Vialed Product were analyzed by SDS-PAGE (4-12% gradient polyacrylamide) with and without reduction and isoelectric focusing. The gels were stained with Coomassie Brilliant Blue for visualization.

^{64}Cu (half-life 12.8 h; 0.18 positrons/decay) was provided by the Mallinckrodt Institute of Radiology, Washington University School of Medicine. Five mg of DOTA-conjugated antibody was incubated with ^{64}Cu for 45 min at 43°C, chased with 1 mM diethylenetriamine pentaacetic acid (DTPA), and purified on a size-exclusion, preparative column (Superdex-200; GE Healthcare Life Sciences). The endotoxin content of the products after labeling was determined using a Charles River's Endosafe™-PTS system. The endotoxin concentration is routinely determined to be < 0.1 endotoxin units/mL. Briefly, the antibody was buffer exchanged into sodium bicarbonate buffer and subsequently conjugated to the metal chelator NHS-DOTA (1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid mono-N-hydroxysuccinimide ester) (Macrocyclics™, Plano Tx), buffer exchanged into 0.25 M ammonium acetate (pH7), then aseptically filtered and vialed. The Dara pre-conjugation, DOTA-Dara post-conjugation and DOTA-Dara mAb Vialed Product were analyzed by SDS-PAGE (4-12% gradient polyacrylamide) with and without reduction and isoelectric focusing. The gels were stained with Coomassie Brilliant Blue for visualization.

Immunoreactivity Assays

CD38-binding assays to the antigen were carried out using ^{64}Cu-DOTA-Dara in the presence or absence of soluble unlabeled CD38-Fc antigen (Chimerigen Laboratories, San Diego Cat#CHI-HF-210CD38) at a 20-fold antigen excess. The immunoreactivity was expressed as a percentage of the ^{64}Cu-DOTA-Dara bound by CD38-Fc antigen vs. unbound ^{64}Cu-DOTA-Dara compared to total as determined by radioactive monitoring of size exclusion HPLC (RIT SOP 0504).

Stability Studies

The ^{64}Cu-DOTA-Daratumumab was formulated in saline and held at room temp for *in vitro* stability studies. Aliquots were taken at 0, 2, 8, 24 and 48 hours and analyzed by radioactive monitoring of size exclusion HPLC. For *in vivo* stability analysis, after the final 2 day PET images were acquired, two mice were bled, serum prepared and analyzed by radioactive monitoring of size exclusion HPLC.

Animal Models

The City of Hope Institutional Animal Care and Use Committee approved all animal studies. MM.1S GFP+/Luc+ and U266 cells were harvested during the logarithmic growth phase and injected intravenously (IV) into NSG mice (0.2 ml/mouse containing 5x10^6 cells in PBS). Seven days later, mice were monitored daily for tumor development by BLI assessment using the LagoX Imaging System (Spectral Imaging, Tucson, AZ) (see BLI experimental method). On day 15, after bioluminescence reached approximately ≥2x10^6 photons/sec/cm^2/sr, mice were injected with ^{64}Cu-DOTA-Dara (3.7 MBq, 10 µg), and PET imaging was performed at 21 and 44 hrs after the injection (see ^{18}F-FDG PET/CT scans experimental method). Tumor-free mice were used as controls.

Bioluminescence imaging (BLI) of tumor growth

Non-invasive, whole body imaging for the assessment of tumor growth was performed weekly using the LagoX Imaging System (Spectral Imaging, Tucson, AZ). Mice were injected i.p. with 100 µl of the D-Luciferin solution at a final dose of 3 mg/20 g mouse body weight (Biosynth, Cat. No. L-82220) and then anaesthetized with isoflurane (Faulding Pharmaceuticals). Images were acquired for 0.5–30 sec (images are shown at 1 sec) from the side angle, and the photon emission transmitted from mice was captured and quantitated in photons/sec/cm^2/sr using AMIView software.

PET Imaging and Biodistribution Studies

Mice bearing MM or tumor-free mice were IV injected with ^{64}Cu-DOTA-Dara (3.7 MBq, 10 µg) or ^{64}Cu-Trast-DOTA (3.7 MBq, 10 µg) or ^{64}Cu-DOTA-Dara (3.7 MBq, 10 µg) + 500 µg of unlabeled Dara (1:50). A total of 13 tumor bearing mice (11 engrafted with MM.1S and 2 engrafted with U266), plus 2 non engrafted mice were used for imaging studies. Static scans were acquired at 0 (10 min scan), 1 (40 min scan), and 2 (60 min scan, with CT) days post-injection using the InVeon PET/CT (Siemens). For biodistribution studies, mice were euthanized at 24hrs, which was considered the best time point for imaging. Various organs were obtained from 4 tumor bearing mice (MM.1S), 4 tumor free mice, and 3 mice bearing CD38+ tumors (MM.1S) but injected with ^{64}Cu-Trast-DOTA. Organs were harvested and wet-weighed, and gamma counting was performed to determine their radioactive content using a WIZARD2 automatic gamma counter (PerkinElmer). Biodistribution studies are presented as a percentage of the injected dose per gram (% ID/g). Additionally, one group of U266 engrafted mice (n = 2) were injected with ^{64}Cu-

DOTA-Dara (3.7 MBq, 10 µg), and 1 group (n=2) of MM.1S engrafted mice were injected with ^{64}Cu-Trast-DOTA (3.7 MBq, 10 µg) and 500 µg unlabeled Dara (1:50), to further map the distribution of nonspecific binding.

^{18}F-FDG PET/CT scans

^{18}F-FDG was supplied by Cardinal Health**.** An InVeon microPET/CT (Siemens Medical Solutions USA, Inc.) was used for imaging. Mice were fasted overnight before the tracer injection. After a tail vein injection of 3.7MBq ^{18}F-FDG in 200 µL of PBS, a 10-min prone PET/CT acquisition scan was performed approximately 60 min after injection. Mice were maintained under isoflurane anesthesia during the injection, accumulation, and scanning periods. Mice were kept warm using a heating pad or heat lamp while under anesthesia to maintain a body temperature of around 38°C. microPET images were reconstructed with the ordered-subsets expectation-maximization algorithm using Fourier Rebinning and 4 iterations with attenuation-correction. The CT acquisition was a full rotation with 120 projections on low magnification with 4x4 binning, voltage 80kV and current 500µA. The reconstruction was done with no downsampling using the Feldkamp algorithm with slight noise reduction, Shepp-Logan filter, and HU calibration.

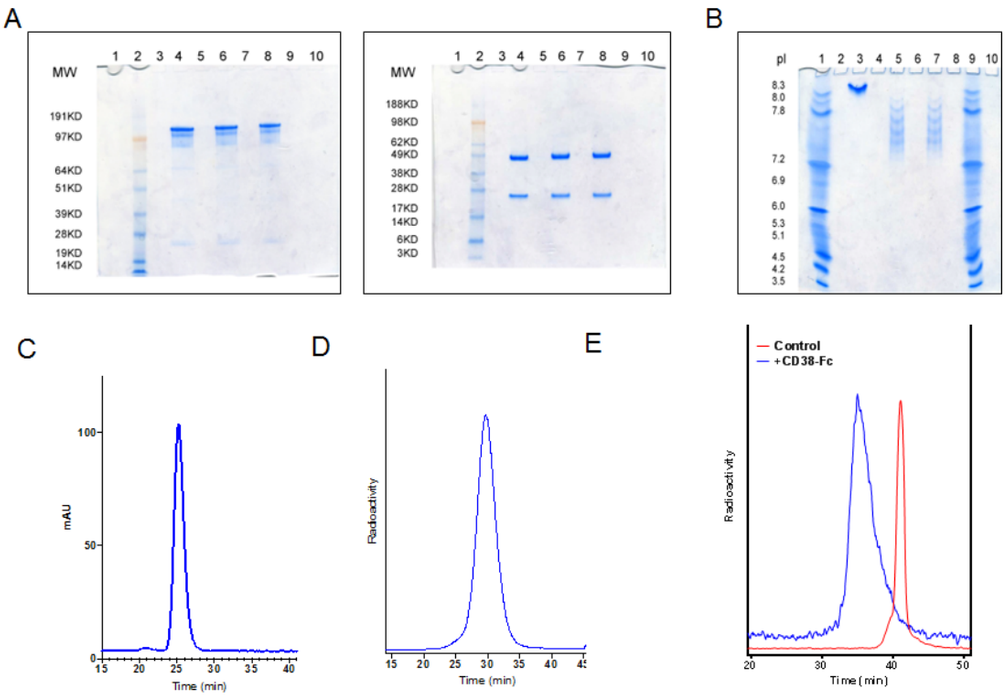
Histology and IHC studies

For pathology studies, mice bearing MM at the very early stage of the disease (10 days after MM cell injection) were assessed by BLI and were intravenously injected the next day with ^{64}Cu-DOTA-Dara (50 µCi, 10 µg) as described above. After imaging, the mice were euthanized, and the bones including tibias and femoral bones were fixed in 10% (v/v) buffered formalin (48 to 72 h at 4°C), followed by 2-3 days of decalcification using a modification of Kristensen's decalcification solution (2 parts 8N formic acid to 8 parts 1N sodium formate). Serial, 5 µm-thick, longitudinal sections were prepared and stained with hematoxylin and eosin (H&E) or with anti-human CD138 antibody. Formalin-fixed, paraffin embedded tissue sections were first deparaffinized using xylene and then stained with the Leica Biosystem anti-CD138 Cat. #PA 0088 (Ready to use antibody). IHC stain was performed on Leica Bond III using Bond Polymer Refine Detection Kit. Analysis was performed on a Zeiss Axio Imager. M2 microscope and photomicrographs were taken using an Axiocam 105 color digital camera and ZEN 2012 (blue edition) software.

Statistical Methods

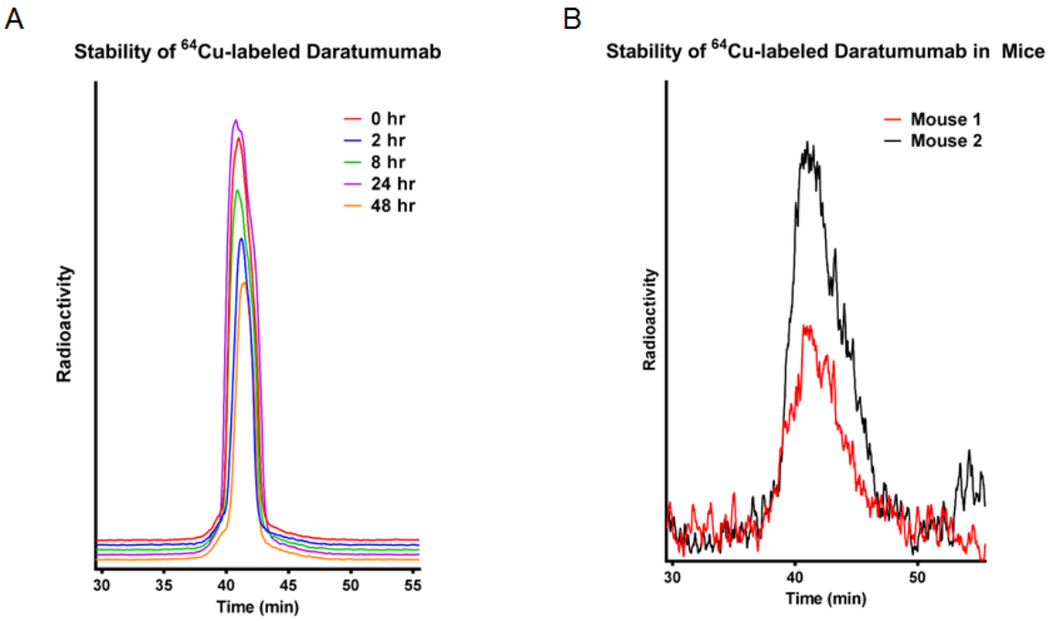
Biodistribution data were analyzed using GraphPad Prism 7 software. Differences between ^{64}Cu-DOTA-Dara injected control free mice (4), MM.1S engrafted mice (4) and ^{64}Cu-DOTA-Trast injected MM.1S engrafted mice (3) were analyzed by the One-way ANOVA test with Tukey's Multiple Comparison post-test: *$p<0.05$, **$p<0.01$, and ***$p<0.001$ values were considered as significant.

SUPPLEMENTARY FIGURES AND LEGENDS



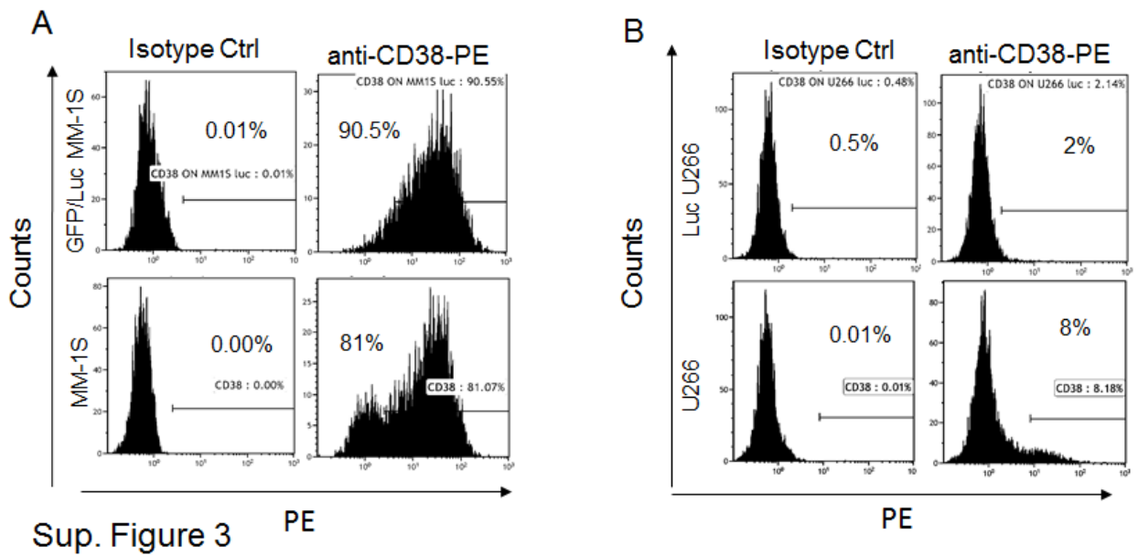
Sup. Figure 1

Figure 1. ^{64}Cu-DOTA-Dara conjugation. A) SDS-PAGE. The Dara pre-conjugation, DOTA-Dara post-conjugation and DOTA-Dara mAb vialed product were analyzed by SDS-PAGE under non-reducing (left) and reducing (right) conditions. The samples are the following: lane 2, Bio-Rad See Blue Plus 2 standards; lane 4, Dara; lane 6, DOTA-Dara post-conjugation and lane 8 DOTA-Dara vialed product. **B)** Isoelectric Focusing: The samples were analyzed on an isoelectric focusing (IEF) gel: lane 1, Invitrogen IEF standards; lane 3, Dara; lane 5, DOTA-Dara post-conjugation; lane 7 DOTA-Dara vialed product and lane 9, Invitrogen IEF standards. The Dara pre-conjugation had a band at the top of the gel with an isoelectric point (pI) of > 8.5 (lane 3). After conjugation, the isoelectric point (pI) of DOTA-Dara (lane 5 and 7) shifted to a more acidic family of bands with a pI range of ~7.8-7.2; **C)** DOTA-Dara analysis by size exclusion chromatography (SEC). Representative SEC chromatogram showing the DOTA-Dara final product as a single peak, corresponding to the expected molecular weight of ~150,000 Daltons, with a purity of 99.1% as determined by $Absorbance_{280}$ peak area; **D)** ^{64}Cu-DOTA-Dara analysis by SEC. Radiochromatogram of the purified ^{64}Cu-DOTA-Dara after ^{64}Cu radiolabeling. The radioactive trace (blue) shows the DOTA-Dara labeled efficiently with Cu-64, with no evidence of aggregates and approximately <1% unincorporated ^{64}Cu CI; **E**) ^{64}Cu-DOTA-Dara immunoreactivity. The purified ^{64}Cu-DOTA-Dara was incubated with soluble CD38-Fc antigen and analyzed by SEC. The ^{64}Cu-DOTA-Dara radioactive trace (red) shows a radioactive molecular size shift (blue) after incubation, demonstrating the ability of the radiolabeled ^{64}Cu-DOTA-Dara to bind to unlabeled CD38-Fc antigen to the extent of >95%.



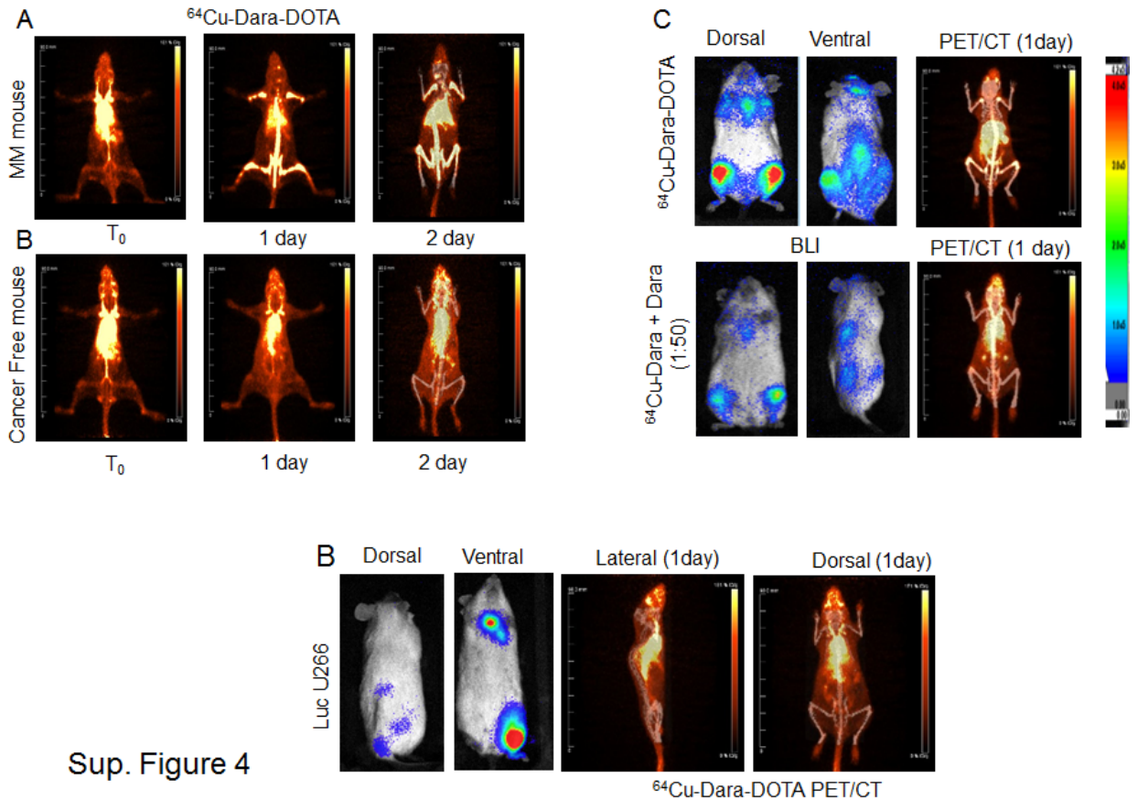
Sup. Figure 2

Figure 2. ^{64}Cu-DOTA-Dara stability in *vitro* and in *vivo*. A) Radiolabeled ^{64}Cu-DOTA-Dara was maintained in saline solution (PBS1X) up to 48hrs (^{64}Cu, half-life) and tested for stability by size exclusion chromatography (SEC) at the different time points as indicated; B) Radiolabeled ^{64}Cu-DOTA-Dara was also injected in two tumor free mice, and after 48hrs the circulating radiolabeled antibody was assessed for stability. In both mice, intact ^{64}Cu-DOTA-Dara was observed.



Sup. Figure 3

Figure 3. CD38 surface expression in MM cell lines. A-B) Flow cytometry analysis showing CD38 expression on the surface of GFP/Luc MM.1S and Luc U266 cells, compared to the parental cell lines.



Sup. Figure 4

Figure 4. ^{64}Cu-DOTA-Dara specifically recognize MM cancer cells. ^{64}Cu-DOTA-Dara images at time zero (T_0), 1 and 2 days after ^{64}Cu-DOTA-Dara injection in a mouse bearing CD38+ MM cells (A), and in a cancer free mouse (B); B-C) Imaging data at 1 day after ^{64}Cu-DOTA-Dara injection show lack of bone signaling when radiolabeled Dara was injected in mice bearing CD38 negative tumors (U266) or in the presence of excess unlabeled Dara (1:50).

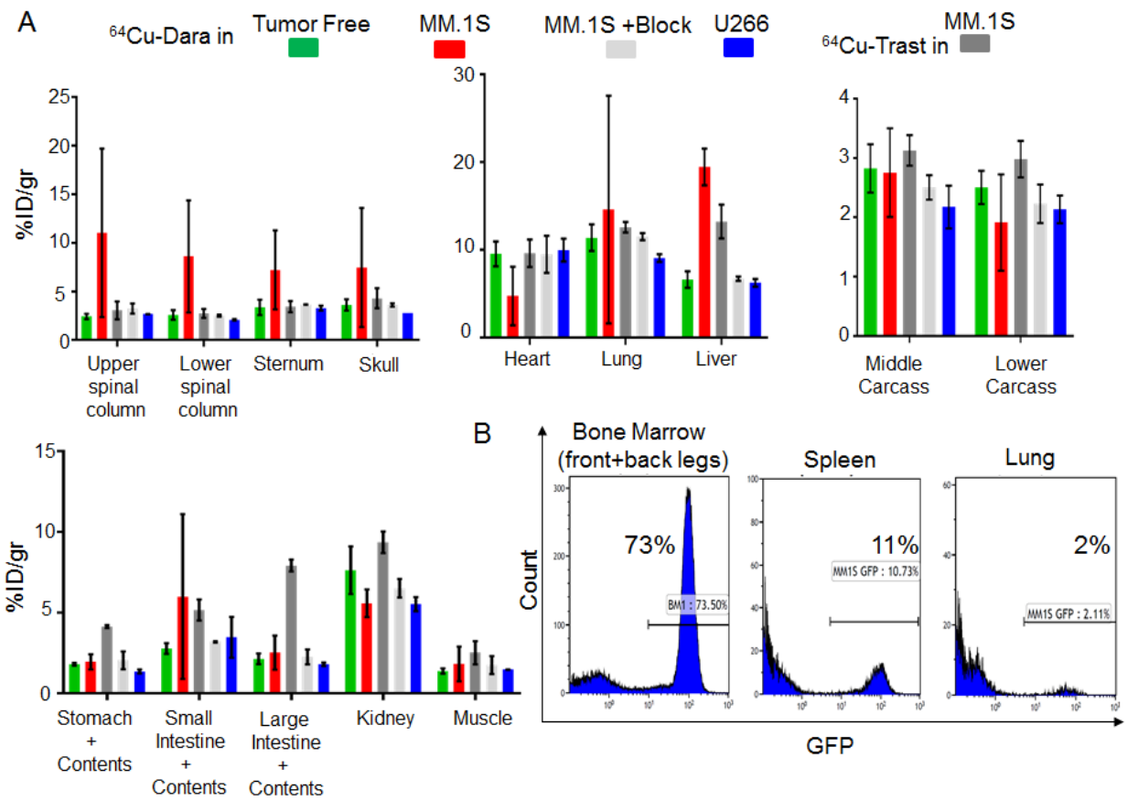


Figure 5. ^{64}Cu-DOTA-Dara bio-distribution studies. A) ^{64}Cu-DOTA-Dara PET/CT positive mice (MM.1S Gfp+/Luc+ engrafted mice [4]) and negative mice (tumor free mice [4]); ^{64}Cu-DOTA-Trast treated mice (3); unlabeled Dara (Block) treated mice (2) and U266 engrafted mice (2) were sacrificed, and organs were harvested at 24 hrs and used for gamma counting to determine their radioactive content as presented in the bar graph as a percentage of the injected dose per gram (% ID/g); B) An Independent group of mice (n=3) engrafted with MM.1S Gfp+/Luc+ were sacrificed and presence of MM.1S cells in each organ was analyzed by flow cytometry analysis for Gfp positive signal. MM cell engraftment was mainly identified in the bone marrow, spleen. Presence of MM cells in the lungs was detected in highly engrafted mice at the terminal disease stage.