EXPERIMENTAL PROCEDURES:

Cell Culture

SKOV3 cells were purchased from the American Type Cell Culture (ATCC) in 2018. The cells were cultured in RPMI McCoy's 5A Medium, modified to contain 1.5 mM L-glutamine, 100 units/mL penicillin G and 100 μg/mL streptomycin and 10% fetal bovine serum. The cells were maintained at 37 °C in water-jacketed incubators and supplied with 5% CO_2. The cell lines were sub-cultured by splitting a T-150 flask (1:5) once per week using 0.25% trypsin/0.53 mM EDTA in Hank's Buffered Salt Solution without calcium and magnesium. In addition to routine testing for mycoplasma, SKOV3 cells were authenticated by STR profiling.

Construction and Expression of Different IgG Variants of the L1CAM-Targeting huE71 Antibody

Anti-human L1CAM monoclonal antibody E71 was developed in Dr Nai-Kong Cheung's lab in MSK and was humanized as detailed in patent application WO2018232188A1. Based on human homologs, CDR sequences of both heavy and light chains of E71 were grafted onto the human IgG1 framework. The combination of H1 and L1 sequences were chosen for production of the version of huE71 used in the current study. The huE71 genes (either wildtype or Fc-modified) were synthesized (Genscript, Piscataway, NY) and incorporated into a mammalian expression vector (Eureka, CA), and transfected into CHO-S cells for stable production. Similarly, human V_H and V_L sequences were fused to human IgG4 constant region (either wildtype or Fc-modified) to produce the IgG4 variants. Lastly, huE71-IgG1 genes were transfected into $GnT1^{-/-}$ CHO cells to produce afucosylated huE71-1 IgG1.

HuE71 producer lines were cultured in OptiCHO serum free medium (Invitrogen, Carlsbad, CA) or PowerCHO-2 (Lonza, Basel, Switzerland) and the mature supernatant was harvested.

Protein A affinity column was pre-equilibrated with 25 mM sodium citrate buffer with 0.15 M NaCl, pH 8.2. Bound huE71 antibody variants were eluted with 0.1 M citric acid/sodium citrate buffer, pH 3.9 and alkalinized (1:10 v/v ratio) in 25 mM sodium citrate, pH 8.5. The resulting eluant was passed through a Sartobind-Q membrane and concentrated to 5-10 mg/mL in 25 mM sodium citrate, 0.15 M NaCl, pH 8.2, and frozen in aliquots at -80°C.

Size Exclusion-High Performance Liquid Chromatography (SE-HPLC)

The purity of the antibodies was evaluated by size-exclusion high-performance liquid chromatography (SEC-HPLC). Sample purity and protein aggregation was analyzed using a TOSOH TSK-GEL G3000SWXL (7.8mm ID × 30cm) stainless column and TOSOH TSK-GEL SWXL (6.0mm ID × 4.0cm) guard column on a HPLC system (Shimadzu Scientific Instruments, Inc.). The eluant used was 0.4 M $NaClO_4$, 0.05 M $NaHPO_4$, pH 6.0. The flow rate was set at 0.5 ml/min and the run time was 40 min. Samples were passed through a 0.22μM filter (Millipore) prior to loading. The area under the curve by OD_{280} was calculated for each protein peak in the HPLC chromatogram. The monomer peak consisted of proteins with MW of 150 kD. The salt peak was excluded from the AUC analysis.

Surface Plasmon Resonance

An SPR assay was set up on the Biacore T200 instrument (GE Healthcare) to determine the Fc-FcγR binding affinities (K_D) for the three L1CAM-binding IgG1 variants. To this end, recombinant mouse FcγRIV (1974-CD-050; R&D Systems) and human FcγRIII-158V isoform were employed as the ligand(s) whilst the L1CAM IgG1 variants were used as the analyte in the assay. The ligands were immobilized on a series S sensor chip CM5 (29401988; GE Healthcare) using an amine coupling kit (BR-1000-50; GE Healthcare) according to the standard procedure prescribed by the application wizard on the Biacore T200. High performance injections of the three

L1CAM IgG1 variants (42–667 nmol/L) were analyzed under the following conditions – 1 minute for association, 3 minutes for dissociation – both performed at a flow rate of 30 μL/min. At the end of each cycle, the chip surface was regenerated using a 60s injection of 10 mM NaOH at a flow rate of 50 μL/min. The kinetic data was analyzed using the Biacore T200 evaluation software. The data was fitted using a two-state reaction model: $A+B = AB = AB^*$, $K_D = (k_{d1}/k_{a1})/(1 + k_{a2}/k_{d2})$.

Antibody Conjugation

The various antibodies used in this study were buffer-exchanged from a solution of 25 mM sodium citrate and 150 mM sodium chloride to chelex-treated phosphate-buffered saline (PBS) by using separate PD-10 desalting columns (17085101, Cytiva Life Sciences) for each variant. The PD-10 columns were pre-equilibrated with chelex-treated PBS pH 7.2 (dead volume of 2.5 mL, elution volume of 2mL). Thereafter, the buffer-exchanged antibodies were concentrated to 2.5 – 4.0 mg/mL using Ultra-2 Amicon 50 kDa molecular weight cutoff (MWCO) filtration spin columns (UFC205024, Millipore). Thereafter, subject to the inventory available for each antibody, 1.2 – 3 mg (at concentrations > 2 mg/mL) of the antibody was aliquoted, and the pH of the antibody solution was adjusted to 8.7-9.0 using 1 M sodium carbonate (Na_2CO_3) prior to the gradual drop-wise addition of 10 molar equivalents of isothiocynato-desferrioxamine (*p*-SCN-Phe-DFO) (B-705; Macrocyclics, Inc.) dissolved in DMSO (41640; Sigma Aldrich) at a concentration of 10 mg/mL in DMSO. Care was taken to ensure that the volume of DMSO in the conjugation reaction mixture was <2% (v/v). The reaction mixture was incubated at 37 degrees Celsius (ºC) for 1 h with constant shaking at 500 rpm in a thermomixer. Thereafter, the antibody-DFO conjugates were purified from excess unconjugated DFO using separate PD-10 desalting columns (17085101, Cytiva Life Sciences) for each variant. The antibody-DFO conjugates collected in the 2 mL elution

fraction were subsequently concentrated to 3-5 mg/mL using Ultra-2 Amicon 50 kDa molecular weight cutoff (MWCO) filtration spin columns (UFC205024, Millipore).

^{89}Zr-radiolabeling

High specific activity zirconium-89 (^{89}Zr-oxalate) was procured from 3D Imaging (Little Rock, AR, USA). ^{89}Zr[Zr]-oxalate was neutralized using 1M sodium carbonate and ^{89}Zr-radioimmunoconjugates were prepared by mixing 44.4 MBq; 1.2 mCi of pH-adjusted ^{89}Zr[Zr]$^{4+}$ with 200 μg of each of the DFO-conjugated antibodies 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 to achieve a radioactivity concentration of 3.6 μCi/μL. The reaction progress was assayed via radio-thin layer chromatography (radio-TLC) on an AR-2000 Bioscan using silica-impregnated paper (ITLC-SG, Varian) with an eluent of 50 mM EDTA; pH 5. After 1 h, the radiolabeling reaction was quenched by adding 1/10 (v/v) 10 mM EDTA. Finally, the radioimmunoconjugates were purified promptly 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 the radioimmunoconjugate preparation was assayed by radio-instant thin layer chromatography (radio-ITLC).

Radioimmunoconjugate Stability

The purified radioimmunoconjugates were tested for antibody stability and demetallation of ^{89}Zr^{4+} by incubating them in human AB-type serum for 6 days at 37 °C and the radiochemical purity was assayed via radio-TLC. Aliquots of each ^{89}Zr-antibody complex (100 μL) were incubated with 900 μL of human AB type serum and agitated constantly on a thermomixer at 37 ºC. Samples were taken from each microcentrifuge tube and analyzed using radio-ITLC at day 0, 1, 3, 5, and 7 in triplicate. The stability of the complexes was measured as the percentage of

radioactivity that was retained at the origin of the radio-ITLC strip. This percentage was reported as % intact.

To investigate the integrity and stability of the various radioimmunoconjugates, SE-HPLC analysis was performed between incubation of the radioimmunoconjugate samples in chelexed PBS (no radioprotectant) and maintained at 37 °C with constant shaking at 400 rpm up to 6 days post-radiosynthesis. For SE-HPLC, a Phenomenex Yarra 3 μm SEC-3000 (300 x 7.8 mm) column was used on an HPLC system (Shimadzu Scientific Instruments, Inc.). The mobile phase used was an aqueous solution containing 100 mM sodium citrate tribasic dihydrate, 100 mM sodium chloride, pH 6.4. The flow rate was set to 1 mL/min and the run time was 20 minutes. Peaks on the 280 nm chromatogram were integrated using LabSolutions Postrun software (Shimadzu Scientific Instruments, Inc.).

Immunoreactivity

The immunoreactive fraction of the ^{89}Zr-DFO-antibodies was determined using a cell binding assay following procedures derived from Lindmo *et al* (*1*).To this end, SKOV3 cells were suspended in microcentrifuge tubes at concentrations ranging from 5.0 x 10^5 – 5.0 x 10^6 cells/mL in 500 μL PBS, 1% BSA (pH 7.4). Aliquots of ^{89}Zr-DFO-antibody (50 μL of 1 μCi/mL stock) were added to each tube to a final volume of 500 μL. The samples were incubated for 60 min on a thermomixer set to 37 °C and 500 rpm. The treated cells were pelleted by centrifugation (1400 rpm for 4 min), the supernatant was aspirated out and the pellet was washed three times with ice-cold PBS before removing the supernatant and counting the radioactivity associated with the cell pellets. The activity data were background-corrected and compared with the total number of counts

in appropriate control samples. Immunoreactive fractions were determined by linear regression analysis of total/bound radioactivity plotted against the inverse of normalized cell concentration.

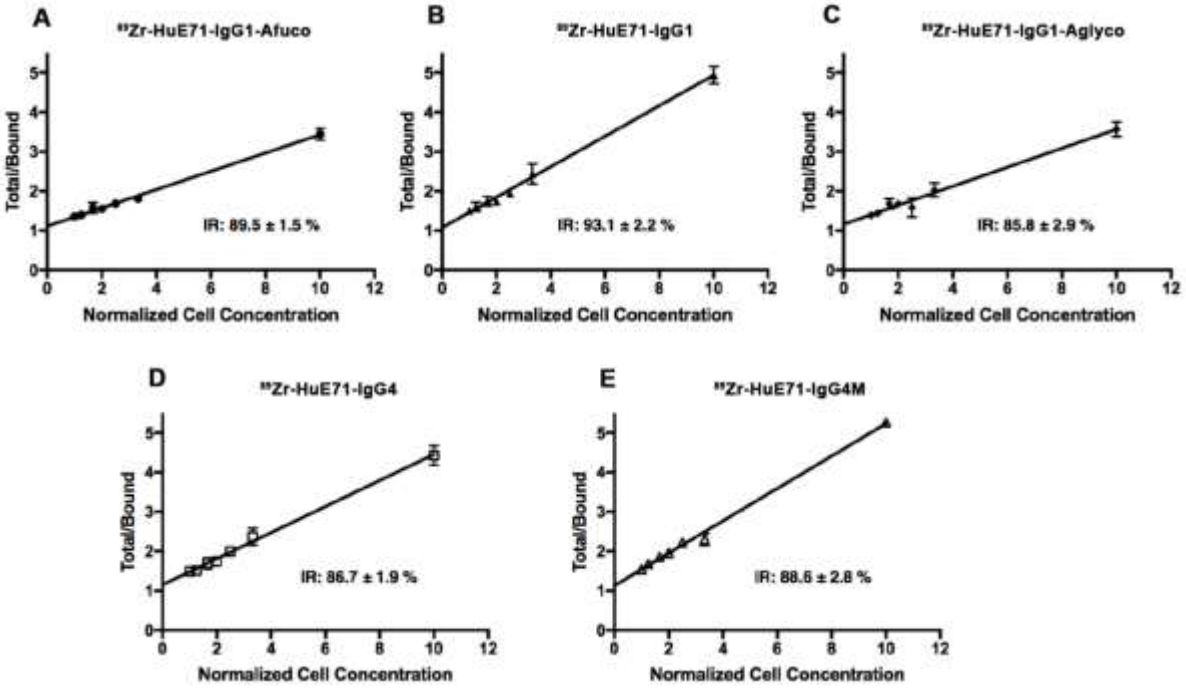
PET-CT Imaging

An energy window of 350-700 keV and a coincidence timing window of 6 ns were used for PET imaging. Acquired data was sorted into 2D histograms by Fourier rebinning, and transverse images were reconstructed by filtered back-projection (FBP) into a 128 × 128 × 63 (0.72 × 0.72 × 1.3 mm^3) matrix. The counting rates in the reconstructed images were converted to activity concentrations (percentage injected dose per gram of tissue, %ID/g) by use of a system calibration factor derived from the imaging of a mouse-sized water-equivalent phantom containing [^{89}Zr]Zr.

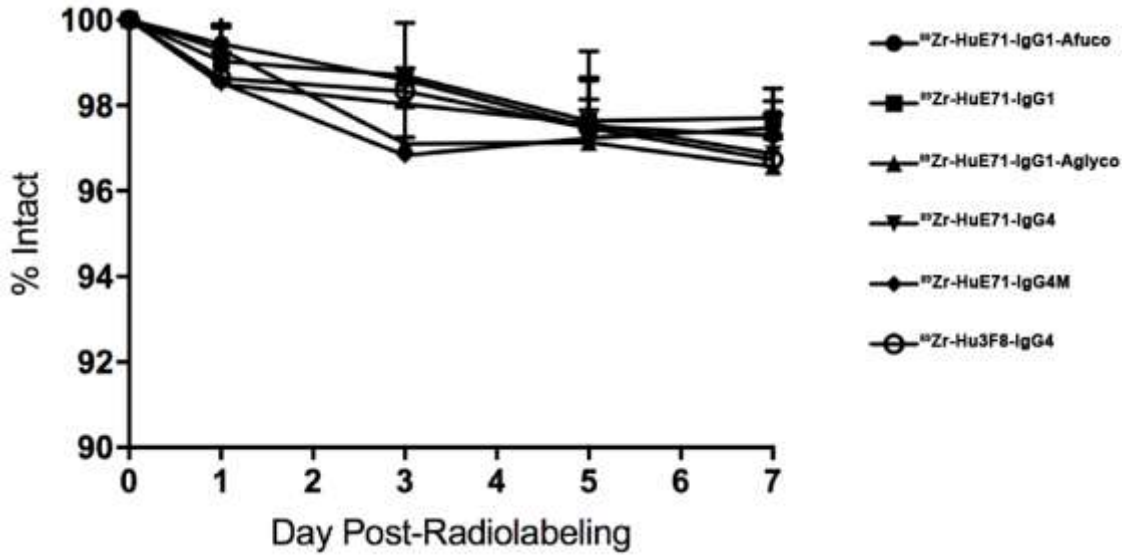
Histopathology

Tissue samples were fixed in 10% neutral buffered formalin and processed for histology. Paraffin embedded blocks were sliced to obtain 5μm sections and slides were stained with hematoxylin and eosin. Histopathologic analysis was performed in a blinded manner by a board-certified veterinary pathologist (AP).

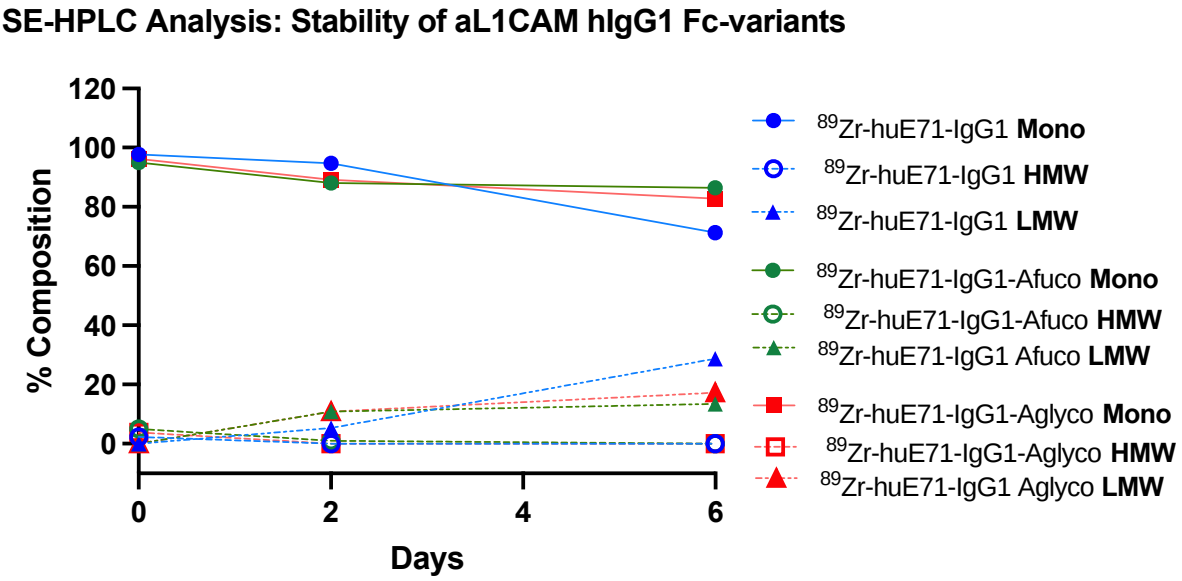
SUPPLEMENTARY FIGURES



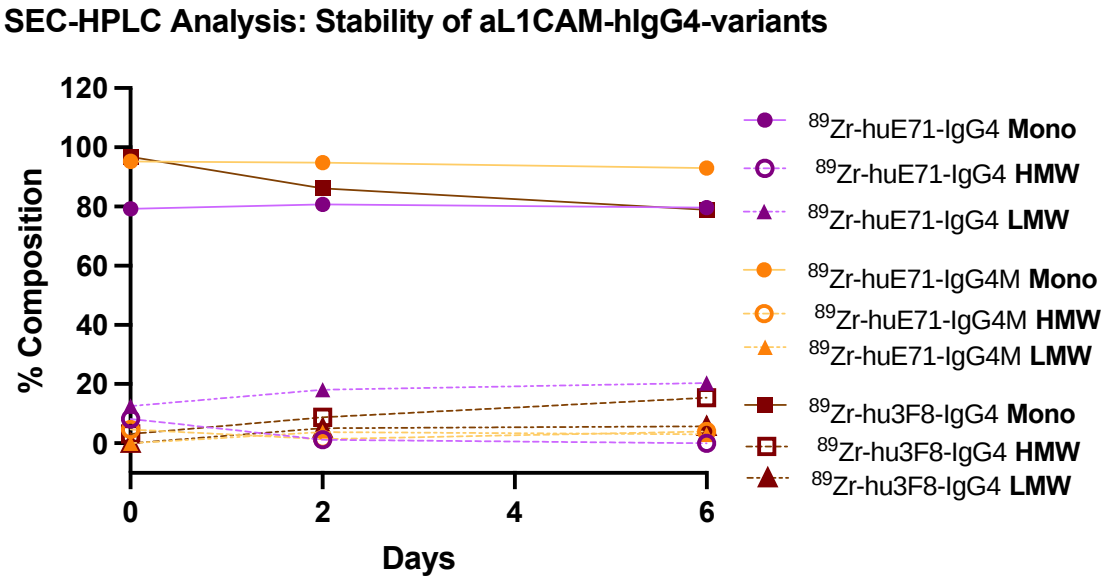
Supplemental Figure 1. Comparable immunoreactive fractions of the ^{89}Zr-labeled L1CAM IgG1 Fc-modified antibodies and IgG4 hinge variants.



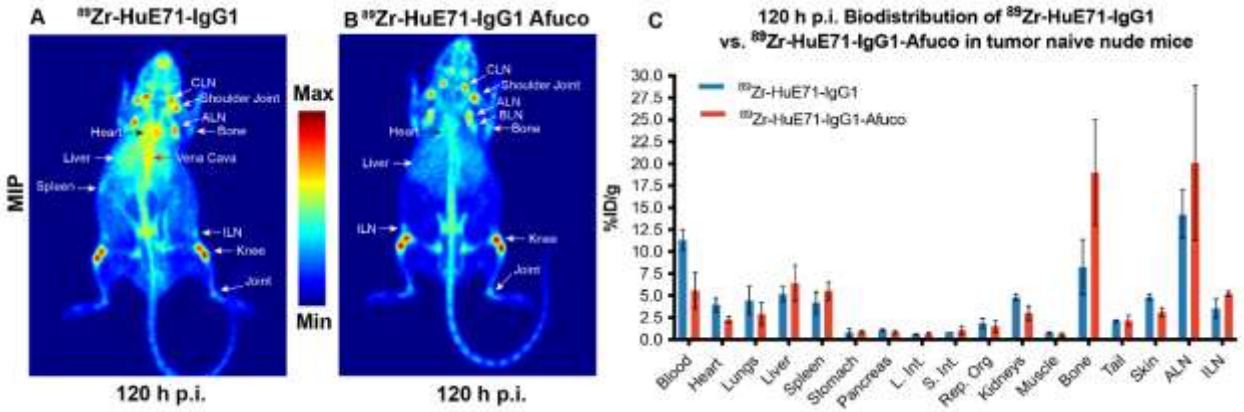
Supplemental Figure 2. Comparable in vitro serum stability of ^{89}Zr-labeled L1CAM antibody variants and isotype control ^{89}Zr-hu3F8-IgG4.



Supplemental Figure 3. SE-HPLC analysis demonstrating stability and integrity of the various anti-L1CAM ^{89}Zr-labeled Fc-modified IgG variants. Upwards of 80% of the IgG1 Fc-modified radioimmunoconjugates were found to be monomeric out to 6 days post-radiosynthesis. A gradual increase in two low molecular weight peaks was identified on the SE-HPLC chromatogram between day 2-6 of incubation in PBS without the presence of a radioprotectant in the solution.



Supplemental Figure 4. SE-HPLC analysis demonstrating stability and integrity of the various anti-L1CAM ^{89}Zr-labeled IgG4 variants. Upwards of 75% of the IgG4 radioimmunoconjugates were found to be monomeric out to 6 days post-radiosynthesis. HuE71-IgG4 was found to have a relatively high percentage of low molecular weight (LMW) fraction from day 0. On the other hand, Hu3F8-IgG4 was found to have a relatively high percentage of high molecular weight (HMW) fraction in it. Nevertheless, the presence of LMW and HMW protein was <20%. The hinge mutated anti-L1CAM IgG4 Ab was found to have the best monomeric profile up to 6 days post-radiosynthesis.



Supplemental Figure 5. Immuno-PET imaging and ex vivo biodistribution analysis of L1CAM-targeted IgG1 variants in tumor naïve athymic nude (Nu/Nu) mice. PET-CT images acquired at 120 hours after intravenous injection of 1.8 mg/kg; 7.95 MBq; 45 µg of (**A**) ^{89}Zr-HuE71-1 – the wildtype IgG1 antibody – showing persistence of radioactivity in the heart, vena cava and jugular veins. High radioactivity concentrations were seen in the liver, joints of the long bones, pelvic bones and vertebrae. Uptake of radioactivity was also seen in the axillary, cervical and inguinal lymph nodes (ALN, CLN and ILN) – most likely owing to the high proportion of ^{89}Zr-HuE71-IgG1 remaining in systemic circulation due to the lack of a target sink (L1CAM-expressing tumor); (**B**) ^{89}Zr-HuE71-IgG1-Afuco – the ADCC-enhanced IgG1 variant – showing relatively less radioactivity persisting in systemic circulation (heart, vena cava and jugular veins). Relatively

low radioactivity concentrations are apparent in the liver, however, the joints of the long bones, pelvic bones and vertebrae showed high uptake of radioactivity. Radioactivity was also seen in the axillary, cervical and inguinal lymph nodes (ALN, CLN and ILN) – plausibly owing to a combination of ^{89}Zr-HuE71-IgG1-Afuco remaining in systemic circulation as well as Fc-FcγR interactions for this IgG1 variant; (**C**) Ex vivo biodistribution profile at 120 h after intravenous injection of 0.25 mg/kg (1.15 MBq; 6.4 µg) of the two ^{89}Zr-labeled L1CAM-targeted IgG1 variants in tumor naïve nude mice. Comparable uptake was found in most tissues except lower radioactivity concentration was found persisting in the blood and heart of tumor-naïve mice injected with ^{89}Zr-HuE71-1 MAGE but slightly higher uptake of radioactivity in the bones was found for this variant.

Supplemental Table 1 Comparative ex vivo biodistribution data (radioactivity concentrations) for the various ^{89}Zr-labeled L1CAM-targeted IgG1 antibody variants dosed in SKOV3 xenografts. The values represent average ± standard deviation (n=3 animals per group).

Conjugate Tissues	^{89}Zr-HuE71-IgG1 (% ID/g)	^{89}Zr-HuE71-IgG1 Blocked (% ID/g)	^{89}Zr-HuE71-IgG1-Afuco (% ID/g)	^{89}Zr-HuE71-IgG1-Afuco Blocked (% ID/g)	^{89}Zr-HuE71-IgG1-Aglyco (% ID/g)	^{89}Zr-HuE71-IgG1-Aglyco Blocked (% ID/g)
Blood	5.2 ± 0.66	6.6 ± 0.66	0.8 ± 0.40	2.9 ± 0.14	3.7 ± 0.37	6.2 ± 0.07
Heart	2.0 ± 0.19	2.6 ± 0.53	0.7 ± 0.20	1.9 ± 0.20	1.7 ± 0.03	2.7 ± 0.21
Lungs	2.2 ± 0.05	2.9 ± 1.37	0.6 ± 0.20	1.4 ± 0.04	1.7 ± 0.14	2.9 ± 0.16
Liver	3.9 ± 1.78	3.8 ± 0.71	10.8 ± 2.10	7.2 ± 1.64	4.6 ± 1.42	4.4 ± 1.07
Spleen	3.5 ± 0.48	5.1 ± 0.25	3.5 ± 0.60	7.7 ± 0.60	3.3 ± 0.19	6.1 ± 1.48
Stomach	0.7 ± 0.14	0.8 ± 0.21	0.2 ± 0.10	0.2 ± 0.11	0.4 ± 0.08	0.5 ± 0.13
Pancreas	0.8 ± 0.21	0.8 ± 0.08	0.3 ± 0.10	0.4 ± 0.06	0.4 ± 0.09	0.6 ± 0.15
L. Intestine	0.7 ± 0.12	0.6 ± 0.23	0.2 ± 0.10	0.3 ± 0.08	0.4 ± 0.10	0.5 ± 0.04
S. Intestine	0.7 ± 0.06	0.9 ± 0.25	0.2 ± 0.10	0.4 ± 0.05	0.5 ± 0.13	0.7 ± 0.05
Rep. Organs	1.9 ± 0.53	2.0 ± 0.40	0.8 ± 0.20	1.0 ± 0.16	1.4 ± 0.66	1.9 ± 0.75
Kidneys	3.2 ± 0.44	3.3 ± 0.35	1.9 ± 0.60	2.6 ± 0.29	2.5 ± 0.28	3.3 ± 0.18
Muscle	0.5 ± 0.10	0.6 ± 0.04	0.3 ± 0.10	0.3 ± 0.07	0.4 ± 0.07	0.5 ± 0.08
Bone	8.3 ± 2.35	8.3 ± 1.81	6.6 ± 2.60	8.6 ± 1.66	3.7 ± 0.75	3.9 ± 1.65
Tail	1.3 ± 0.21	1.5 ± 0.33	1.0 ± 0.30	1.4 ± 0.44	0.7 ± 0.11	1.1 ± 0.26
Skin	2.8 ± 0.18	2.9 ± 0.64	1.9 ± 0.30	3.9 ± 1.23	2.0 ± 0.85	3.5 ± 0.23
ALN	14.1 ± 2.80	24.8 ± 6.36	25.5 ± 10.00	32.7 ± 2.95	6.1 ± 1.18	10.5 ± 1.89
ILN	3.2 ± 1.08	4.8 ± 0.81	4.2 ± 2.60	6.6 ± 1.39	2.4 ± 0.57	3.9 ± 1.33
Tumor	20.9 ± 2.22	6.8 ± 0.15	7.7 ± 2.00	3.0 ± 0.87	14.7 ± 4.53	4.9 ± 0.46

Supplemental Table 2. Comparative ex vivo biodistribution data (percentage of injected dose) for the various ^{89}Zr-labeled L1CAM-targeted IgG1 antibody variants dosed in SKOV3 xenografts. The values represent average ± standard deviation (n=3 animals per group).

Conjugate Tissues	^{89}Zr-HuE71-IgG1 (% ID	^{89}Zr-HuE71-IgG1 Blocked (% ID)	^{89}Zr-HuE71-IgG1-Afuco (% ID	^{89}Zr-HuE71-IgG1-Afuco Blocked (% ID)	^{89}Zr-HuE71-IgG1-Aglyco (% ID)	^{89}Zr-HuE71-IgG1-Aglyco Blocked (% ID)
Blood	4.8 ± 0.27	5.8 ± 0.65	0.7 ± 0.45	2.3 ± 0.52	3.9 ± 0.63	6.3 ± 0.03
Heart	0.3 ± 0.01	0.4 ± 0.06	0.1 ± 0.02	0.3 ± 0.04	0.3 ± 0.02	0.4 ± 0.11
Lungs	0.5 ± 0.05	0.5 ±0.03	0.2 ± 0.05	0.3 ± 0.01	0.4 ± 0.06	0.6 ± 0.19
Liver	4.8 ± 0.35	5.4 ± 0.37	17.6 ± 3.39	11.3 ± 0.82	7.8 ± 2.56	7.7 ± 1.75
Spleen	0.4 ± 0.03	0.6 ± 0.06	0.4 ± 0.08	0.6 ± 0.15	0.3 ± 0.07	0.6 ± 0.11
Stomach	0.2 ± 0.04	0.3 ± 0.03	0.1 ± 0.04	0.2 ± 0.04	0.2 ± 0.05	0.3 ± 0.03
Pancreas	0.2 ± 0.00	0.3 ± 0.01	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.03	0.2 ± 0.06
L. Intestine	1.0 ± 0.16	1.1 ± 0.07	0.4 ± 0.08	0.6 ± 0.10	0.9 ± 0.21	0.9 ± 0.01
S. Intestine	1.5 ± 0.21	1.8 ± 0.21	0.6 ± 0.13	0.9 ± 0.11	1.3 ± 0.10	1.7 ± 0.36
Rep. Organs	0.9 ± 0.11	0.6 ± 0.08	0.3 ± 0.05	0.3 ± 0.10	0.5 ± 0.29	0.6 ± 0.33
Kidneys	1.5 ± 0.07	1.5 ± 0.01	0.8 ± 0.23	1.0 ± 0.25	1.1 ± 0.06	1.5 ± 0.31
Muscle	0.1 ± 0.01	0.1 ± 0.01	0.04 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.02
Bone	0.3 ± 0.03	0.4 ± 1.05	0.2 ± 0.07	0.2 ± 0.07	0.2 ± 0.01	0.1 ± 0.02
Tail	0.9 ± 0.11	1.0 ± 0.29	0.6 ± 0.08	1.0 ± 0.31	0.6 ± 0.05	0.8 ± 0.26
Skin	0.1 ± 0.02	0.1 ± 0.05	0.1 ± 0.01	0.2 ± 0.05	0.1 ± 0.05	0.2 ± 0.03
ALN	0.7 ± 0.24	1.1 ± 0.03	1.3 ± 0.68	1.4 ± 0.29	0.4 ± 0.14	0.5 ± 0.13
ILN	0.1 ± 0.07	0.2 ± 0.05	0.2 ± 0.09	0.2 ± 0.07	0.1 ± 0.01	0.2 ± 0.05
Tumor	7.1 ± 1.52	1.7 ± 0.13	7.5 ± 2.47	2.4 ± 0.53	11.0 ± 2.90	4.5 ± 0.72

Supplemental Table 3. Comparative ex vivo biodistribution data (radioactivity concentrations) for the two ^{89}Zr-labeled L1CAM-targeted IgG4 antibody variants and the isotype control IgG4 antibody dosed in SKOV3 xenografts. The values represent average ± standard deviation (n=3 animals per group).

Conjugate Tissues	^{89}Zr-HuE71-IgG4 (% ID/g)	^{89}Zr-HuE71-IgG4 Blocked (% ID/g)	^{89}Zr-HuE71-IgG4M (% ID/g)	^{89}Zr-HuE71-IgG4M Blocked (% ID/g)	^{89}Zr-Hu3F8-IgG4 (% ID/g)
Blood	1.8 ± 1.33	3.5 ± 0.59	3.8 ± 1.05	8.1 ± 1.00	2.5 ± 0.42
Heart	1.1 ± 0.33	1.7 ± 0.24	1.9 ± 0.69	2.6 ± 0.39	1.3 ± 0.09
Lungs	1.3 ± 0.68	2.0 ± 0.40	2.1 ± 0.70	3.0 ± 0.76	1.3 ± 0.30
Liver	6.0 ± 2.70	11.1 ± 0.06	4.0 ± 0.07	5.0 ± 1.69	4.9 ± 0.37
Spleen	2.6 ± 0.85	3.9 ± 0.46	3.4 ± 0.74	5.7 ± 2.44	3.4 ± 0.47
Stomach	0.2 ± 0.08	0.5 ± 0.23	0.3 ± 0.07	0.4 ± 0.01	0.2 ± 0.06
Pancreas	0.3 ± 0.11	0.5 ± 0.01	0.5 ± 0.10	0.5 ± 0.04	0.4 ± 0.01
L. Intestine	0.4 ± 0.06	0.7 ± 0.36	0.5 ± 0.13	0.6 ± 0.22	0.5 ± 0.05
S. Intestine	0.3 ± 0.07	0.4 ± 0.09	0.4 ± 0.11	0.6 ± 0.07	0.4 ± 0.07
Rep. Organs	0.9 ± 0.27	3.0 ± 0.76	0.9 ± 0.50	2.3 ± 0.86	1.2 ± 0.69
Kidneys	7.4 ± 2.32	10.2 ± 4.61	2.5 ± 0.48	3.1 ± 0.23	15.2 ± 5.14
Muscle	0.3 ± 0.08	0.3 ± 0.11	0.4 ± 0.07	0.5 ± 0.14	0.3 ± 0.03
Bone	4.0 ± 0.74	3.5 ± 0.78	4.3 ± 1.30	4.2 ± 0.92	3.0 ± 0.53
Tail	0.7 ± 0.16	0.8 ± 0.14	1.2 ± 0.38	1.1 ± 0.16	0.8 ± 0.17
Skin	1.5 ± 0.42	3.0 ± 0.68	2.3 ± 0.61	3.1 ± 1.03	2.1 ± 0.28
ALN	10.6 ± 3.72	7.5 ± 1.06	13.4 ± 1.73	27.3 ± 3.95	12.2 ± 3.53
ILN	2.7 ± 0.78	2.5 ± 0.22	3.0 ± 0.23	6.7 ± 1.82	3.6 ± 0.83
Tumor	4.4 ± 0.84	2.6 ± 0.79	17.3 ± 4.21	5.5 ± 1.64	3.6 ± 1.76

Supplemental Table 4. Comparative ex vivo biodistribution data (percentage injected dose) for the two ^{89}Zr-labeled L1CAM-targeted IgG4 antibody variants and the isotype control IgG4 antibody dosed in SKOV3 xenografts. The values represent average ± standard deviation (n=3 animals per group).

Conjugate Tissues	^{89}Zr-HuE71-IgG4 (% ID/g)	^{89}Zr-HuE71-IgG4 Blocked (% ID/g)	^{89}Zr-HuE71-IgG4M (% ID/g)	^{89}Zr-HuE71-IgG4M Blocked (% ID/g)	^{89}Zr-Hu3F8-IgG4 (% ID/g)
Blood	1.6 ± 1.28	2.8 ± 0.86	3.7 ± 1.33	6.9 ± 1.19	2.0 ± 0.93
Heart	0.1 ± 0.06	0.2 ± 0.06	0.3 ± 0.12	0.3 ± 0.04	0.2 ± 0.03
Lungs	0.3 ± 0.27	0.5 ± 0.11	0.5 ± 0.21	0.7 ± 0.15	0.3 ± 0.12
Liver	9.3 ± 4.09	14.9 ± 1.61	6.4 ± 1.08	8.1 ± 3.27	8.4 ± 1.59
Spleen	0.3 ± 0.08	0.3 ± 0.02	0.3 ± 0.03	0.5 ± 0.12	0.5 ± 0.08
Stomach	0.2 ± 0.07	0.2 ± 0.04	0.2 ± 0.06	0.3 ± 0.05	0.3 ± 0.11
Pancreas	0.1 ± 0.02	0.1 ± 0.03	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.01
L. Intestine	0.7 ± 0.21	1.0 ± 0.23	0.8 ± 0.09	1.1 ± 0.33	0.9 ± 0.08
S. Intestine	0.7 ± 0.23	1.0 ± 0.21	1.0 ± 0.27	1.4 ± 0.30	1.0 ± 0.17
Rep. Organs	0.3 ± 0.06	0.7 ± 0.27	0.3 ± 0.17	0.5 ± 0.43	0.3 ± 0.17
Kidneys	3.0 ± 1.1	4.1 ± 2.13	1.1 ± 0.20	1.3 ± 0.25	6.4 ± 1.89
Muscle	0.04 ± 0.01	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.03	0.1 ± 0.01
Bone	0.2 ± 0.04	0.1 ± 0.02	0.2 ± 0.04	0.2 ± 0.02	0.1 ± 0.01
Tail	0.5 ± 0.13	0.5 ± 0.12	0.8 ± 0.25	0.7 ± 0.12	0.5 ± 0.07
Skin	0.1 ± 0.03	0.1 ± 0.03	0.1 ± 0.03	0.1 ± 0.03	0.1 ± 0.05
ALN	0.6 ± 0.26	0.3 ± 0.13	0.6 ± 0.18	1.2 ± 0.26	0.5 ± 0.3
ILN	0.1 ± 0.05	0.1 ± 0.002	0.2 ± 0.03	0.2 ± 0.05	0.1 ± 0.08
Tumor	5.4 ± 2.05	3.1 ± 0.84	12.4 ± 2.80	3.9 ± 1.18	3.4 ± 2.25

REFERENCES

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