Supplemental data for:

Pharmacokinetic analysis reveals limitations and opportunities for nanomedicine targeting of endothelial and extravascular compartments of tumors

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Supplemental Methods

Fitting of nanoparticle PK data into the model: We used data from a recent report by Goel *et al* (1) wherein 150-nm silica-shell nanoparticles modified with Zr-89 and endothelium-targeting ligand anti-CD105 ([^{89}Zr]bMSN-PEG5k-TRC105) were used for PET imaging of neovasculature in a 4T1 breast tumor allograft. The authors report percent-injected dose per gram tissue (%ID/g) in the tumor over time for targeted and non-targeted (control PEGylated) particles as well as elimination the half-life for targeted particles. The reported blood volume fraction for 4T1 tumors is ~9.5% (5% plasma volume fraction (2)). The blood half-life of non-targeted particles was assumed to be the same as for targeted particles.

Staining of tumor for VEGFR2 and endothelial volume fraction: Mouse studies were performed under the University of Colorado IACUC-approved protocol to DS according to the institutional guidelines for animal care. Female BALB/c mice (8 weeks old) were inoculated with 4T1 cells (0.5 million per mouse) into mammary fat pads. Tumors were harvested when they reached a size of 250-300mm^3 and then formalin fixed. Tumor histological sections were stained with anti-mouse VEGFR2 antibody (BioLegend, San Diego CA) and the corresponding secondary fluorescent antibody. The fluorescent images were taken at 100x magnification and analyzed with ImageJ for percent of area occupied by VEGFR2 positive endothelial cells (determined with color thresholding tool). It was assumed that area percentage scales in all 3 dimensions, so that 5% endothelial area means 5% endothelium volume fraction.

Supplemental Table 1
Tumor accumulation values (perfused values – blood pool subtracted) derived from Goel *et al* (1). The values were extracted from graphs using Plot Digitizer software. Left part, non-targeted silica particles; right part, CD105 targeted silica particles.

Time (Minutes)	%ID per g
30	1.6000
240	2.7000
1440	3.7000
2880	3.5000

Time (Minutes)	%ID per g
30	4.4000
240	11.3000
1440	11.5000
2880	11.3000

Supplemental figures

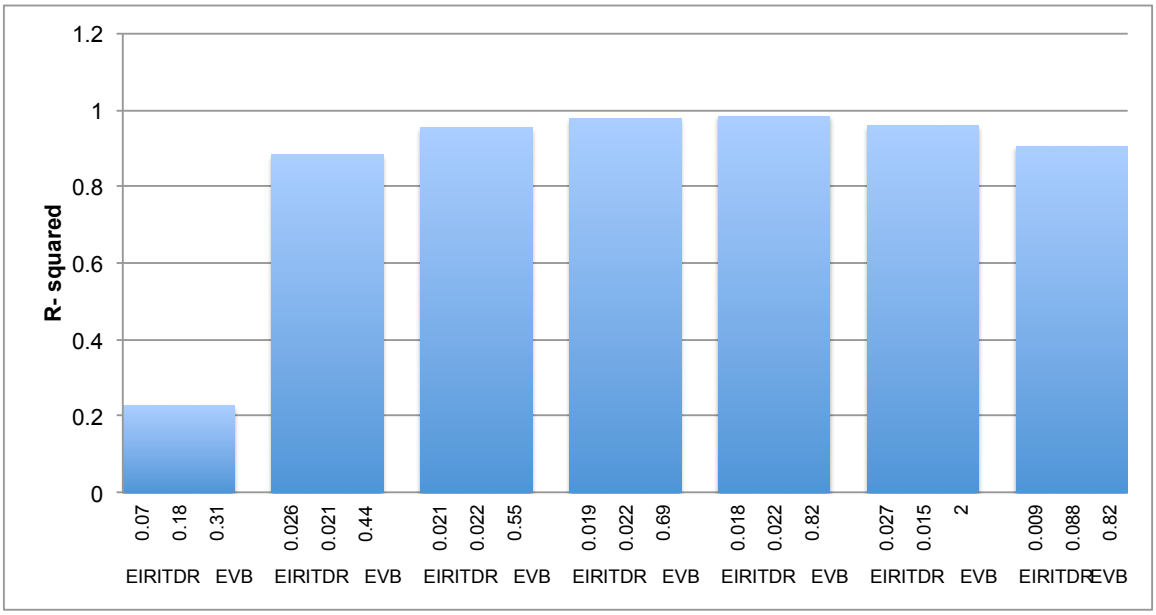

Fig. S1. Fitting of the data of non-targeted SWCNTs with the model.

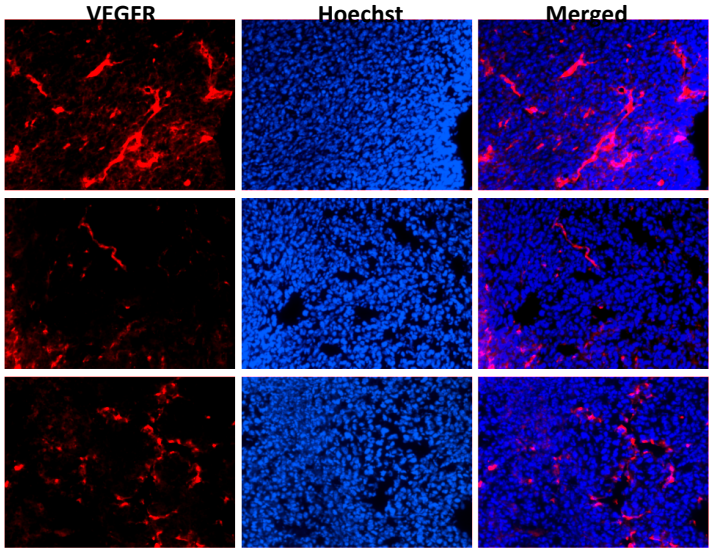

Fig. S2. Immunostaining of 4T1 tumors for neovasculature: Tumors were stained with anti-VEGFR2 antibody and the endothelial volume fraction was quantified.

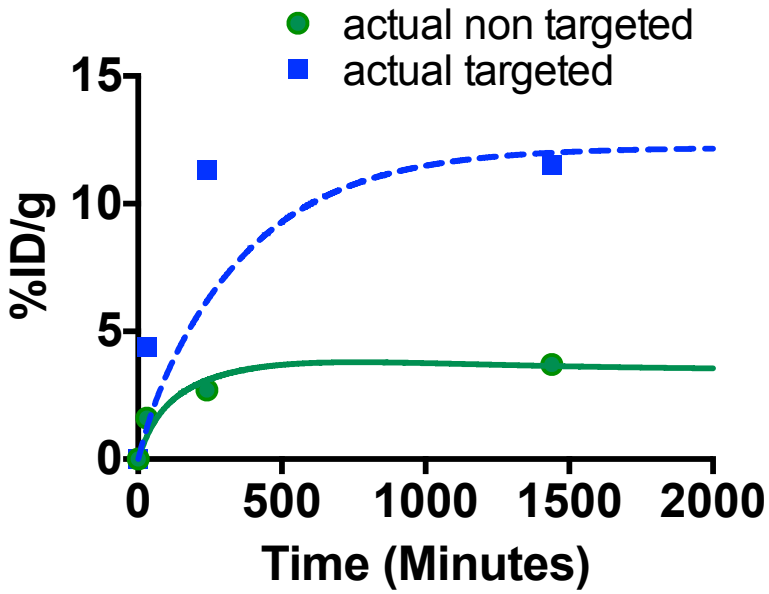

Fig. S3. Actual and fitted pharmacokinetic profiles in tumor based on data from Goel *et al* (1)**.** The fitting was performed as described in Fig. 2. Green trace, non-targeted particles; green circles, actual non-targeted data; blue trace, neovasculature-targeted particles; blue squares, actual targeted data. The curve fit for CD105-targeted silica particles was not as good as for targeted carbon nanotubes in Fig. 2D, possibly due to differences in EIR, ITDR, or EVB between targeted and non-targeted silica, which was not accounted for in the fitted data.

Supplemental references

1. Goel S, Chen F, Luan SJ, Valdovinos HF, Shi SX, Graves SA, et al. Engineering Intrinsically Zirconium-89 Radiolabeled Self-Destructing Mesoporous Silica Nanostructures for In Vivo Biodistribution and Tumor Targeting Studies. Adv Sci. 2016;3(11).
2. Proulx ST, Luciani P, Alitalo A, Mumprecht V, Christiansen AJ, Huggenberger R, et al. Non-invasive dynamic near-infrared imaging and quantification of vascular leakage in vivo. Angiogenesis. 2013;16(3):525-40.