Antibody labeling with ^{124}I and ^{131}I

Clinical PET-CT Studies: Antibody huA33 was labeled with ^{124}I for clinical PET-CT imaging. Briefly, 0.7 mL of huA33 (10mg/mL) was added to 500μL of 0.2 M phosphate buffer (pH 7.4) and 150-520 MBq (4-14 mCi) ^{124}I in 20-40 μL of 0.05M NaOH in an iodogen tube. The solution was left at ambient temperature for 10min then passed over an equilibrated 1 g AG1-X8 ion exchange column (BioRad Laboratories, Hercules, Ca) and eluted with 1% HSA/0.2 M phosphate buffer (pH 7.4). The purified material was sterile filtered with a 0.22 μm filter and made up to 10mg A33 prior to final formulation in 5% HSA/isotonic saline. The radiochemical purity of ^{124}I-huA33 was assessed using ITLC-SG strips (5 x 100 mm, Pall, East Hills, NY) with an eluant of 10% trichloroacetic acid.

Saturation Binding Studies: Antibody huA33 was labeled with ^{131}I for *ex-vivo* saturation binding studies. Briefly, 20μL of huA33 (10mg/mL) was added to 200μL of 50mM phosphate buffer (pH 7.4) and 45-55MBq (1.2-1.5mCi) ^{131}I in 20μL of 0.05M NaOH in an iodogen tube. The solution was left at ambient temperature for 10min then separated by size exclusion chromatography using a 10mL P6 column (BioRad Laboratories, Hercules, Ca) with an eluant of 1% BSA/PBS. The radiochemical purity of the ^{131}I-huA33 was assessed using ITLC-SG strips (10 x 100 mm, Pall, East Hills, NY) with an eluant of 10% trichloroacetic acid.

huA33 concentration in plasma, tumor and colon

In order to examine the projected kinetic behavior of huA33 in plasma, tumor and colon, a previously described (ref 27) compartmental model was simulated numerically. Parameter estimates were taken from the original report and uptake values in tumor and colon were normalized to correspond to 0.052 and 0.03%ID/ml respectively at 7 days (168 hr) post-

administration, i.e. the average DAR-derived uptake measurements for antigen-positive regions in the current study. The kinetic evolution of antibody concentration in the three compartments, based on this simulation, is shown in fig S1(A). This illustrates that the concentration of antibody in the colon is anticipated to decrease slowly for T >~60 hr. As the concentration of antigen in the colon is continually renewed and thus fixed, the ratio of bound antibody to antigen density will also decrease at the same rate.

Multi-step targeting

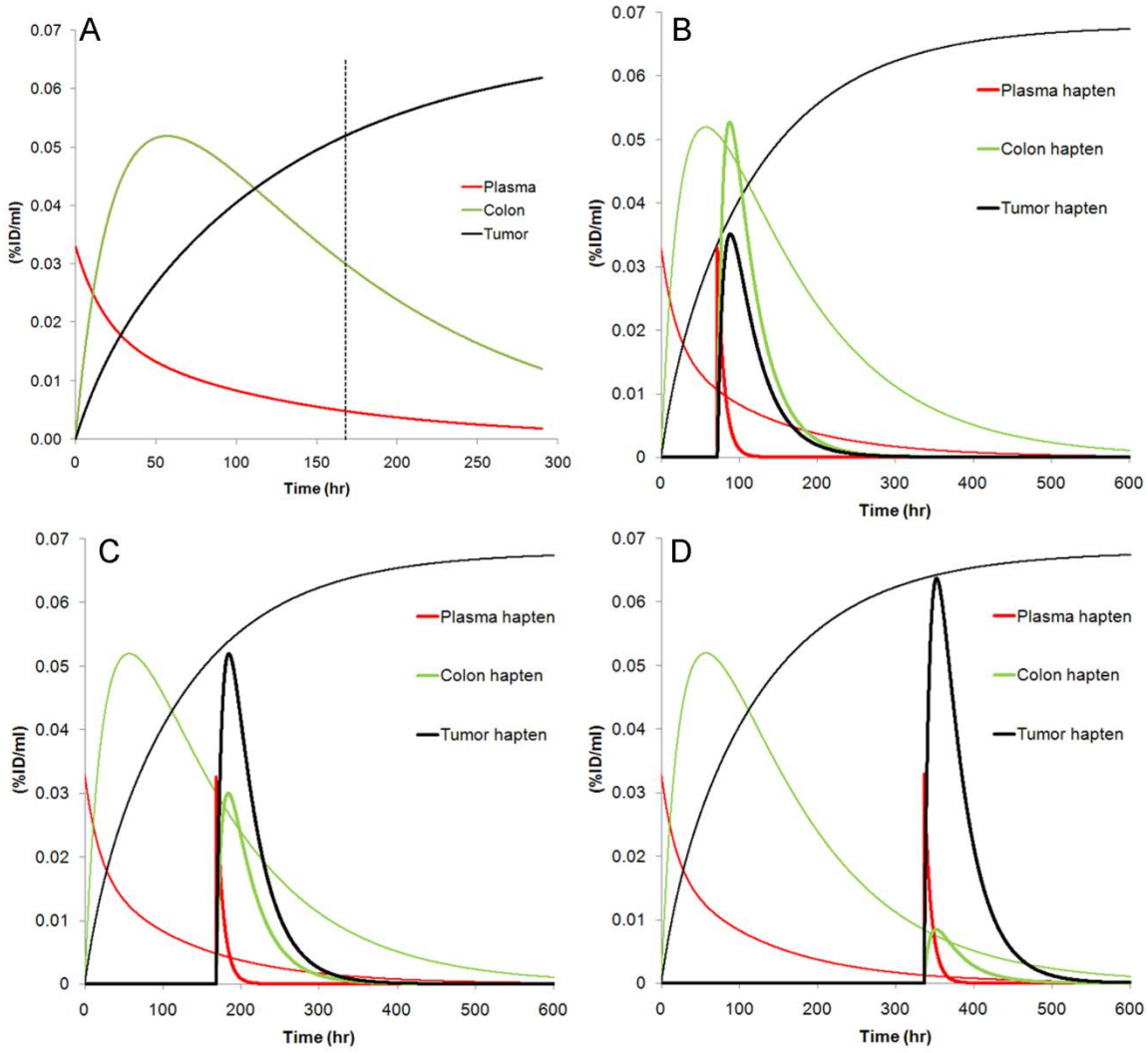
A similar approach was used to illustrate a hypothetical multi-step approach, where the initial administration of a huA33 antibody-based construct with additional specificity for some small molecule is followed after a delay by administration of the small molecule (denoted here as hapten). The following assumptions were made to facilitate the simulation:

1. The kinetics of the hypothetical construct are identical to those of huA33 IgG.
2. The plasma concentration of hapten may be represented by a mono-exponential function. For purposes of illustration a clearance half-time of 6 hours was used.
3. The rate of hapten-construct association in tumor and colon compartments is proportional to the compartmental concentration of construct and the plasma concentration of hapten. For purposes of illustration, half-times for hapten-construct association and dissociation of 3 and 24 hours were used for both tumor and colon.

Figure S1(B-D) shows the projected kinetic behavior of hapten concentration in plasma, tumor and colon for administration at three different times (3, 7 and 14 days) after construct administration. In broad terms hapten uptake in tumor and colon is related to the respective concentrations of construct at the time of hapten administration. At early times (exemplified by 3

days) colon uptake is greater than tumor, as the projected colon concentration of construct is greater at this time. In contrast at late times (exemplified by 14 days) tumor uptake is greater, as now the projected concentration of construct is greater in tumor. The ratio of radiation absorbed dose for a radiolabeled hapten is related to the respective ratio of areas under the hapten concentration-time curve. For any given radionuclide the kinetic curves would have to be adjusted to take into account the physical decay of the radionuclide and this is not considered here, however the principle remains the same. The numerical values of the ratio of tumor AUC to colon AUC are 0.68, 1.77 and 7.69 for hapten administration at 3, 7 and 14 days respectively. This illustrates that a multi-step approach based on the huA33 system has the potential to greatly improve the dosimetric differential between tumor and colon. It is also noteworthy that although the actual curves shown in the figure are dependent on the assigned numerical parameters, in qualitative terms the findings are independent of these values.

Not considered here is the possible impact of administering a clearing agent to rapidly remove construct from the circulation. This would have the effect of reducing or eliminating the instantaneous uptake of construct into tumor and colon, depending on the efficiency of the clearing agent. In the context of the compartmental model used here, after administration of clearing agent tumor concentration would be expected to remain approximately constant while colon concentration would decrease more rapidly at a rate determined by cellular turnover.



A: Projected compartmental kinetics of huA33 based on the model of Scott (27) and normalized to 0.052 and 0.03 %ID/ml for tumor and colon respectively at 168 hr (indicated by the vertical dashed line).

B-D: Projected compartmental kinetics of a hypothetical multi-step approach: a huA33 antibody-based construct (faint curves) with additional specificity for some hapten is followed after variable delays by hapten administration (bold curves).

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