Supplemental Data

Data Sets

The bio-distribution studies analyzed here were reported in reference (*6*) and are summarized below. Forty 11-week old female athymic mice were implanted subcutaneously with 5×10^6 A2058 human melanoma cells (ATCC, Manassas, VA) in each of two abdominal sites and used for experiments 14 days later. For pharmacokinetic experiments, 36 athymic mice bearing A2058 cell-derived melanoma tumors were given 30 μCi ^{188}Re-6D2 (total amount of 6D2 150 μg/100 μL) and sacrificed at 5 min, 2 h, 4, and 24 h; or 120 μCi ^{188}Re-6D2 (total amount of 6D2 150 μg/100 μL) and sacrificed at 48 hr. Blood clearance samples (2 μL) were collected at 5 min, 1 h, 2, 4, 24 and 48 h from the dorsal tail vein and counted in a sodium iodide gamma well counter (Packard, Downers Grove, IL). The concentration of radioactivity in each organ was expressed as %ID/g.

Data Analysis

We normalized the pharmacokinetics data by using the radioactivity concentrations at 5 minutes after administration $C_i(j, 5)$ as normalization factors. The variability of these concentrations was substantial, but not dramatic. For example, for blood the mean %ID/g at 5 minutes was 67.8, the standard deviation was 16.7, and the range was 50.7–142.6. Based on prior experience, the shapes of the radioactivity concentration vs. time curves were similar regardless of the initial ^{188}Re dose, supporting the assumption that first-order (not second-order) kinetics were dominating in pharmacokinetics of the radioactivity.

Mathematical Models

To describe the data, we used seven mechanistically-plausible models for pharmacokinetics of radioactivity, which are summarized in Table 1 and were described briefly in the main text (Materials and Methods). We discuss these models in more detail here.

A very simple model for pharmacokinetics of radioactivity from the selected organ/tissue involves a single first-order elimination rate. This mono-exponential (ME) model is described by the following equation, where $P_{ME}(t)$ is the predicted normalized radioactivity at decay time t, and τ_1 is an adjustable parameter for biochemical decay:

$$P_{ME}(t) = g(t) \exp(-t/\tau_1) \qquad \text{Supplemental Eq. 1}$$

In some situations a portion of radioactivity can be "trapped" in an organ. This is represented by the following equation for the mono-exponential with added constant (MEC) formalism, w_1 is the fraction of un-trapped radioactivity and τ_2 is the biochemical decay process:

$$P_{MEC}(t) = g(t) (w_1 \exp[-t/\tau_2] + 1 - w_1) \qquad \text{Supplemental Eq. 2}$$

A more complex model which involves the sum of two first-order elimination rates is the following commonly used bi-exponential (BE) formalism, where τ_3 and τ_4 are fast and slow biochemical decay processes, and w_2 is the fraction of biochemical decay which proceeds by the fast process:

$$P_{BE}(t) = g(t) [w_2 \exp(-t/\tau_3) + (1 - w_2) \exp(-t/\tau_4)] \qquad \text{Supplemental Eq. 3}$$

A simple model which assumes an underlying CPD of pharmacokinetic rates (rather than the sum of two discrete rates) is the stretched exponential (SE) function (*4, 5, 9*). The SE model is

described by the following equation, where τ_5 is an adjustable parameter (with units of time) and γ_1 is an exponent parameter:

$$P_{SE}(t) = g(t) \exp[-(t/\tau_5)^{\gamma 1}] \quad \text{Supplemental Eq. 4}$$

We also used a modified version of the SE model, which we call the MSE model here (*5*). The MSE model is described by the following equation, where τ_6 is an adjustable parameter (with units of time) and γ_2 is an exponent parameter:

$$P_{MSE}(t) = g(t) \exp[1 - (1 + t/\tau_6)^{\gamma 2}] \quad \text{Supplemental Eq. 5}$$

The underlying probability distribution of pharmacokinetic rates for the SE model is a single-peaked function which sometimes resembles the log-normal distribution. It can be calculated by inverse Laplace transform using the Bromwich integral (*5*). However, a log-normal distribution of first-order rates has no analytical solution (*4*), making its use for describing radiopharmaceutical pharmacokinetics less convenient.

This rate distribution for the SE model ($PDF_{SE}(k)$) can be expressed in terms of elementary functions for the special case where $\gamma_1 = ½$ as follows, where k is the elimination rate (*5*):

$$PDF_{SE}(k) = (\tau_5/(2\pi^{1/2}(k\tau_5)^{3/2})) \exp[-1/(4k\tau_5)] \quad \text{Supplemental Eq. 6}$$

The probability distribution of elimination rates for the MSE model ($PDF_{MSE}(k)$) is a simple modification of the one for the SE model (*5*):

$$PDF_{MSE}(k) = (\tau_6/(2\,\pi^{1/2}\,(k\tau_6)^{3/2})) \exp[-1/(4k\tau_6) + 1 - k\tau_6] \quad \text{Supplemental Eq. 7}$$

Because of these convenient features of the special cases where the exponent parameters for the SE and MSE models equal ½, we produced simplified 1-parameter versions of these models (called SSE and SMSE) as follows, where τ_7 and τ_8 are adjustable parameters:

$$P_{SSE}(t) = g(t)\exp[-(t/\tau_7)^{1/2}],$$

$$P_{SMSE}(t) = g(t)\exp[1-(1+t/\tau_8)^{1/2}]$$

Supplemental Eq. 8

Model Fitting Procedure

The mathematical models were fitted to the normalized data $F_i(j,t)$ for each organ/tissue separately, by maximizing the log likelihood, using optimization routines in Maple 17® software. The probability of finding the global maximum (rather than local maxima) was enhanced by using 100 random initial conditions for the model parameters, and by checking the results manually (for example, for 2-parameter models the dependence of the log likelihood function on parameter values could be plotted in 3 dimensions and visualized directly). All parameters were restricted to positive values to maintain mechanistic plausibility.

We assumed a Gaussian error distribution with constant variance for all data points. This assumption was reasonable because the instrument-related errors introduced during measurement of the initial dose of radioactive material administered to each mouse, which were likely to be the main contributors to the errors in $F_i(j,t)$, were not estimated explicitly and were likely to be the same for all data points. Poisson-distributed errors introduced during counting of radioactive decays were likely to be much smaller (*16*). The log likelihood function ($LL_{M,i}$) for the M-th model and for the *i*-th organ/tissue, under assumption of constant variance, is described by the

following equation, where M indicates one of the seven models and N(i) is the total number of data points for the i-th organ/tissue (106 for blood, 23 for bone marrow, and 24 for kidneys, liver, and lungs):

$$LL_{M,i} = -\tfrac{1}{2}N(i) \times (\ln[\textstyle\sum_j \sum_t [(P_{M,i}(t) - F_i(j,t))^2]/N(i)] + \ln(2\pi) + 1) \qquad \text{Supplemental Eq. 9}$$

The constant term $\ln(2\pi)+1$ was included for completeness, but had no effect on the comparison of model performances and on parameter estimation.

The likelihood function in Supplemental Eq. 9 is equivalent to nonlinear least squares with a single parameter set fitted across subjects (mice). Notably, the stretched exponential formalisms (SE, MSE, SSE, SMSE) are closed form functions in which fitted parameters enter nonlinearly, and the probabilistic component (i.e. the CPD shape) refers to the underlying derivation, not to the data fitting procedure per se.

Absolute goodness of fit (GOF) for the analyzed models under assumption of constant variance was assessed by exploratory data fitting using three methods: (1) visual inspection of model fits and the data; (2) calculation of the coefficient of determination, R^2; (3) linear regression of model predictions $P_{M,i}(t)$ vs. data points $F_i(j,t)$. Implementation of these approaches showed that one or more of the tested models performed reasonably well when fitted to data from each organ/tissue: the model prediction curves visually passed through most of the data clusters, R^2 values were generally > 0.7 (and in some cases > 0.98), and the 95% confidence intervals (CIs) for the regression of model predictions vs. data included 0 for the intercept and 1 for the slope.

In contrast, analogous exploratory calculations showed that assumption of Gaussian errors with magnitudes proportional to the values of $F_i(j,t)$, which resulted in replacement of the term

$[P_{M,i}(t) - F_i(j,t)]^2$ in Supplemental Eq. 9 by the term $[P_{M,i}(t) - F_i(j,t)]^2/F_i(j,t)^2$, produced much worse GOF for each of the tested models. This occurred because outlier data points with small values of $F_i(j,t)$ strongly affected the fit, causing model predictions to underestimate the majority of other data points. Consequently, the assumption of constant variance (implemented in Supplemental Eq. 9) was used for the subsequent data analysis presented below.

Estimation of Model Parameter Uncertainties

Uncertainties (95% CIs) for best-fit model parameter values were estimated by profile likelihood (*17*) as follows: 10,000 Monte-Carlo-generated parameter values in the vicinity of the best-fit values were used to estimate the critical contour of the log likelihood function, which is based on the asymptotic χ^2 behavior of the log likelihood distribution.

Information Theoretic Model Selection

As mentioned in the main text, sample size-corrected Akaike information criterion (AICc) was used to rank models by relative GOF. The equation for AICc for the M-th model and for the *i*-th organ/tissue ($AICc_{M,i}$) is given below, where K_M is the number of model parameters:

$$AICc_{M,i} = -2\,LL_{M,i} + 2\,K_M + 2\,K_M\,(K_M + 1)/(N(i) - K_M - 1) \qquad \text{Supplemental Eq. 10}$$

Here, no additional parameter was used to represent the errors because they were not estimated from the data, but considered to be constant for all data points. Consequently, the numbers of adjustable parameters (K_M) for each of the seven models were: $K_{ME} = 1$ (τ_1), $K_{MEC} = 2$ (w_1, τ_2), $K_{BE} = 3$ (w_2, τ_3, τ_4), $K_{SE} = 2$ (τ_5, γ_1), $K_{MSE} = 2$ (τ_6, γ_2), $K_{SSE} = 1$ (τ_7), $K_{SMSE} = 1$ (τ_8) (Table 1).

One of the most convenient features of using AICc is that it allows the evidence for multiple structurally distinct models to be compared simultaneously, without the need for models to be "nested" or to belong to the same class. The relative likelihood of the M-th model and for the *i*-th organ/tissue, called the evidence ratio ($ER_{M,i}$), can be expressed as:

$$ER_{M,i} = \exp[-\tfrac{1}{2}\Delta AICc_{M,i}], \text{ where } \Delta AICc_{M,i} = AICc_{M,i} - AICc_{min,i} \quad \text{Supplemental Eq. 11}$$

Here $AICc_{min,i}$ is the lowest AICc value generated by the set of models being compared. If $\Delta AICc_{M,i} > 6$, then the evidence ratio $ER_{M,i} < 0.05$, suggesting that the M-th model has poor support from the data for the *i*-th organ/tissue, relatively to the best-ranking model in the set of models being compared.

The Akaike weight, $W_{M,i}$, is another useful quantity – it represents the probability that the M-th model would be considered the best-ranking model for the *i*-th organ/tissue upon repeated sampling of the data. It is a normalized evidence ratio, i.e. the evidence ratio for the tested model divided by the sum of the evidence ratios for all the models being compared:

$$W_{M,i} = ER_{M,i} / \sum_M ER_{M,i} \quad \text{Supplemental Eq. 12}$$

Multi-model Inference for the Time Integral of Radioactivity

We used multi-model inference to produce a model-averaged time integral of radioactivity as follows. First, we calculated the normalized time integral, $NTI_{M,i}$, for the *i*-th organ/tissue using the following equation, where $P_{M,i}(t)$ are the best-fit predictions for the M-th model as function of decay time *t*, and $P_{BE,i}(t)$ are the best-fit predictions for the BE model:

$$NTI_{M,i} = \int_{t=0}^{\infty} P_{M,i}(t)dt \Big/ \int_{t=0}^{\infty} P_{BE,i}(t)dt \quad \text{Supplemental Eq. 13}$$

Next, we calculated the Akaike weighted normalized time integral, $WNTI_{M,i}$, using the expression below, where $W_{M,i}$ is the Akaike weight of the M-th model:

$$WNTI_{M,i} = W_{M,i}NTI_{M,i} \quad \text{Supplemental Eq. 14}$$

Finally, we calculated the model-averaged normalized time integral, $MMTI_i$:

$$MNTI_i = \sum_M WNTI_{M,i} \quad \text{Supplemental Eq. 15}$$

Here the normalization of the time integrals for all models by the value predicted by the BE model was used for convenience, to emphasize potential differences between model predictions, rather than the absolute values of the time integrals, which have no importance within the scope of this paper. Consequently, $MNTI_i$ is the model-averaged time integral of radioactivity, relative to the prediction of the BE model. If $MNTI_i$ is smaller/greater than 1, then accounting for the evidence supporting alternative formalisms other than the BE model results in reduction/inflation of the estimated time integral, i.e. using the BE model alone would overestimate/underestimate the time integral.