Supporting information for Xue *et al*. (2002) *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.182209699.

Supporting Text

Calculation of Cumulated Dose from a Uniformly Distributed Radionuclide in the LS174T Growing Tumor. The calculation of the average dose (cGy) to a mass of tissue in which a radionuclide resides for a period of time is generally carried out using the MIRD formalism (1). In this approach, we need to know only two parameters: (*i*) the absorbed dose per unit cumulated activity (cGy/μCi·h; 1 Ci = 37 GBq) for the radionuclide in question, known as the S value, which has been calculated and tabulated for various tissue masses (1, 2), and (*ii*) the experimentally determined radioactivity time curve from which the cumulative dose (μCi·h) is calculated. The volume of the organ/tissue is assumed to be constant over time. In the current work, however, LS174T tumor cells were injected subcutaneously into mice, where they grew into sizeable tumors. Under these circumstances, the radioactivity and the S value both change because of an additional factor, the time-dependent change of tumor size. Consequently, dose calculations for a growing tumor must take into account the effective half-lives with which the intratumoral radioactivity (t_R) and the S value (t_S) decline over the observation period. It is important to note that when the ratio of radiolabeled cells to unlabeled cells in a cluster is less than 1:10, i.e., as in the current experiments, the same absorbed dose is obtained when classical MIRD (1) or microdosimetric methods are used (3–6).

Total Dose Calculations. In calculating the total dose absorbed by the subcutaneous LS174T tumors, the following assumptions are made: (*i*) the radiolabeled cells are distributed uniformly throughout the growing tumor (note that even lower doses would be obtained if, in fact, the radioactivity were nonuniformly distributed throughout the growing tumor), (*ii*) the cells are spherical (a diameter of 28 μm has been observed), (*iii*) the cells and interstitial spaces are of unit density, and (*iv*) the tumor grows as a sphere that is made of a closely packed collection of spherical cells, a configuration in which

each cell is in contact with 12 other cells and the cells occupy 74% of the tumor mass volume, i.e., 26% is interstitial space (7).

Determination of t_R. Mice were injected subcutaneously in both legs with a mixture of 5-[^{125}I]iodo-2'-deoxyuridine (^{125}IUdR)-labeled and unlabeled tumor cells and killed over a 12-day period. The radioactivity within their limbs was determined, and the data were plotted as a function of time (Fig. 3). These results demonstrate that the disappearance of radioactivity is biphasic with a $t_{R(d0–1)}$ of 0.65 days and a $t_{R(d1–15)}$ of 3.85 days.

Determination of t_S. ^{125}IUdR-labeled (1×10^6, 2×10^5, 1×10^5) and unlabeled (1×10^6) LS174T tumor cells were mixed and injected subcutaneously into mice. To derive tumor diameters (D, μm), the volume of each injectate (LS174T cell diameter = 28 ± 7 μm; the cells occupy 74% of the "tumor mass" volume), and the consecutive volumes after each doubling in size were calculated. S values for calculating self-absorbed doses to spheres of varying diameters that contain uniformly distributed ^{125}I radioactivity, previously published by Goddu *et al.* (2), were then plotted versus tumor diameter obtained after each doubling time, and the data points were fitted by linear regression

$$S = antilog\ (2.623 - 2.952 \log D),$$

and the parameters of this equation were used to determine the S values (cGy per decay) that correlate with tumor diameters after each doubling in volume. Finally, these S values were plotted as a function of time, using the doubling times (d.t.) for each tumor growth curve (Fig. 1 in article), i.e., after 1 d.t., $2 \times$ d.t., $3 \times$ d.t., etc., the data points were fitted (linear regression), and the equation parameters were used to obtain the $T_{1/2}$ of the S values (i.e. t_s).

Definitions. *Radioactivity (pCi).*

$$R(t) = R_{d0} * e^{\left(-0.693 \frac{t}{t_{R(d0-1)}}\right)} \quad \text{for } 0 < t < 1 \text{ in days,}$$

where R_{d0} is the initial radioactive content of the tumor at day zero and $t_{R(d0–1)}$ = 0.65 days (Fig. 3). Similarly,

$$R(t) = R_{d1} * e^{\left(-0.693 \frac{t}{t_{R(d1\text{-}15)}}\right)} \quad \text{for } 1 < t < 15 \text{ in days,}$$

where R_{d1} is the activity remaining in the tumor after the first day and $t_{R(d1–15)}$ = 3.85 days (Fig. 3).

Dose per unit cumulated activity (cGy/decay).

$$S(t) = S_{d0} * e^{\left(-0.693 \frac{t}{t_{S(d0\text{-}1)}}\right)},$$

where S_{d0} is the S value on day zero (i.e., when the tumor is composed of the injected tumor cells) and $t_{S(d0–1)}$ is the effective half-life with which the S value declines over time (derived from each of the fits described above). Similarly,

$$S(t) = S_{d1} * e^{\left(-0.693 \frac{t}{t_{S(d1\text{-}15)}}\right)},$$

where S_{d1} is the S value on day 1 and $t_{S(d1–15)}$ is the effective half-life with which the S value declines over time.

General Equations. *Radiation dose (cGy).* The loss of radioactivity from within the tumors was clearly biphasic in nature (Fig. 3). To simplify our calculation, the total dose (D_T) to the tumor was therefore obtained individually for the two time periods (days 0–1 and 1–15) and then summed.

$$D_T = \int_{d0}^{d15} S(t) * R(t) dt = D_{(d0\text{-}1)} + D_{(d1\text{-}15)},$$

where $D_{(d0–1)}$ is tumor dose cumulated in the first day and $D_{(d1–15)}$ is tumor dose cumulated from day 1 to day 15. Substituting the analytical expressions for S(t) and R(t)

$$D_{(d0\ -\ 1)} = \int_{d0}^{d1} S(t)*R(t)dt = \int_{d0}^{d1} S_{d0}*e^{-\frac{0.693*t}{t_{S(d0-1)}}}*R_{d0}*e^{-\frac{0.693*t}{t_{R(d0-1)}}}dt =$$

$$S_{d0}*R_{d0}*\int_{d0}^{d1} e^{-0.693*t*\left(\frac{1}{t_{S(d0-1)}}+\frac{1}{t_{R(d0-1)}}\right)}dt =$$

$$-\left[\frac{\left[S_{d0}*R_{d0}\right]}{\left[0.693*\left(\frac{1}{t_{S(d0\ -\ 1)}}+\frac{1}{t_{R(d0\ -\ 1)}}\right)\right]}\right]*\left[\left[e^{-0.693*t*\left(\frac{1}{t_{S(d0-1)}}+\frac{1}{t_{R(d0-1)}}\right)}\right]_0\Big/^1\right]=$$

$$\left[\frac{\left[S_{d0}*R_{d0}\right]}{\left[0.693*\left(\frac{1}{t_{S(d0\ -\ 1)}}+\frac{1}{t_{R(d0\ -\ 1)}}\right)\right]}\right]*\left[1-e^{-0.693*1*\left(\frac{1}{t_{S(d0-1)}}+\frac{1}{t_{R(d0-1)}}\right)}\right] \qquad \textbf{Eq. 1}$$

$$D_{(d1\ -\ 15)} = \int_{d1}^{d15} S(t)*R(t)dt = \int_{d1}^{d15} S_{d1}*e^{-\frac{0.693*t}{t_{S(d1-15)}}}*R_{d1}*e^{-\frac{0.693*t}{t_{R(d1-15)}}}dt =$$

$$S_{d1}*R_{d1}*\int_{d1}^{d15} e^{-0.693*t*\left(\frac{1}{t_{S(d1-15)}}+\frac{1}{t_{R(d1-15)}}\right)}dt =$$

$$-\left[\frac{\left[S_{d1}*R_{d1}\right]}{\left[0.693*\left(\frac{1}{t_{S(d1\ -\ 15)}}+\frac{1}{t_{R(d1\ -\ 15)}}\right)\right]}\right]*\left[\left[e^{-0.693*t*\left(\frac{1}{t_{S(d1-15)}}+\frac{1}{t_{R(d1-15)}}\right)}\right]_1\Big/^{15}\right]=$$

$$\left[\frac{\left[S_{d1}*R_{d1}\right]}{\left[0.693*\left(\frac{1}{t_{S(d1\ -\ 15)}}+\frac{1}{t_{R(d1\ -\ 15)}}\right)\right]}\right]*\left[1-e^{-0.693*14*\left(\frac{1}{t_{S(d1-15)}}+\frac{1}{t_{R(d1-15)}}\right)}\right] \qquad \textbf{Eq. 2}$$

By defining $T_{d0–1}$ and $T_{d1–15}$ such that

$$\frac{1}{T_{(d0\ -\ 1)}} = \frac{1}{T_{S(d0\ -\ 1)}} + \frac{1}{T_{R(d0\ -\ 1)}}$$

and

$$\frac{1}{T_{(d1\ -\ 15)}} = \frac{1}{T_{S(d1\ -\ 15)}} + \frac{1}{T_{R(d1\ -\ 15)}},$$

Eqs. **1** and **2** reduce to

$$D_T = \text{total dose in rad} = D_{d0\,-\,1} + D_{d1\,-\,15} =$$

$$\left[\frac{\left[S_{d0} * R_{d0}\right]}{\left[0.693 * \left(\frac{1}{T_{(d0\,-\,1)}}\right)\right]}\right] * \left[1 - e^{-0.693 * 1 * \left(\frac{1}{T_{(d0-1)}}\right)}\right] +$$

$$\left[\frac{\left[S_{d1} * R_{d1}\right]}{\left[0.693 * \left(\frac{1}{T_{(d1\,-\,15)}}\right)\right]}\right] * \left[1 - e^{-0.693 * 14 * \left(\frac{1}{T_{(d1-15)}}\right)}\right] \qquad \textbf{Eq. 3}$$

Evaluation. Using Eq. **3**, we have calculated the doses absorbed by the tumors shown in Fig. 1 where 1×10^6, 2×10^5, and 1×10^5 ^{125}IUdR-labeled cells containing 0.19 pCi per cell were mixed with 1×10^6 unlabeled cells, and the diameter of the LS174T cell, as measured from histology sections from 14-day tumors, is 28 μm. Conversion of units and substitution of values in the above equation yields:

Case 1: Number of cell injected: 1×10^6 ^{125}IUdR-labeled cells + 1×10^6 unlabeled cells

Day 0–1

$S_{d0} = 1.05 \times 10^{-8}$ cGy per decay

R_{d0} = 190,000 pCi × 2.22 dpm/pCi/60 sec = 7,030 decays per sec

$t_{S(d0–1)}$ = 2.35 days ; $t_{R(d0–1)}$ = 0.65 days

$1/T_{d0–1} = 1/t_{S(d0–1)} + 1/t_{R(d0–1)} = 1.96$

Therefore, $T_{d0–1}$ = 0.51 days = 43,992 sec

$D_{d0-\infty} = 1.05 \times 10^{-8} * 43{,}992 * 7{,}030/0.693 = 4.7$ cGy

and $D_{d0–1} = 4.7 * (1 - e^{(-0.693)*(1\text{ d} / 0.51\text{ d})}) = 3.5$ cGy

Day 1–15

$S_{d1} = 7.82 \times 10^{-9}$ cGy per decay

R_{d1} = 65,422 pCi × 2.22 dpm/pCi/60 sec = 2,421 decays per sec

$t_{S(d1–15)}$ = 2.35 days ; $t_{R(d1–15)}$ = 3.85 days

$1/T_{d1–15} = 1/t_{S(d1–15)} + 1/t_{R(d1–15)} = 0.69$

Therefore, $T_{d1–15}$ =1.46 days = 126,081 sec

$D_{d1-\infty} = 7.82 \times 10^{-9} * 126{,}081 * 2{,}421/0.693 = 3.4$ cGy

and $D_{d1–15} = 3.4 * (1 - e^{(-0.693)*(14\ d\ /\ 1.46)}) = 3.4$ cGy

Therefore, D_T, the total dose over 15 days, = 3.5 + 3.4 = 6.9 cGy

Case 2: Number of cell injected: 2×10^5 ^{125}IUdR-labeled cells + 1×10^6 unlabeled cells

Day 0–1

$S_{d0} = 1.74 \times 10^{-8}$ cGy per decay; R_{d0} = 38,000 pCi

$t_{S(d0–1)}$ = 2.46 days; $t_{R(d0–1)}$ = 0.65 days

$D_{d0–1}$ = 1.2 cGy

Day 1–15

$S_{d1} = 1.31 \times 10^{-8}$ cGy per decay; R_{d1} = 13,085 pCi

$t_{S(d1–15)}$ = 2.46 days; $t_{R(d1–15)}$ = 3.85 days

$D_{d1–15}$ = 1.2 cGy

Therefore, D_T = 1.2 + 1.2 = 2.4 cGy

Case 3: Number of cell injected: 1×10^5 ^{125}IUdR-labeled cells + 1×10^6 unlabeled cells

Day 0–1

$S_{d0} = 1.90 \times 10^{-8}$ cGy per decay; R_{d0} = 19,000 pCi

$t_{S(d0–1)}$ = 2.52 days; $t_{R(d0–1)}$ = 0.65 days

$D_{d0–1} = 0.9 * (1 - e^{(-0.693)*(1\ d\ /\ 0.52\ d)}) = 0.6$ cGy

Day 1–15

$S_{d1} = 1.44 \times 10^{-8}$ cGy per decay; R_{d1} = 6,542 pCi

$t_{S(d1–15)}$ = 2.52 days; $t_{R(d1–15)}$ = 3.85 days

$D_{d1–15}$ = 0.7 cGy

Therefore, $D_T = 0.6 + 0.7 = 1.3$ cGy

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