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# Dosimetric Comparison of Bolus and Continuous Injections of CC49 Monoclonal Antibody in a Colon Cancer Xenograft Model

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**BACKGROUND.** Improved understanding of dose and effective dose calculations may contribute to the optimization of fractionated radioimmunotherapy.

**METHODS.** Comparison three-dimensional tumor dosimetry was performed on athymic nude mice bearing established LS174T human colon carcinoma xenografts. Mice were given bolus intraperitoneal injections of 300  $\mu\text{Ci}$   $^{131}\text{I}$ -labeled CC49 monoclonal antibody once (Day 0) or three times (Days 0, 3, and 7) or continuous intraperitoneal infusion with miniosmotic pumps over 7 days. Serial section autoradiography was used to reconstruct tumor activity density distributions for Days 3, 4, 7, 10, and 11 (single injection); Days 3, 4, 7, 8, and 11 (3 injections); and Days 4, 7, 10, and 13 (pump). At least three tumors were reconstructed at each time point. Uptakes in blood and tumor were measured up to 14 days (single injection), 11 days (3 injections), or 16 days (pump) after injection.

**RESULTS.** Average dose values calculated from total activity uptake data only (assuming no energy loss external to the tumor) yielded 102 Gy (single injection), 158 Gy (three injections), and 47 Gy (pump). Average doses using three-dimensional dose calculations were 88 Gy, 139 Gy, and 40 Gy, respectively. The nonuniformity of dose deposition affects treatment outcome, because cell loss is an exponential function of dose. Using the linear quadratic model with fractional cell survival to define an effective dose,  $D_{\text{eff}}$  were calculated to be 20 Gy, 23 Gy, and 14 Gy, respectively. Cell proliferation affects outcome for variable dose-rate treatments. With cell proliferation parameters set to reproduce single-fraction  $^{60}\text{Co}$  recurrence results,  $D_{\text{eff}}$  (for local control endpoint) were 8.9 Gy, 12.8 Gy, and 3.9 Gy, respectively. Three bolus injections compared with a single bolus injection were relatively less efficient in tumor uptake. However, three bolus injections resulted in a more uniform dose rate over a longer period, resulting in a 50% improvement in  $D_{\text{eff}}$ . The slower dose delivery for pump infusion resulted in a significantly lower  $D_{\text{eff}}$ , although dose-rate distributions were more uniform compared with the single bolus injection.

**CONCLUSIONS.** Improvement in dose-rate nonuniformities was observed for fractionated and continuous radiolabeled monoclonal antibody injections. Fractionated injections produced superior dosimetric results compared with single bolus or continuous injections. *Cancer* 1997;80:2567–75. © 1997 American Cancer Society.

**KEYWORDS:** dosimetry, radioimmunotherapy,  $^{131}\text{I}$ -labeled monoclonal antibodies, effective dose, fractionation, serial section autoradiography, beta dosimetry.

**T**reatment fractionation has been historically proven to be advantageous for external beam radiotherapy, primarily due to the relative sparing of normal tissues allowing higher dose levels to be delivered to the target volume. Fractionation in radioimmunotherapy (RIT) was

also expected to benefit due to this relative sparing effect. Delivery of higher doses for equivalent toxicity was observed for fractionated RIT, allowing a gain in therapeutic advantage.<sup>1-3</sup>

The interpretation of dose estimates for RIT has been studied by several groups. Experiments have been performed to relate external beam radiotherapy (EBRT) and RIT dosimetry in terms of outcome, typically tumor recurrence delay. A review of the literature concluded that the RIT/external beam radiotherapy relative effectiveness for equivalent reported doses varied by factors ranging from 0.3 to 3.<sup>4</sup> Our previous dosimetry studies have reconciled this difference for a colon carcinoma xenograft model by correcting for known effects of nonuniform changing dose rates, exponential dose response, energy deposited outside of tumor volume, cell proliferation rate, and hypoxic/necrotic fractions of the tumors.<sup>5</sup> An essential part of the analysis was the measurement of the average space-time dependence of the dose rate.

This article reports the results of a study of the spatial and temporal dependences of CC49 monoclonal antibody (MoAb) uptake in LS174T xenografts and accompanying dosimetric implications for fractionated and continuous injections. Of primary interest was the investigation of dose-rate distribution effects, such as the predicted effect of delivering a higher dose rate to the well-perfused cells,<sup>6</sup> particularly through multiple bolus or continuous infusion injections compared with a single bolus injection.

## MATERIALS AND METHODS

Athymic nude mice bearing 7-day established subcutaneous LS174T human colon carcinoma xenografts received 300  $\mu\text{Ci}$  single bolus intraperitoneal injections (1X) on Day 0 (equal to 7 days after transplant), 300  $\mu\text{Ci}$  each triple bolus intraperitoneal injections (3X) on Days 0, 3, and 7, or continuous intraperitoneal infusion via Alzet miniosmotic pumps (Model 2001; Alza, Palo Alto, CA) over 7 days (Days 0-7) of <sup>131</sup>I-labeled CC49 MoAb. MoAb CC49 has a high affinity ( $1.6 \times 10^{10} \text{ M}^{-1}$ ) and binds to the mucinous antigen TAG-72, which is excreted in the extracellular space.<sup>7</sup> The triple bolus injections were prepared in two labelings of approximately 10 mCi/mg. The first labeling was used for the Day 0 and 3 injections.

The miniosmotic pumps were filled aseptically according to the manufacturer's instructions with <sup>131</sup>I-antibody diluted in phosphate-buffered saline containing 5% human serum albumin. The pumps were loaded with excess activity so that the difference between the initial and residual activity levels approximated the nominal activity. The injected activity was calculated using

$$\text{Injected Activity} = \frac{A_i - A_f \exp(\lambda T)}{\lambda T} [1 - \exp(-\lambda T)]$$

where  $A_i$  and  $A_f$  are the initial and final measured activities in the pump,  $\lambda$  is the decay constant for <sup>131</sup>I, and  $T$  is the pumping time ( $\leq 7$  days). The formula corrects for radioactive decay while the labeled antibody remained in the pump. Average values of  $A_i$  and  $A_f$  for  $T \geq 7$  days were 389  $\mu\text{Ci}$  and 76  $\mu\text{Ci}$ , respectively. The average actual injected activity achieved over 7 days was 204  $\mu\text{Ci}$  with a sample standard deviation of 21%.

Tumors were resected, frozen, and sectioned at Days 3, 4, 7, 10, and 11 (1X); Days 3, 4, 7, 8, and 11 (3X); and Days 4, 7, 10, and 13 (pump) following initial radiolabeled antibody injection. A minimum of three tumors were reconstructed per time point. The numbers of tumors used to determine the uptake curves, the average tumor masses, and the subset reconstructed at each time point are given in Table 1. Bolus Day 1-3 tumors were used for both 1X and 3X data sets. Uptake in blood and tumor was measured up to 14 days (1X), 11 days (3X), and 16 days (pump), as shown in Figure 1. Autoradiographs of the tumor sections were taken and digitized using a laser densitometer (Lumysis, Sunnyvale, CA) at 100  $\mu\text{m}$  resolution. The tumor section images were registered to form three-dimensional reconstructions of the tumor MoAb uptake. Gelatin calibration standards with known activity densities were prepared, frozen, sectioned, and autoradiographed to convert optical density to three-dimensional activity density matrices.<sup>8</sup> Three-dimensional dose-rate distributions were calculated from the activity density distributions folded with a voxel kernel derived from a dose point kernel for <sup>131</sup>I,<sup>9</sup> using fast Fourier transforms. The serial section images were used to determine tumor section contours and tumor surfaces.<sup>10</sup>

Each tumor has a unique dose-rate distribution. However, the dose-rate distributions have some common characteristics,<sup>11</sup> particularly in the presence of a large time dependence of the distribution of uptake. The LS174T tumor model mimics a tumor nodule with primarily surface vascularity.<sup>8,12</sup> The dose-rate nonuniformity for each tumor was characterized by calculating dose-rate volume histograms as a function of radius. The equivalent radius of each voxel was determined by taking the ratio of the distance from the tumor center of mass to the voxel and the distance from the tumor center of mass to the surface, passing through the voxel.<sup>13</sup> The radial histograms represent the magnitude of dose-rate nonuniformity for each tumor. Histograms for tumors reconstructed for the same experimental conditions (same injection scheme

**TABLE 1**  
**Number of Tumors, Average Masses, and Subset Reconstructed versus Time after Injection**

Time (days)	1X			3X			Pump		
	No. of tumors	Mass (g)	3D	No. of tumors	Mass (g)	3D	No. of tumors	Mass (g)	3D
1	6	0.47 (0.10) <sup>a</sup>	—	6 <sup>b</sup>	0.47 (0.10) <sup>b</sup>	—	—	—	—
2	7	0.45 (0.05)	—	7 <sup>b</sup>	0.45 (0.05) <sup>b</sup>	—	7	0.48 (0.04)	—
3	12	0.44 (0.05)	4	12 <sup>b</sup>	0.44 (0.05) <sup>b</sup>	4 <sup>b</sup>	—	—	—
4	10	0.42 (0.09)	4	8	0.72 (0.21)	8	12	0.54 (0.05)	4
5	7	0.52 (0.06)	—	—	—	—	—	—	—
6	7	0.53 (0.15)	—	—	—	—	—	—	—
7	10	0.53 (0.08)	4	4	0.37 (0.14)	4	21	0.46 (0.08)	12
8	6	0.16 (0.05)	—	9	0.28 (0.05)	9	—	—	—
10	12	0.44 (0.09)	5	—	—	—	11	0.46 (0.10)	3
11	8	0.20 (0.04)	3	13	0.62 (0.14)	13	10	0.53 (0.10)	—
12	—	—	—	—	—	—	9	0.43 (0.09)	—
13	—	—	—	—	—	—	18	0.61 (0.07)	12
14	5	0.29 (0.13)	—	—	—	—	—	—	—
16	—	—	—	—	—	—	5	0.48 (0.25)	—

3D: three-dimensional.

<sup>a</sup> Mean and standard deviation, 0.47 ± 0.10.

<sup>b</sup> Same data as for 1X.

and time after injection) were normalized and averaged to represent an average nonuniformity.

The averaged radial histograms for each time point were normalized to the average tumor uptake. The space-time dependent description was represented by the uptake curve augmented by the dose-rate nonuniformity histograms. To complete the time description of the three-dimensional uptake, histograms for each radius were matched assuming the approximation of maximum heterogeneity (equal volumes summed according to dose-rate rank order).<sup>5</sup> The uptake description includes the time dependence of the dose-rate nonuniformity.

**Dosimetry**

Tumor dose and effective dose calculations were performed using various assumptions. The uniform-isotropic model of the Medical Internal Radiation Dose Committee<sup>14</sup> was used to calculate dose. The area under the uptake curves, summed using the trapezoidal rule, represented the time-activity integrals per unit mass (A/m). For a mean energy per nuclear transition of Δ and an absorbed fraction (Φ) of unity, the calculated dose (D) for the uniform-isotropic model was

$$D = A\Delta\Phi/m \tag{1}$$

The dose-rate nonuniformities described by the radial histograms were normalized to the activity densities obtained from the tumor uptake curves. The ra-

tio of the average dose rate from the histograms to the theoretical maximum average dose rate assuming the uniform-isotropic model provided the time-dependent absorbed fractions, Φ(t). These were used to calculate the actual mean dose estimates,

$$D = \frac{\Delta}{m} \int A(t)\Phi(t) dt \tag{2}$$

The effective dose, D<sub>eff</sub>, was defined as the equivalent uniform absorbed dose required to produce the same fractional cell survival, S, as for a dose response with linear coefficient α,

$$D_{\text{eff}} \equiv 1/\alpha \ln(S) \tag{3}$$

For a varying dose rate and assuming the linear-quadratic dose-response model,

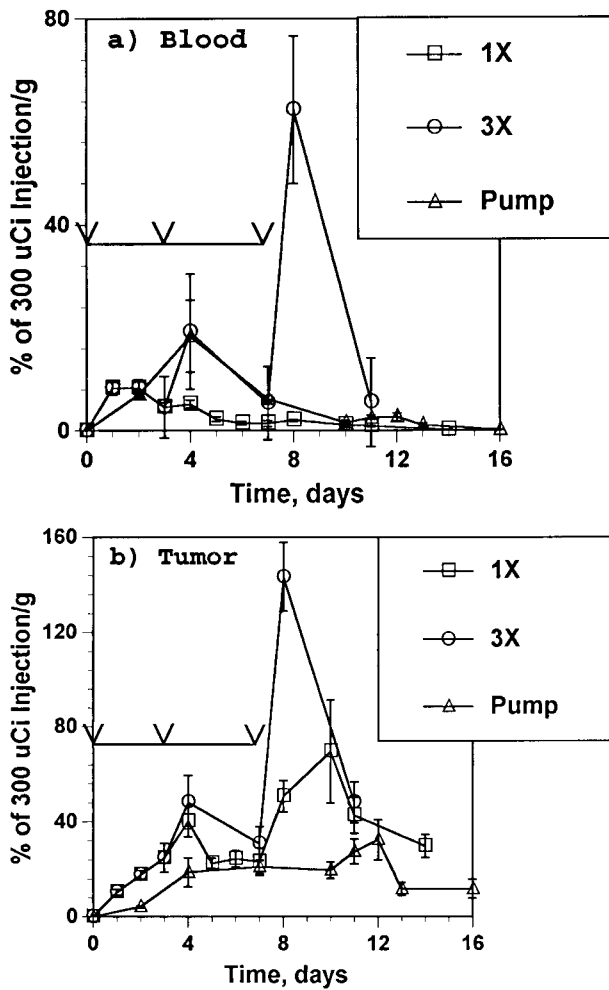
$$S(t) = \frac{1}{V} \int_v \exp[-\alpha D(\vec{r},t) \text{Re}(\vec{r},t)] d^3r \tag{4}$$

where<sup>15</sup>

$$\text{RE}(\vec{r},t) = 1 +$$

$$\left( \frac{2 \int_0^t R(\vec{r},t) [\int_0^{t'} R(\vec{r},t'') dt''] dt'}{D(\vec{r},t)} \right) \tag{5}$$

is the relative effectiveness describing the dose-rate response according to the linear-quadratic model, and



**FIGURE 1.** Uptake in (a) blood and (b) tumor for injections of <sup>131</sup>I-labeled CC49 MoAb given as a single fraction (1X) of 300  $\mu$ Ci, three fractions (3X) of 300  $\mu$ Ci each, and continuous infusion (pump) of 300  $\mu$ Ci over 7 days. The mean and standard deviations of the observed MoAb uptake are plotted as a percent of a 300  $\mu$ Ci injection per gram of sample. Injection times for the 3X and pump schemes are indicated with arrows and a bar, respectively.

$$D(\vec{r},t) = \int_0^t R(\vec{r},t') dt' \quad (6)$$

$R(\vec{r},t)$  is the local dose rate as a function of time,  $\mu$  is the cell repair constant, and  $\alpha$  and  $\beta$  are parameters of the linear-quadratic model. The three-dimensional spatial dependence of  $R(\vec{r},t)$  was represented by the radial histograms,  $R(\vec{r},t) = R(r,\Delta V,t)$ . The integral over  $d^3r$  was reduced to a sum over  $\Delta V$ . Equation 4 reduced to:

$$S(t) = \frac{1}{V} \sum_V \exp[-\alpha D(r,\Delta V,t) RE(r,\Delta V,t)] dV \quad (4a)$$

with a similar simplification to equation 5.

The effects of hypoxic and necrotic tissues on the effective dose were estimated. The dependence of the oxic, hypoxic, and necrotic tissues on vascular density was calculated assuming local equidistant blood vessels and nominal distances from the nearest blood vessel for hypoxic and necrotic tissues to form. The average radial dependence of vascular density was assumed equal to the uptake at Day 1 after injection for a low-affinity antibody (17-1A), as verified using hematoxylin and eosin staining.<sup>8</sup> The normalization of the radial dependences of the tumors was constrained to match the measured average fractional volume of necrotic tissue (30%). This yielded a hypoxic fraction of 10%. The linear-quadratic parameters were converted to  $\alpha_o, \beta_o,$  and  $\alpha_H, \beta_H$  for oxic and hypoxic subpopulations. The ratio of oxic to hypoxic parameters was set to reproduce an oxygen enhancement ratio of 2, appropriate for low dose rates.<sup>16</sup> The total S value was the weighted sum of the oxic and hypoxic S values.

Cell proliferation was assumed to be exponential. The S value was multiplied by  $\exp[(\ln 2)t/t_d]$ , where  $t_d$  was the tumor doubling time.

Parameters used in the calculation were  $\alpha = 0.3 \text{ Gy}^{-1},$ <sup>17</sup>  $\alpha/\beta = 25 \text{ Gy},$ <sup>18</sup> and  $\mu = 0.46 \text{ h}^{-1}.$ <sup>17</sup> The tumor doubling time ( $t_d = 3.4$  days) was chosen to reproduce the observed regrowth doubling time delay for a 6-Gy <sup>60</sup>Co exposure of  $15 \pm 1$  day.<sup>19</sup> This value was consistent with the value measured by Leith et al.<sup>20</sup> for LS174T of 3.3 days. The oxic and hypoxic parameters were adjusted so that the time to recurrence for the 6-Gy <sup>60</sup>Co data was reproduced.

Doses to bone marrow were calculated using the area under the blood uptake curves, summed using the trapezoidal rule. The uptake in bone marrow relative to blood used was 0.24.<sup>21</sup> The absorbed fraction for <sup>131</sup>I was estimated to be 63%.<sup>22,23</sup> Because these factors remain constant for the injection technique comparison presented here, relative values for blood and bone marrow remained constant. Effective dose calculations for bone marrow assumed a cell kill exponent linear in dose and constant cell proliferation. Parameter values were  $\alpha = 1.1 \text{ Gy}^{-1}$ <sup>24,25</sup> and  $t_d = 3.2$  days.<sup>26</sup>

## RESULTS

The primary data for the dose calculations were the uptake curves, shown for blood and tumor in Figure 1. Calculated tumor doses and effective doses are presented in Table 2. The calculated values are normalized to 300  $\mu$ Ci,  $3 \times 300 \mu$ Ci (900  $\mu$ Ci), and 300  $\mu$ Ci for the single bolus (1X), triple bolus (3X), and pump injection, respectively. Dose calculations using the uniform-isotropic model (an absorbed fraction of unity) are given in the first row of Table 2. The second

**TABLE 2**  
**Tumor Dose and Effective Dose for <sup>131</sup>I-Labeled CC49 MoAb Injection of 300 μCi Bolus (1X), 3 × 300 μCi Bolus (3X), and 300 μCi 7-Day Continuous Pumping (Pump)**

Dose model	1X	3X	Pump
Uniform-Isotropic Three-dimensional average	102 (9) <sup>a</sup>	158 (16)	47 (7)
Nonuniformity <sup>b</sup>	88	139	40
Proliferation <sup>d</sup>	20.4 <sup>c</sup>	23.4 <sup>c</sup>	13.7 <sup>c</sup>
Cell oxygenation status	8.9 <sup>c</sup>	12.8 <sup>c</sup>	3.9 <sup>c</sup>
	9.7 <sup>c</sup>	13.8 <sup>c</sup>	4.5 <sup>c</sup>

<sup>a</sup> Doses are in Gy. Uncertainties derived from standard deviations of tumor uptake curve data are shown in parentheses.

<sup>b</sup> Linear quadratic parameters:  $\alpha = 0.3 \text{ Gy}^{-1}$ ,  $\alpha/\beta = 25 \text{ Gy}$ .

<sup>c</sup> Effective dose.

<sup>d</sup> Cell doubling time,  $t_d = 3.4 \text{ days}$ .

row of Table 2 lists average tumor doses using the radial histograms to calculate the absorbed fractions. This calculation includes energy loss external to the tumor. Any further use of the space-time dependence of the dose distribution must employ an effective dose calculation to relate the expected outcome of RIT to a standard. The standard chosen was a uniform exposure with a logarithmic cell loss response linear in dose, the equivalent uniform dose. It must be emphasized that effective dose calculations are useful for comparisons of predicted outcome. The absolute values depend on the assumptions and endpoint specified.

The effective dose calculation given in row 3 of Table 2 includes the additional effects of exponential cell loss and dose rate assuming the linear-quadratic model of dose response.<sup>17</sup> The loss of effectiveness compared with the uniform equivalent dose was striking. This was primarily due to the nonuniform dose rates.

Tumor cell proliferation over the treatment interval may require a significant correction to the effective dose, depending on the comparison of tumor doubling time and the effective half-life of the radioactive isotope. In the present case, assuming tumor cure as the endpoint, the effective doses are significantly lower when proliferation is included (row 4, Table 2). It was assumed that all dose deposited after the nadir in the fraction of surviving tumor clonogens was not useful.

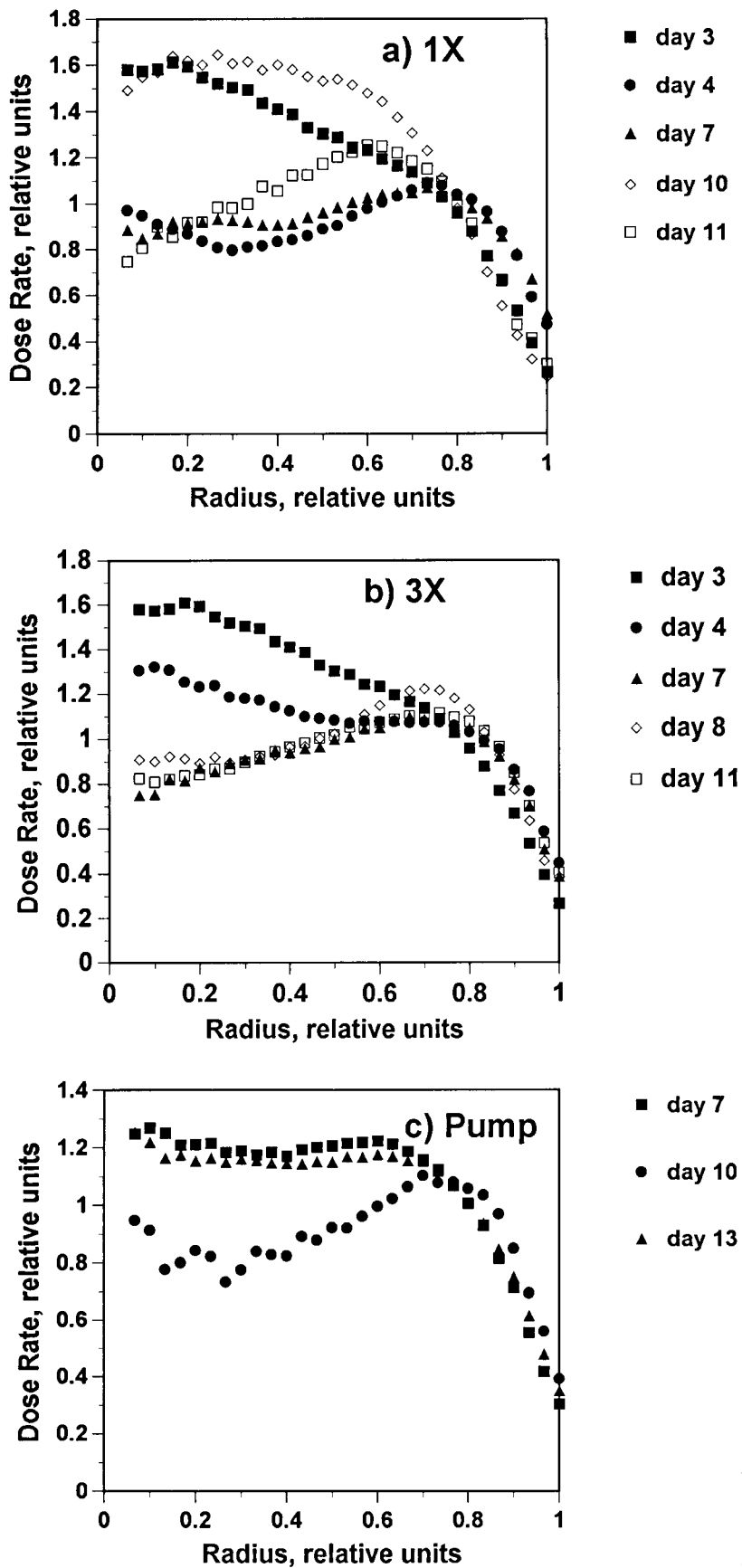
Finally, the effect of the hypoxic and necrotic cell fractions was included but made little relative difference. The general effect of the inclusion of hypoxic/necrotic cell fractions was to give more emphasis to the surface regions where there are more well-perfused cells.

Although the radial histograms were used to cal-

culate the dose and effective dose values, the average radial dose rates were calculated from the radial histograms to help analyze the uptake trends. Figure 2 shows radial dose-rate curves as a function of time after injection. For a single bolus injection (1X), the surface dose rates (relative radius  $\geq 0.8$ ) peak at Days 4–7 and then decline (Fig. 2a). For the 3X data, the surface dose rates remain consistently higher from Day 4 onward (Fig. 2b). For the pump data, the surface dose rates are again more consistent over time (Fig. 2c). The comparison is shown more explicitly in Figure 3, in which late radial dose rate curves for the three injection techniques are compared. Both the 3X and pump injection techniques produced higher surface dose rates compared with the 1X technique.

The effective dose calculations presented in the last two rows of Table 2 assume maximum cell loss (potential for local control) as the endpoint. Time to tumor recurrence would yield a different (higher) set of effective doses. As illustrated in Figure 4, the surviving fraction of cells experiences a minimum value (nadir) for all curves. Any dose deposited after reaching the minimum is of no value if local control is the desired endpoint. Note that the 3X (compared with 1X) therapy is more effective in terms of maximum cell loss than relative time delay to recurrence. The predicted time to recurrence for the 300 μCi single bolus injection was approximately 32 days. Recurrence delay times for therapy trials using a single 600 μCi injection varied, because average growth rates were slower for tumor recurrence than for the untreated controls. The average observed delay was approximately 27 days.<sup>2</sup> The effective dose model as presented predicts a somewhat greater recurrence delay for this scenario.

Bone marrow doses and effective dose are given in Table 3. Uncertainties were derived from the observed variations in the uptake data. The average doses included the loss of energy external to the marrow and the contribution from the whole body. Representative geometries for the bone marrow configuration were used.<sup>22,23</sup> The proliferation model assumed constant proliferation and used the cell survival nadir to determine the effective dose. Because the effective dose calculation is nonlinear in injected activity, effective dose values do not scale with greater injected activities. The 3X calculated bone marrow dose exceeded three times the single bolus value. The relatively larger bone marrow calculated dose for the 3X case was primarily due to the large blood uptake following the third injection (Fig. 1a). The survival analysis for the present mouse/human-tumor model found that the 3 × 300 μCi injection and a single 600 μCi bolus injection produced approximately equivalent results.<sup>2</sup> Assuming that the uptake curve scales with injected ac-



**FIGURE 2.** Radial dose-rate curves for (a) single fraction (1X), (b) three fractions (3X), and (c) continuous infusion (pump) as a function of time after injection. The radial curves were generated from the average values of each of the radial histograms.

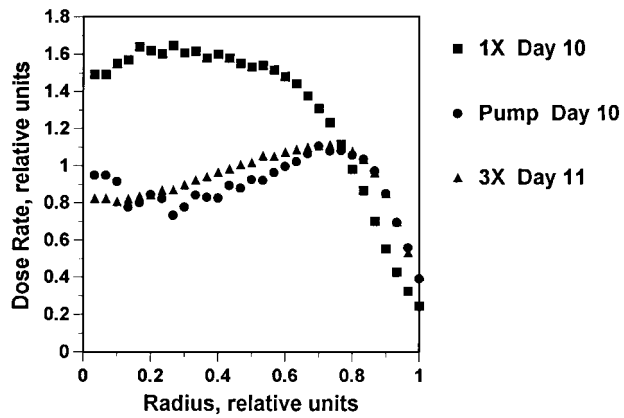


FIGURE 3. Radial dose-rate curves for 1X, 3X, and pump injections for late postinjection times.

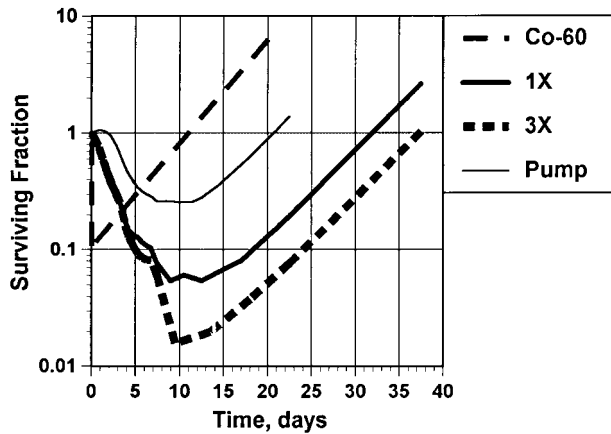


FIGURE 4. Calculated surviving fractions of clonogenic cells versus time for a single fraction <sup>60</sup>Co exposure (6 Gy), single fraction (1X) of 300 μCi, three fractions (3X) of 300 μCi each, and continuous infusion (pump) injections of 300 μCi <sup>131</sup>I-labeled CC49 MoAb.

tivity, a greater average dose appears to be tolerated with the 3X compared with the 1X injection scheme. Analysis using the proliferation effective dose model did not explain this greater tolerance.

**DISCUSSION**

For the triple bolus injection (3X), each injection was maintained at the same activity level as the single bolus injection (1X). In this way, the relative effect of the later injections could be studied. The 3 × 300 μCi injection approximated the tolerance level for the triple injection. Because this injection scheme provided the most promising results,<sup>2</sup> it was retained for the three-dimensional reconstruction study. A 900-μCi single bolus injection exceeded treatment tolerance and could not be studied. Continuous pumping was

TABLE 3  
Bone Marrow Dose and Effective Dose for <sup>131</sup>I-Labeled CC49 Injection of 300 μCi Bolus (1X), 3 × 300 μCi Bolus (3X), and 300 μCi 7-Day Continuous Pumping (Pump)

Dose model	1X	3X	Pump
Average dose	1.24 (0.06) <sup>a</sup>	5.2 (0.6)	2.6 (0.4)
Proliferation <sup>b</sup>	0.14	3.0	0.8

<sup>a</sup> Doses in Gy. Uncertainties in parentheses were derived from standard deviations of the blood uptake data.  
<sup>b</sup> α = 1.1 Gy<sup>-1</sup>, t<sub>d</sub> = 3.2 days.

performed as an extrapolation to an infinite number of injections. The (nominal) 300-μCi pumped injection was chosen for comparison with the 300-μCi single bolus study.

The effective dose model used recurrence results for single fraction <sup>60</sup>Co to determine cell loss and proliferation parameters. All other parameters were taken from the literature or measured for the LS174T xenograft model. The effective dose model predicted results for CC49 that slightly overestimated measured times to recurrence. The magnitude of cell loss was underestimated by assuming a constant proliferation rate. A tumor size-dependent proliferation rate would imply a higher α coefficient, leaving the effective dose comparison approximately intact.

Tumor dose rates for single fraction injections decreased over time at the surface (Fig. 3a), where the well-oxygenated, rapidly proliferating cells predominated. This was due to a decrease in the activity per gram at the surface relative to the tumor center. This may be due to either clearance of MoAb from the well-perfused tissues or to the continuing proliferation of the cells in these regions. The clearance mechanism is less likely because the total tumor uptake shows a maximum at the time of significant reduction in surface dose rate. However, the greater uptake in the surface regions demonstrated by the 3X and pump injections is consistent with increased uptake in the well-perfused regions. Esteban et al.<sup>12</sup> reported that in the LS174T xenograft model using a lower-affinity TAG-72 targeted antibody (B72.3), the increased uptake in the necrotic center was due to radiation-induced cell loss and accompanying increase in the necrotic regions, rather than movement of antibody, while new growth occurred predominantly in well-perfused regions. These interpretations agree with our observations and explain why the dose-rate distribution was more consistent over time (Day 4 and beyond) for the 3X and pump injection techniques than for the 1X technique. The present observation is an example of dosimetry

for a dynamic tumor mass, discussed theoretically by Goddu et al.<sup>27</sup>

The single bolus injection technique suffered from an accelerated decrease in the activity density probably due to cell proliferation in the well-perfused regions. The tumor surface was most vulnerable to this effect because the dose rates tended to be lower due to symmetry. That is, the surface regions are irradiated by only one side, with the depth of surface dose rate reduction dependent on the beta particle ranges.<sup>28</sup>

The pump injection technique had several disadvantages in the effective dose model calculation. The model predicted a significant amount of tumor growth before the dose rates escalated to therapy levels. The disadvantage is apparent in Figure 4, in which the calculated number of cells actually increased for a time after therapy initiation. In addition, because blood activity levels were maintained at higher values over longer periods, dose to bone marrow was predicted to be greater than for a single bolus injection. Although the nominal injected values were difficult to achieve with pumping, the percent uptake and three-dimensional distribution data were expected to be representative of the nominal 300  $\mu\text{Ci}$  value. For this reason, the dose and effective dose calculations were normalized to the nominal value.

The 3X fractionated injection scheme had the advantage of an early, significant therapeutic dose together with later injections that preferentially increased dose rates in the well-perfused tissues, thus improving the spatial distribution. However, the uptake in tumor was ultimately not three times the single injection value, but only 50% more. This could have been due to several causes. Antibody may have more difficulty crossing into tissue following a therapeutic dose.<sup>29</sup> Also, there may be less target antigens or viable vessels late in therapy, decreasing the available target volume.

The differences in the results given in Table 2 for the three injection schemes were statistically significant, due both to the uptake curve and the three-dimensional distribution differences. The nonuniformity of dose rates had a dominant effect on the dose interpretation, requiring an effective dose analysis. The validity of this type of analysis for comparison of expected treatment outcome remains to be fully tested.

Issues of optimum fractionation schedules may be investigated using the effective dose model. Predicted results could be checked via therapy trials. Therapy trials currently being conducted for the injection schemes discussed here will be used to adjust the model and model parameters, in preparation for optimization calculations.

The effective dose model, as described here, simplistically presumes constant proliferation although radiation is known to have cell-cycle modifying effects.<sup>17</sup> Because the results of the model are relatively close to therapy trial results, the cumulative impact of cell-cycle effects (e.g., G2 block) was probably minor for this model. Experiments designed to verify this may be justified as an important component of effective dose model verification.

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