^{131}I Radioimmunotherapy and Fractionated External Beam Radiotherapy: Comparative Effectiveness in a Human Tumor Xenograft

Els C. Barendswaard, Joseph A. O'Donoghue, Steven M. Larson, Jörg Tschmelitsch, Sydney Welt, Ronald D. Finn and John L. Humm

Nuclear Medicine Service, Department of Medical Physics, and Clinical Immunology Service, Memorial Sloan-Kettering Cancer Center, New York; and New York Branch, Ludwig Institute for Cancer Research, New York, New York

This article compares the effectiveness of radiation delivered by a radiolabeled monoclonal antibody, ^{131}I-labeled A33, that targets colorectal carcinoma, with that of 10 fractions of conventional 320 kV_p x-rays. **Methods:** Human colorectal cancer xenografts (SW1222) ranging between 0.14 and 0.84 g were grown in nude mice. These were treated either with escalating activities (3.7–18.5 MBq) of ^{131}I-labeled A33 or 10 fractions of 320 kVp x-rays (fraction sizes from 1.5 to 5 Gy). Tumor dosimetry was determined from a similar group of tumor-bearing animals by serial kill, tumor resection and counting of radioactivity in a gamma counter. The relative effectiveness of the two radiation therapy treatment approaches was compared in terms of tumor regrowth delay and probability of tumor cure. **Results:** The absorbed dose to tumor per MBq administered was estimated as 3.7 Gy (±1 Gy; 95% confidence interval). We observed a close to linear increase in tumor regrowth delay with escalating administered activity. Equitumor response of ^{131}I monoclonal antibody A33 was observed at average radiation doses to the tumor three times greater than when delivered by fractionated external beam radiotherapy. The relationship between the likelihood of tumor cure and administered activity was less predictable than that for regrowth delay. **Conclusion:** The relative effectiveness per unit dose of radiation therapy delivered by ^{131}I-labeled A33 monoclonal antibodies was approximately one third of that produced by fractionated external beam radiotherapy, when measured by tumor regrowth delay.

Key Words: radioimmunotherapy; radiotherapy; colon cancer xenograft; tumor response; monoclonal antibody A33

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The determination of dose-response relationships for radioimmunotherapy (RIT) is more problematic than for external beam radiotherapy (XRT), due to uncertainties in the estimation of absorbed dose to tumor. Langmuir et al. (*1*) reviewed six studies in which the effectiveness of single fraction (SF) or multiple fractions (MF) of XRT was compared with RIT. These studies were conducted with a range of tumor models, antibodies, radiolabels and irradiation procedures, making direct comparisons difficult. RIT was more effective per unit absorbed dose than SF-XRT in two of five studies, less effective in two studies and of similar effectiveness in one study. Compared with MF-XRT, RIT was more effective in two of three studies and less effective in one.

A large number of biophysical factors may account for the variability in tumor response. These include initial tumor size at the start of therapy, differences in tumor vascularity and antigen density, the properties of the antibody or fragment used (e.g., affinity, biodistribution and kinetics), the mass of antibody administered, the radiolabel used, the specific activity and the absorbed dose rates achieved. Direct comparisons are further complicated by variations in the methods by which the dose was measured or calculated.

In theory, the main advantage of RIT is that radiation can be delivered selectively to subclinical tumors that are too small to be treated locally but are too large to be eradicated by chemotherapy (*2,3*). However additional advantages include the "boost" dose that RIT may provide to local disease being treated by conventional radiotherapy (*4,5*) and the delivery of radiation to regions of local dissemination in the vicinity of disease boundaries estimated by imaging modalities. Several animal model studies have shown that combining RIT with XRT can increase the therapeutic effect without increasing normal tissue toxicity (*6–8*). However, to evaluate the utility of combined modality therapy, it is necessary first to examine independently the response of the experimental systems to each modality.

This article describes the efficacy of ^{131}I-labeled monoclonal antibody A33 therapy of human tumor xenografts derived from the colorectal carcinoma cell line SW1222 grown in nude mice. This was compared to the therapeutic effects produced by a course of 10 daily fractions of XRT.

MATERIALS AND METHODS

Antibody/Tumor Xenograft Model

A33 is a murine monoclonal antibody (IgG2a) that detects a heat-stable protease- and neuraminidase-resistant, periodate/

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For correspondence or reprints contact: Els C. Barendswaard, MD, Leukemia Service, Memorial Sloan-Kettering Cancer Center, 1275 York Ave., New York, NY 10021.

reduction-sensitive epitope. The antigen is expressed homogeneously by more than 95% of colon cancers and in normal colon mucosa but not in other epithelial tissues (*9*). The A33 antibody is internalized by antigen positive cells through cytoplasmic vesicles, is transported to perinuclear regions and is subsequently exteriorized in an intact form, thus enabling the process of uptake to repeat (*10,11*). The A33 antibody was used in association with a human colon carcinoma cell line, SW1222, that was obtained from the cell culture bank at the Sloan-Kettering Institute (SKI; New York, NY). This cell line has approximately 8×10^5 antigen binding sites per cell (*12*). The internalized (acid resistant) fraction of cell surface binding A33 was 37% after 2 h incubation (*12*).

Four- to 6-wk-old female Swiss (nu/nu) athymic mice (20–25 g body weight) from our nude mouse facility were injected with $8–12 \times 10^6$ tumor cells intramuscularly in the left hind leg. After 1 wk, mice with tumors of between 200 and 800 mg were selected for the dose-response experiments.

Labeling of Monoclonal Antibodies

Monoclonal antibody A33 and corresponding control (kappa, mouse myeloma protein IgG2a) (Sigma Chemical Co., St. Louis, MO) were labeled with ^{131}I using the Iodogen method (Pierce Chemical Co., Rockford, IL). Specific activities ranged from 760 to 1,110 MBq/mg antibody for ^{131}I A33 (average 930 MBq/mg) and an average of 400 MBq/mg for the IgG2a control antibody. Assessment of immunoreactivity for ^{131}I A33 was performed as described previously (*12*). Briefly, 3.7 kBq radiolabeled antibody were added to a pellet containing 2×10^7 antigen-positive cells. After incubation for 1 h at room temperature, the pellet was washed twice with phosphate buffered saline (PBS) and was counted in a gamma counter. A pellet containing the same number of cells saturated with unlabeled antibody served as a control. Counts due to specific uptake were divided by total added counts to yield immunoreactivities from 35.9% to 55.1% (average 46.8%).

External Beam Therapy

Clinical radiotherapy typically entails the delivery of 25–35 fractions, each between 1.8–2.5 Gy, delivered at a rate of 5 fractions per week. We therefore attempted to irradiate the mouse tumors in a comparable manner. External beam fractions were delivered once per day over a period of 10 consecutive days. Total doses were determined by varying the fraction size between 0.5 Gy and 5.0 Gy. This experimental design was a compromise between using a clinically relevant fractionation schedule and eliminating variations in animal-handling procedures. Under this protocol, all animals experienced equivalent anesthesia and numbers of fractions. The only difference in the procedures was the irradiation times, which varied between 0.33 and 3.3 min per fraction.

Mice were anesthetized with 0.5 mL of 66 mmol/L 2,2,2-tribromoethanol (Pfaltz and Bauer, Inc., Waterbury, CT) in a 1.25% solution of 3-methyl-1-butanol (Sigma Chemical Co.) by intraperitoneal injection. Anesthetized mice (4–9 per group) were immobilized, with the tumor-bearing leg extended and taped in the light field of a 320 kVp x-ray unit (Philips model MG 324; Philips, Eindhoven, The Netherlands). The radiation beam was filtered by 2 mm of copper and was delivered at a dose rate of 150 cGy/min at 50 cm source-to-skin distance (SSD). The x-ray unit was calibrated using a Holt ionization chamber of 0.06-mL volume, 8-mm outer diameter, with a 1-mm build-up cap. Field flatness was within ±3%, as confirmed by Kodak V film.

Radioimmunotherapy

Four to six mice per study group were injected intravenously in the retroorbital plexus with radiolabeled monoclonal antibody in a volume of 250 μL PBS. Administered activities ranged from 0.925 to 18.5 MBq (3–24 μg) for ^{131}I-labeled A33 and from 0.925 to 14.8 MBq (2–42 μg) for ^{131}I-labeled control IgG. Tumor dosimetry was estimated from a biodistribution study in a separate group of tumor-bearing animals. This study used 1.1–2.2 MBq (0.5–2.4 μg) ^{125}I-labeled A33. Animals (average five per time point) were killed at times ranging from 1 to 170 h after injection, and tumors were excised, weighed and counted in a gamma counter (Wallac LKB model 1282; Wallac, Turku, Finland). The corresponding percentage injected dose (%ID) per gram of tumor for ^{131}I was calculated at each time point, and a biexponential function was fitted to the transformed data using nonlinear regression analysis (SAAM II; University of Washington, Seattle, WA). Previous work (*12*) examined the uptake of 1 μg ^{125}I-labeled A33 antibody mixed with increasing amounts of cold antibody in SW1222 xenografts in nude mice. This indicated that there was no significant difference in tumor uptake when 10 μg cold antibody was administered before radiolabeled material. Moreover, saturation of antigen-binding sites was not achieved by doses as high as 100 μg cold antibody, although uptake was reduced by a factor of three at this level. The maximum amount of antibody used in the current study was 24 μg. We have therefore made the assumption that there is little or no variation in mean tumor uptake for antibody doses within the range used. The corollary of this is that activity in the tumor (and thus absorbed radiation dose) scales linearly with administered activity of ^{131}I A33. The observation of a linear relationship between administered activity and tumor regrowth delay acts, to some extent, as a retrospective justification of this assumption.

Tumor Volume Measurements and Toxicity Evaluation

Perpendicular dimensions of tumors were measured by vernier calipers every 2–3 d after initiation of treatment, and the volume was calculated assuming elliptical geometry. Mice were killed when the tumor size exceeded 2 cm^3 or when signs of walking discomfort occurred. Mice were checked daily for signs of toxicity, such as petecchiae, weight loss or skin damage. All treatments were well tolerated.

RESULTS

The %ID per gram of tumor for ^{131}I-labeled A33 was obtained from data measured for ^{125}I-labeled A33. This, together with the fitted biexponential function, is shown in Figure 1. Absorbed doses to tumors were estimated assuming absorbed fractions of 1 for the beta emissions of ^{131}I and 0 for the penetrating photon emissions (*13*) and using an equilibrium dose constant of 0.111 g Gy/MBq h. Cumulative specific activities were calculated from the areas under the tumor uptake curves. The average absorbed dose to tumor per MBq injected was estimated as 3.7 Gy with 95% confidence intervals (CIs) (2.7–4.7 Gy).

Tumor growth curves for treatments by XRT are shown in Figure 2. Figure 3 shows the corresponding growth curves for RIT using ^{131}I A33. These graphs plot the median tumor size for each treatment group normalized to initial tumor size versus time from the onset of treatment. Tumor regrowth delay exhibited a linear relationship with both

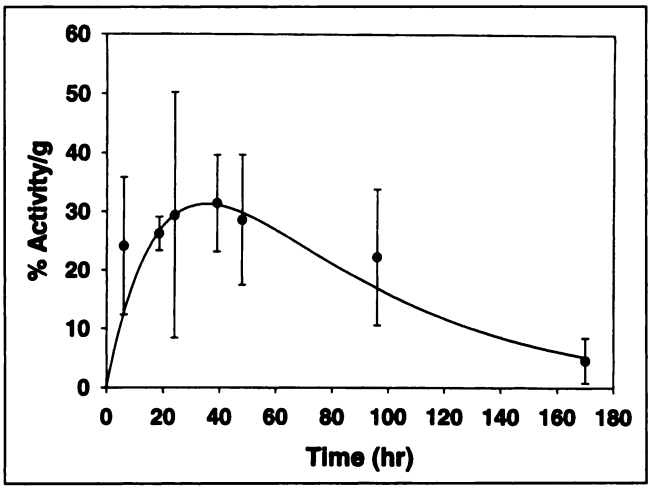

FIGURE 1. Projected tumor uptake of ^{131}I A33 in SW1222 xenografts based on measured data for ^{125}I A33. Animals were killed at various times after injection and tumors were excised, weighed and counted. Biexponential function was fitted to transformed data using nonlinear regression. Tumor absorbed dose was calculated from area under curve. This was estimated as 3.7 Gy (±1 Gy; 95% CI).

increasing external beam dose (r^2 = 0.98) and administered activity (r^2 = 0.97; Fig. 4).

Tumors were considered cured when no regrowth occurred over the period of observation (12 mo). XRT of SW1222 yielded a relationship between tumor cure probability and radiation dose with a well-defined sigmoidal shape (Table 1 and Fig. 5). A logistic function was fitted to these data by nonlinear regression. The XRT dose that produced a 50% cure probability was estimated as 19.2 Gy (SE = 0.2 Gy). For RIT, the dose-cure relationship was less predictable than for XRT (Table 1), and we were unable to generate a meaningful functional fit to the data. RIT gave occasional tumor cures at intermediate administered activities of ^{131}I A33 (3.7–7.4 MBq) corresponding to estimated mean absorbed tumor doses of 14–27 Gy. In contrast, higher administered activities (11.1–14.8 MBq) did not produce any tumor cures. The highest activity administered (18.5 MBq) resulted in tumor cures in all four mice in the group.

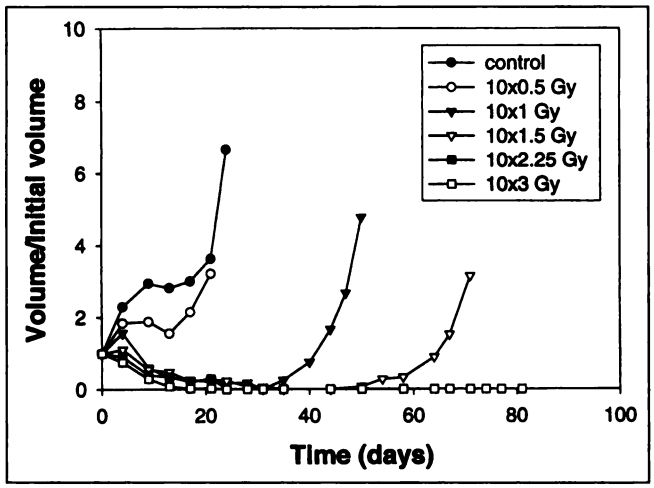

FIGURE 2. SW1222 tumor response to 10 fractions of XRT. Data plotted is median tumor size normalized to initial tumor size for each treatment group versus time from start of treatment.

It is possible to estimate the relative efficacy of tumor doses delivered by RIT or XRT by matching corresponding tumor regrowth delays or cure probabilities. For SW1222, the relationship between regrowth delay and radiation dose was approximately linear for both XRT and RIT. The relative efficacy in terms of regrowth delay was calculated as the ratio of slopes of the regrowth delay curves. The numerical values of these slopes were 4.1 d/Gy for XRT and 1.2 d/Gy for RIT. RIT of SW1222 xenografts with ^{131}I A33 was thus approximately 0.3 times as effective as XRT on a dose-for-dose basis and this was essentially independent of the dose level. Given the uncertainty associated with the estimate of tumor Gy/MBq, we estimate the 95% CIs on the relative efficacy to be 0.2–0.4. In terms of tumor cure probability, the relative efficacy could not be accurately determined due to the unpredictable relationship between RIT dose and tumor cure.

DISCUSSION

It is possible to compare RIT with XRT in two ways. One approach is to relate equi-tumor response between an administered activity of radiolabeled antibody with an accurately known absorbed dose of XRT. The advantage of this approach is that no assumptions are made about the radionuclide dosimetry. Instead, the tumor doses from RIT are ascribed values equivalent to those obtained for equi-tumor response after XRT. For example, in this article, the slope of the regrowth delay versus activity curve for RIT with ^{131}I A33 was 4.4 d/MBq. The corresponding value for XRT was 4.1 d/Gy. This means that the tumor absorbed dose according to this procedure would be approximately 1 Gy/MBq. The disadvantage of this procedure is that it presupposes that tumor irradiations by RIT and by XRT are exactly equivalent, but is incapable of testing the validity of this assumption.

The second approach, which has been used here, is an attempt to determine tumor dosimetry for RIT and to express the effectiveness of RIT versus XRT as the ratio of the absorbed doses required for an equivalent tumor response. The accuracy of this procedure is limited by the accuracy with which tumor absorbed dose can be estimated. A significant degree of variation in the tumor uptake of ^{125}I A33 was observed between the experimental animals in the biodistribution study. We must therefore assume that the absorbed doses to tumors in the therapy study were also significantly variable from one animal to another. However, an estimate of the uncertainty in tumor dose in animal studies is usually not included. For example, Langmuir et al. (*1*) discussed several studies comparing RIT with XRT, but they provided no data as to the uncertainties associated with the dosimetry. One study that did address this question was

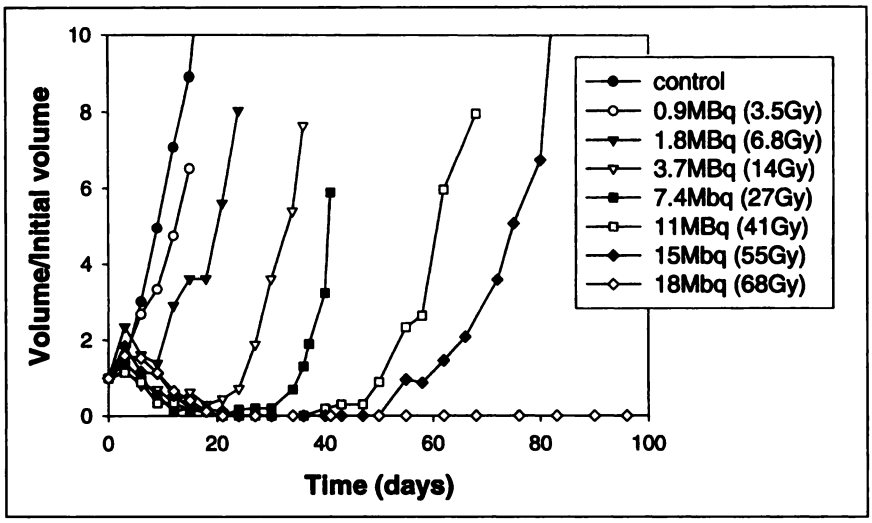

FIGURE 3. SW1222 response to increased administered activities of ^{131}I-radiolabeled A33. Data plotted is median tumor size normalized to initial tumor size for each treatment group versus time from start of treatment.

performed by Buchsbaum et al. (*14*). Their study found an SD for the absorbed dose to tumor (LS174T grown in nude mice) per 300 μCi activity of ^{131}I-labeled antibody 17-1A to be 9.5%. The dose estimates per administered activity in our article were greater, with an SD of approximately 13.5% for the individual variability in tumor uptake between mice (Fig. 1). When this uncertainty is compounded with the variability in tumor regrowth delay, we estimate the relative efficacy (RIT/XRT) to be 0.3 (±0.1) with a 95% CI. The apparent linearity of the dose-response relationships for both XRT and RIT suggests that there was little fractionation effect at the low XRT doses-per-fraction used. It also indicates that antigen saturation (leading to a plateau of antibody uptake in tumors) did not occur and that our assumption of a linear relationship between administered activity and tumor absorbed dose was reasonable.

These considerations, together with the requirement for a large number of animals to establish the biodistribution (average of five per time point), suggest that the use of image-based dosimetry would be appropriate for these types of studies. Although the accuracy with which ^{131}I activity in small tumors in animals can be measured is limited, such an approach would have the advantage of providing direct dosimetry estimates for the same tumors that are being assessed for response. In future studies, with this experimental system, we plan to investigate the use of image-based methods for estimating tumor doses.

Although there was a well-defined sigmoidal relationship between tumor cure probability and external beam dose, this was not seen with RIT. One possible reason for this

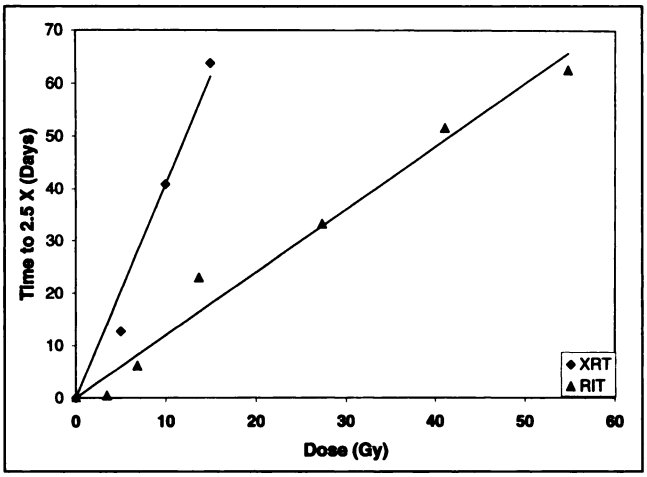

FIGURE 4. Median tumor regrowth delay as function of absorbed dose for external beam therapy (XRT) or radioimmunotherapy (RIT) using ^{131}I A33. Data were fitted by linear regression. Calculated slopes were 4.1 d/Gy for XRT (r^2 = 0.98) and 1.2 d/Gy (r^2 = 0.97) for RIT. Relative efficacy of RIT compared with XRT, calculated as ratio of slopes, was 0.29.

TABLE 1
Tumor Responses, in Terms of Regrowth Delay or Number of Cures, for SW1222 Xenografts Treated by Either External Beam Radiotherapy (XRT) or Radioimmunotherapy (RIT) with ^{131}I-Labeled A33 Antibody

Treatment	Time to 2.5 × initial size (d)*	Cures/total
XRT		
Control	5.6	0/7
10 × 0.5 Gy	18.3	0/7
10 × 1.0 Gy	46.5	0/7
10 × 1.5 Gy	69.4	1/8
10 × 2.25 Gy	N/A	7/9
10 × 3.0 Gy	N/A	7/7
RIT (MBq)		
Control	5.1	0/7
0.925	5.5	0/6
1.85	11.2	0/5
3.7	28.1	2/6
7.4	38.4	1/5
11.1	56.7	0/5
14.8	67.7	0/5
18.5	N/A	4/4

*Medians of four to six animals per group.
N/A = not applicable.

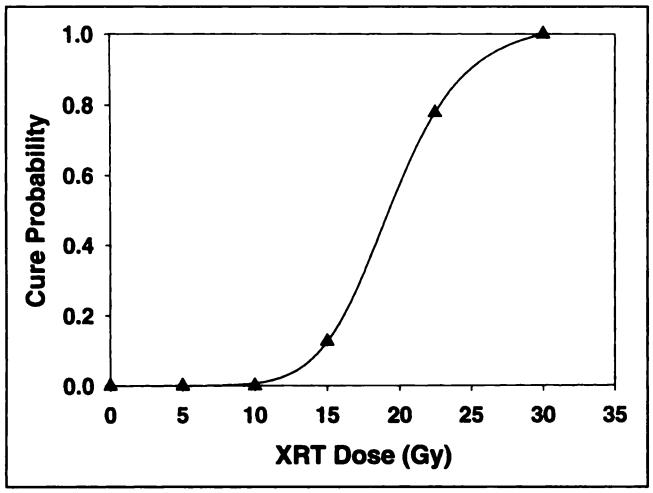

FIGURE 5. Relationship between external beam radiotherapy (XRT) dose and tumor cure probability had well-defined sigmoidal shape. Data were fitted by logistic function. XRT dose that produced 50% cure probability was estimated as 19.2 Gy with SE of 0.2 Gy.

observation is nonuniformity of absorbed dose within the tumor consequent to heterogeneity in antibody uptake. Data from a previous study (*12*) using this model system, but using humanized A33 antibody (to enable immunohistochemical staining), indicate a heterogeneous pattern of tumor uptake with antibody doses of 50 μg. Close-to-homogeneous uptake was observed with 200 μg antibody, an amount 10 times higher than the maximum used in our study. The beta particles emitted by ^{131}I have an average range of 0.37 mm and may thus contribute "crossfire" radiation to tumor regions of low uptake. This will reduce the heterogeneity of the dose distribution relative to the activity distribution but will not eliminate it. To cure a tumor, the number of clonogenic tumor cells must be reduced to zero (or close to zero). Variations in tumor microvasculature and architecture will have an impact on antibody distribution, and even microscopic tumor regions that experience a reduced dose may give rise to recurrence. The cure endpoint is thus determined on a microscopic level and may be less predictable for RIT than for external beam treatments, where doses are essentially uniform. Conversely, growth-regrowth delay is a macroscopic phenomenon and will be less affected by the microscopic pattern of energy deposition.

In most previous studies, RIT has been compared with an SF of XRT. Although this is technically simpler to perform, it does not mimic the reoxygenation, repair and regrowth conditions of clinically used fractionated radiation therapy. Our long-term goal is to systematically study the factors that influence the effectiveness of biologically targeted radionuclide therapy and to develop a scientific rationale for combining this with XRT in a clinical setting. Our study presented here represents a preliminary step in this direction.

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