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Volume Reduction versus Radiation Dose for Tumors in Previously Untreated Lymphoma Patients Who Received Iodine-131 Tositumomab Therapy

Conjugate Views Compared with a Hybrid Method

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BACKGROUND. A Phase II study of previously untreated patients with malignant low grade follicular lymphoma given a combination of unlabeled tositumomab and tositumomab labeled with iodine-131 has recently been completed. The responses of these patients have been characterized, and for some of them tumor dosimetry during therapy has been estimated not only by pretherapy tracer conjugate views but also by a hybrid method.

METHODS. Available patients were studied if they had had a pelvic or abdominal tumor evaluation by single photon emission computed tomography (SPECT) and achieved a partial response. A tumor outlined on the iodine-131 conjugate-view images was called a composite tumor. Its volume estimate came from multiple, not necessarily contiguous, regions of interest (ROI) on the pretherapy computed tomography (CT) scan. Its radiation dose was estimated from the weeklong series of pretherapy images and standard Medical Internal Radiation Dose methods. Computed tomography ROI were also grouped into smaller, contiguous volumes that defined individual tumors. Their radiation doses were estimated by the hybrid method. This method employed the activity measured for each individual tumor by a single intratherapy SPECT scan, as well as the tumor's volume, to individually normalize the composite time-activity curve as appropriate. The individual normalization factors then converted the composite radiation dose to radiation doses for individual tumors. Reduction in tumor volume was calculated for both composite and individual tumors at 12 weeks posttherapy.

RESULTS. For 14 composite tumors in 10 patients, the median pretherapy volume was 170 cm^3. Application of a sigmoidal curve function to the plot of volume reduction versus radiation absorbed dose resulted in degeneration of the curve into a straight line with a negative slope. There was no statistical significance in the relationship ($P = 0.73$). For 43 individual tumors, the median pretherapy tumor volume was 26 cm^3. The plot of volume reduction versus dose was fairly well fit by a sigmoidal curve, and the relationship approached statistical significance ($P = 0.06$). The representation assigned 56% of the shrinkage to the effects of unlabeled tositumomab. For the subset of individual tumors with a pretherapy volume less than 10 cm^3 from 6 patients ($n = 15$), the relationship was significant ($P = 0.03$). The sigmoidal representation assigned only 12% of the shrinkage to unlabeled tositumomab, as contrasted with 72% for tumors with pretherapy volume greater than 10 cm^3.

CONCLUSIONS. For patients who attained a partial response, analysis of individual tumors by a hybrid dosimetric method led to a dependence between volume reduction at 12 weeks and radiation dose that tended to be significant. The same

was not true with dosimetry of composite tumors based on pretherapy conjugate views alone. It appeared that volume reductions from both unlabeled antibody and radiation dose were important in tositumomab therapy of lymphoma patients, with unlabeled antibody relatively more important for larger tumors. ***Cancer* 2002; 94:1258–63.** © *2002 American Cancer Society.*
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KEYWORDS: non-Hodgkin lymphoma, follicular lymphoma, radioimmunotherapy, iodine-131, radiation dose, dose response, monoclonal antibody.

Radioimmunotherapy of lymphoma patients with iodine-131 (^{131}I) tositumomab has been under investigation at the University of Michigan Medical Center for over 10 years. Details of the Phase I experience with tositumomab at this center have been summarized.[1] A confirmatory, multicenter trial of chemotherapy-relapsed/refractory low-grade and transformed low-grade non-Hodgkin lymphoma (NHL) patients has reported an overall response rate of 57% and a complete response rate of 32%.[2] Results from a recently completed Phase II study of previously untreated low-grade NHL patients have also been published.[3,4] An abstract about this study has reported the correspondence between degree of response and not only radiation dose estimates from pretherapy conjugate views but also disease characteristics.[5] For a subset of these patients, an initial report on the correspondence between degree of response and radiation dose from a hybrid computed tomography–single photon emission computed tomography (CT-SPECT) conjugate-view method[6] has also been published.[7] In the current article, we report an analysis of tumor volume reduction in a subset of previously untreated low-grade NHL patients who eventually attained a partial response. For the patients, we estimated volume reduction of tumor at a single time point after therapy, rather than at all time points at which CT scans were taken. The results from the hybrid method for individual tumors are compared with those from conjugate views for composite tumors. The terms *composite* and *individual,* as applied to tumors, are defined.

PATIENTS AND METHODS

The two major criteria for entrance into the Phase II study were that patients be 1) previously untreated, and 2) diagnosed with malignant low-grade follicular lymphoma.

Baseline Computed Tomography and Administered Activity for Evaluation and Treatment

The patients received a baseline CT scan. This scan was usually obtained sometime during the week prior to the administration of a trace dose of ^{131}I–labeled tositumomab. Patients then underwent pretherapy conjugate-view imaging with a tracer dose of ^{131}I-labeled tositumomab. In all cases, it was the first time the patients were administered tositumomab and also the first time they were undergoing an intervention for their lymphoma. For the 1-week tracer evaluation, patients were given a 450-mg predose of unlabeled tositumomab infused over 1 hour to optimize biodistribution and then an infusion of tositumomab labeled with about 185 MBq (5 mCi) of ^{131}I. This evaluation was then followed by the administration on Day 7 of a therapy dose. This dose consisted of an infusion of the same amount of predose as used in the evaluation, followed by the administration of a higher activity of labeled tositumomab. This higher activity had been calculated to give a 75-centigray (cGy) total-body radiation absorbed dose based on the tracer measurements. The patients also underwent intratherapy SPECT imaging. The time point for the single scan was usually between 2 and 3 days after the therapy administration.

Estimates of Tumor Volume and Radiation Dose

Disease involvement shown on the baseline CT scan was documented by a radiologist (I. R. F.), who drew regions of interest (ROI) outlining the cancerous lymph nodes plane by plane.

A tumor outlined on the ^{131}I conjugate-view images was called a composite tumor. Its volume estimate came from multiple, not necessarily contiguous, ROI on the baseline CT scan. The association of many particular ROI into one composite tumor volume of interest (VOI) was due to the projective nature of the conjugate-view images and to the relatively poor resolution of the nuclear medicine camera-collimator system. The CT ROI were also grouped into smaller contiguous volumes that defined what were called individual tumors. This grouping was accomplished by the principal author using the CT image set, which had higher resolution and was three-dimensional. The criterion for starting an individual tumor was that the included ROI had to touch in a given plane or had to be in adjacent planes and overlap. Once an individual tumor was started, another ROI touching at least one

of its components or overlapping at least one of them from an adjacent plane was also included as part of the tumor. It was also possible for an individual tumor to consist of a single ROI.

The dosimetry of the composite tumors from conjugate views was fairly standard; details of the method have been summarized previously.[6] Dosimetric estimation for individual tumors with the hybrid method depended on four features: 1) the baseline CT was used to delineate the tumor boundary; 2) sequential tracer conjugate views for a composite tumor were used to estimate the shape of the therapy time-activity curve; 3) an intratherapy SPECT was performed to set the activity level for each tumor at that time point during therapy; and 4) the dosimetric estimates for individual tumors were obtained by appropriately multiplying the conjugate-view radiation dose estimate. The details of the method were given in two recent publications.[6,7]

After therapy with tositumomab, the patients underwent follow-up CT scans at 6, 12, and 25 weeks and every 13 weeks thereafter, usually for 1 year and for up to 2 years.

Choice of Patient and Time of Evaluation, and Criteria for a Tumor Remnant

Because of limited resources, we could not evaluate all the patients who had undergone a SPECT scan. Previous results indicated that abdominal and pelvic tumors have similar dosimetry, with axilla tumors in a different category due to either technical or, more likely, physiologic causes.[6] Since they had a greater range in their dosimetric estimates, we chose to analyze the patients with abdominal and pelvic SPECT scans. To reduce the numbers further, as well as to have a more homogeneous group, we needed to analyze either the patients who eventually attained a complete response (CR) or those with a partial response (PR), classified by standard criteria. For the analysis presented in this article, we have included only those patients who eventually were classified as having achieved a PR. The reasoning was that we wanted to assure that at least some tumors had undergone a volume reduction of less than 100%. Among 47 patients who underwent a SPECT scan covering the abdomen or pelvis, 10 were classified as having achieved a PR, and these were analyzed. An examination of the 37 CR patients in the future would also be of interest.

To further conserve resources, we also chose to evaluate tumor volume reduction from only the CT scans taken at 12 weeks posttherapy, rather than at all time points at which patients were evaluated. The rationale for this particular choice was that all patients had achieved their PR response status by this time. In the future, investigation of the degree of volume reduction at earlier and/or later time points would also be of interest. The same radiologist who evaluated the baseline CT scans also evaluated the 12-week CT scans and drew ROI for the remaining cancerous tissue. These ROI were grouped into remnants for the composite and for the individual tumors by the principal author. To be part of a given tumor remnant, ROI had to fall within the transverse and longitudinal extent of the pretherapy tumor. However, ROI did not have to meet the more stringent criteria for originally being classified as an individual tumor.

Calculation of Volume Reduction and Fitting

The number of pixels in all ROI belonging to a tumor or tumor remnant were summed. Volume was computed by multiplying by the volume of a single voxel. Percent volume reduction, R, was computed as follows:

$$R = 100 \times \frac{\text{original volume} - \text{volume at 12 weeks}}{\text{original volume}}$$

The data points for R versus radiation dose, D, were fit by a probit function.[8] This function is the sigmoidal curve that is commonly used to represent dose-response relationship. It was given by the following equation:

$$R(x) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{x} e^{-u^2/2} du$$

where

$$x = a + b \times D$$

Here, a and b were parameters to be returned by the fitting. With this function, if the variable a has a large negative value, then the curve has a sigmoidal shape beginning at the origin. If the variable a is less negative, or positive, only part of the sigmoid is present and there is a positive intercept, i.e., there is a positive volume reduction even with zero radiation dose. This latter case can represent a volume reduction from unlabeled antibody. Choosing the probit function is appropriate because it is already known that unlabeled tositumomab, administered by itself, causes lymphoma tumors to respond[9] (Knox SJ, personal communication).

RESULTS

For 10 PR patients, 14 composite tumors in the abdomen or pelvis were identified, had dose estimates

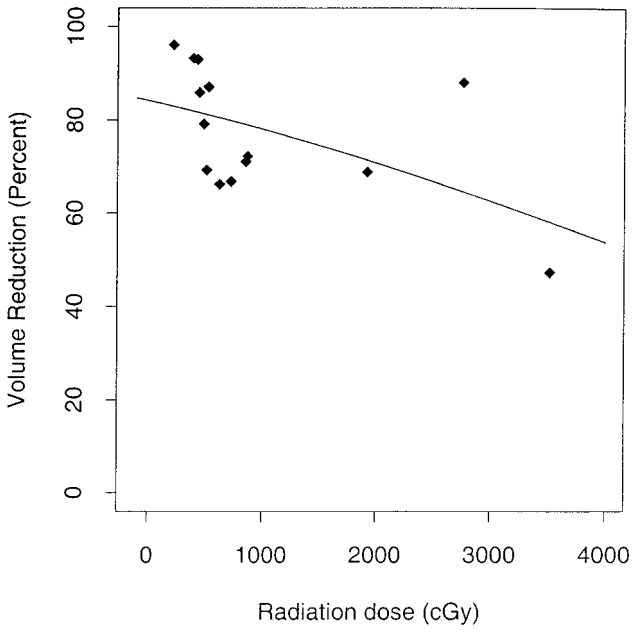

FIGURE 1. This plot shows the percentage of volume reduction versus radiation dose for 14 composite tumors. Radiation dose was estimated by pretherapy tracer conjugate views alone. The line is best fit to the data by a probit function. The usual sigmoidal shape for the function has degenerated into an almost-straight line with a negative slope.

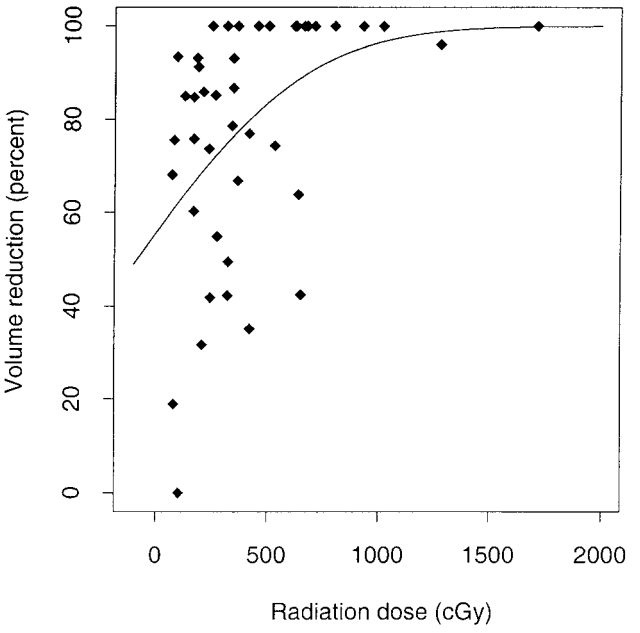

FIGURE 2. This plot shows the percentage of volume reduction versus radiation dose for 43 individual tumors. Radiation dose was estimated by hybrid computed tomography–single photo emission computed tomography conjugate views. The line is best fit to the data by a probit function. Only the upper half of the usual sigmoidal shape appears, because of a 55% volume reduction (see positive intercept) with zero radiation dose, presumably due to unlabeled antibody alone. Data points have considerable scatter about the fit, possibly due to experimental error in the estimates of both radiation dose and volume reduction.

based on conjugate views alone, and were evaluated for volume reduction. The range in pretherapy volume for these 14 composite tumors was 42 cm^3 to 847 cm^3. The mean volume was 263 $\pm$ 63 cm^3 and the median volume was 170 cm^3. (The value after $\pm$ is the standard error.) As seen from CT, a given composite tumor was composed of 1–9 individual tumors. The probit fit for the reduction-versus-dose data for composite tumors degenerated into an almost-straight line with a negative slope, as shown in Figure 1. There was no statistical significance in the relationship ($P = 0.73$).

The 14 composite tumors were comprised of a total of 43 individual tumors. These individual tumors had radiation dose estimates from hybrid CT intratherapy SPECT conjugate views. The range of pretherapy volume for these tumors was 2.0 cm^3 to 847 cm^3. The mean volume was 88 $\pm$ 25 cm^3 and the median volume was 26 cm^3. Volume reduction for these 43 individual tumors, versus radiation dose, is plotted in Figure 2. The data were fit by the probit function, with a = 0.141 $\pm$ 0.403 and b = $1.67 \times 10^{-3} \pm 1.3 \times 10^{-3} cGy^{-1}$; the dependence tended toward a significant level ($P = 0.06$). Data points had considerable scatter about the returned function, possibly due to experimental errors in the estimates of both radiation dose and volume reduction. The Pearson correlation coefficient (r) for R from the fit versus the measured R was 0.44. The volume-reduction intercept at zero radiation dose was 55.6%. We interpreted this value as the average fractional volume reduction at 12 weeks caused by the unlabeled tositumomab.

Furthermore, data for individual tumors were divided into two subsets based on pretherapy tumor volume. The probit fit for the 15 individual tumors with pretherapy volume less than 10 cm^3 distributed over 6 patients is shown in Figure 3. The fit was quite good. Compared with the relationship for tumors of all volumes, the significance of the relationship increased to $P = 0.029$, the r value increased to 0.83, and the amount of volume reduction ascribed to unlabeled antibody decreased to only 11.6%.

Tumors in the subset with volume greater than 10 cm^3 were not represented as well by the probit function, and the relationship was not significant at the 5% level of confidence ($P = 0.307$). Details of all four analyses are given in Table 1.

DISCUSSION

The result for volume reduction versus radiation dose from pretherapy conjugate views for composite tu-

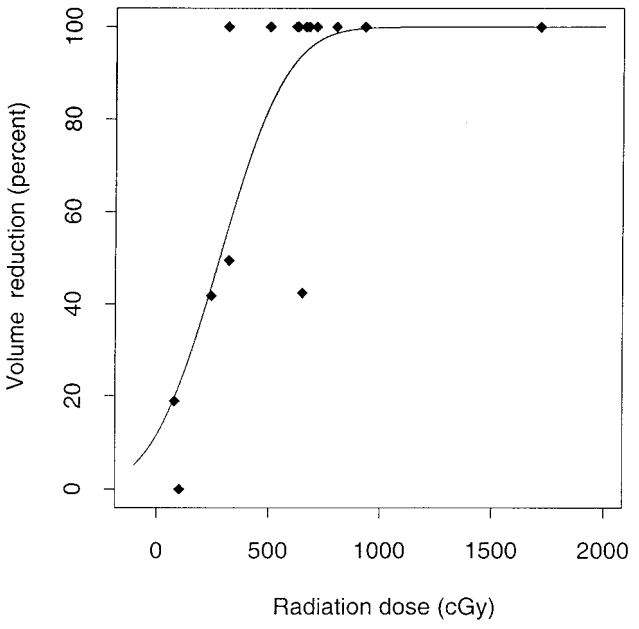

FIGURE 3. This plot shows the percentage of volume reduction versus the radiation dose for the 15 individual tumors evaluated by hybrid computed tomography–single photon emission computed tomography conjugate views. These tumors had a pretherapy volume of less than 10 g. The line is best fit to the data by a probit function. Data points match the function quite well. Only 11% of the volume shrinkage (note positive intercept) is attributed to effects from the unlabeled antibody for these small tumors. This shrinkage compares with 71.6% for tumors with a volume greater than 10 g (data and fit not shown).

mors was much less satisfactory than that from hybrid CT intratherapy SPECT conjugate views for individual tumors. That is, it did not show the reduction to be larger when the measured radiation dose was greater, as expected. An obvious reason is the possibility for cancerous lymph nodes that have different responses to therapy to be combined into a single composite tumor simply because of the accident of their alignment in an anterior-posterior projection. Another reason might be the importance of sampling the tumor activity during therapy rather than estimating it from pretherapy scanning.

The overall rationale for the method used to estimate dosimetry for the individual tumors by the hybrid method was as follows: 1) The use of sequential tracer conjugate views saves the effort needed to acquire and analyze a time sequence of SPECT image sets. 2) The intratherapy SPECT may improve accuracy by employing SPECT and by having a measurement during therapy. 3) The use of CT to outline the tumor VOI for activity assessment increases resolution compared with that available with SPECT, and even more so compared with conjugate views. Relying on CT for the outline does require a fusion of the CT image set to the SPECT image set, however. 4) Individual tumor dosimetry by multiplication of the composite tumor dose by an appropriate factor reduces effort. Some error in the gamma-ray component of the radiation dose results, but this component is less than 5% of the total, and so the error is judged to be acceptable.

The current results from the hybrid method of dosimetry need further verification. Perhaps the most reliable result was that from all tumors, since it involves the most data.

If the division into two subsets based on pretherapy volume yields two basically correct relationships, a further conclusion is that cold tositumomab has a greater effect relative to ^{131}I-labeled tositumomab for tumors with initially larger volumes compared with smaller volumes. This conclusion is supported by an argument based on accessibility. The argument rests on a decreased likelihood that the entire tumor volume will be irradiated if it is large. That is, for the larger tumors, one assumes that only the outer rim is accessible to the antibody for the 8-day time period during which most radioactive decays occur. The entire tumor is presumably accessible to antibody by further diffusion after a period longer than 8 days. Thus, radiation plays less of an overall role, and the cold antibody, by default, plays a relatively larger role. To make this argument, it helps to assume that the residence time of cold antibody in the tumor is longer than 8 days, which is unproven, although certainly possible (the tumor residence time is not necessarily equal to the blood residence time, which is shorter than 8 days). The above argument is supported by the observation that larger tumors absorb slightly less radiation than smaller tumors.[6] If the above argument is correct, the results reported are indirect evidence that the tumor residence time for tositumomab is longer than 8 days.

The time course of tumor volume reduction has not been followed. Different tumors may shrink at different rates. All patients had achieved their PR status by the time of our evaluation at 12 weeks. Some or all of the tumors may still have been shrinking, however. It is possible that all the evaluated tumors eventually reached 100% volume reduction. The patient would still meet PR criteria if tumors in a body region not scanned by SPECT remained. On the other hand, by evaluating the CT images at other time points, it may be possible to obtain a plateau value for the greatest tumor volume reduction. A plot of this plateau value versus radiation dose might yield a functional fit with better adherence of individual values to the fit. It might also be possible to establish an initial

TABLE 1
Dosimetric Method, Number of Tumors and Patients, and Results from Probit Fits

Method	Tumors included	*P* value[a]	r value[b]	Volume reduction from unlabeled antibody (%)
Hybrid CT SPECT conjugate views	All (43 tumors, 10 patients)	0.056	0.44	55.6
Conjugate views	All (14 tumors, 10 patients)	0.73	0.53	84.2
Hybrid CT SPECT conjugate views	V > 10 gm (28 tumors, 10 patients)	0.31	0.18	71.6
Hybrid CT SPECT conjugate views	V < 10 gm (15 tumors, 6 patients)	0.029	0.83	11.6

CT: computed tomography; SPECT: single photon emission computed tomography.

[a] *P* values are for the significance of the given relationship relative to no dose response relationship (i.e., a test of the probit equation with the chosen values of a and b relative to that equation with a = 0 and b = 0, which is volume reduction = 50% independent of dose).

[b] r value is the Pearson correlation coefficient for % volume reduction from fit vs. measured % volume reduction.

rate of shrinkage for the tumors. This rate itself may be correlated to the radiation dose. These possibilities are matters for future research.

The sample selection in which only partial responders were analyzed may affect our conclusions. For previously untreated patients who underwent tositumomab therapy, partial responders represented less than 30% of all patients treated (the vast majority were complete responders). To our knowledge, the volume reduction of tumors in patients who eventually obtained a CR has not been investigated in a similar analysis. According to standard criteria used to define CR, these tumors all eventually reach 100% volume reduction. However, at 12 weeks they may or may not follow the same probit function as the tumors in patients who achieve a PR. Again, this is a matter for further research.

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