^{90}Y-daclizumab, an anti-CD25 monoclonal antibody, provided responses in 50% of patients with relapsed Hodgkin's lymphoma

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Despite significant advances in the treatment of Hodgkin's lymphoma (HL), a significant proportion of patients will not respond or will subsequently relapse. We identified CD25, the IL-2 receptor alpha subunit, as a favorable target for systemic radioimmunotherapy of HL. The scientific basis for the clinical trial was that, although most normal cells with exception of Treg cells do not express CD25, it is expressed by a minority of Reed–Sternberg cells and by most polyclonal T cells rosetting around Reed–Sternberg cells. Forty-six patients with refractory and relapsed HL were evaluated with up to seven i.v. infusions of the radiolabeled anti-CD25 antibody ^{90}Y-daclizumab. ^{90}Y provides strong β emissions that kill tumor cells at a distance by a crossfire effect. In 46 evaluable HL patients treated with ^{90}Y-daclizumab there were 14 complete responses and nine partial responses; 14 patients had stable disease, and nine progressed. Responses were observed both in patients whose Reed–Sternberg cells expressed CD25 and in those whose neoplastic cells were CD25^{-} provided that associated rosetting T cells expressed CD25. As assessed using phosphorylated H2AX (γ-H2AX) as a bioindicator of the effects of radiation exposure, predominantly nonmalignant cells in the tumor microenvironment manifested DNA damage, as reflected by increased expression of γ-H2AX. Toxicities were transient bone-marrow suppression and myelodysplastic syndrome in six patients who had not been evaluated with bone-marrow karyotype analyses before therapy. In conclusion, repeated ^{90}Y-daclizumab infusions directed predominantly toward nonmalignant T cells rosetting around Reed–Sternberg cells provided meaningful therapy for select HL patients.

radioimmunotherapy | ^{90}Y-daclizumab | Hodgkin's lymphoma | myelodysplastic syndrome

Treatment with combination chemotherapy, radiation, and hematopoietic stem cell transplantation has increased the disease-free survival in Hodgkin's lymphoma (HL) from less than 5% in 1963 to more than 80% at present (1–6). Recently the US Food and Drug Administration approved brentuximab vedotin for the treatment of relapsed HL (7). Furthermore the anti-PD1 agent pembrolizumab has shown promising results in classic HL (8). Nevertheless, a significant fraction of patients do not respond to treatment or subsequently relapse. To date more than 30 different mAb preparations directed toward antigens expressed by malignant Reed–Sternberg cells have been studied (6). These include mAbs linked to drugs or toxins targeting CD25 or CD30 expressed on Reed–Sternberg cells (6–11). Brentuximab vedotin, an anti-CD30 antibody drug conjugate, has induced a significant number of responses in refractory HL (7, 11). Although other antibody immunotoxins have demonstrated some clinical efficacy, they have yielded few complete responses (CRs) (6, 9, 10). An alternative strategy has been to arm mAbs with radionuclides. Radioimmunotherapy using ^{90}Y–anti-ferritin and ^{131}I–anti-CD30 antibodies has resulted in partial (PRs) and CRs in HL (12–15). Deficiencies with these approaches reflect the lack of tumor specificity of ferritin-targeted antibodies and the small number of CD30-expressing Reed–Sternberg cells in the tumor.

As an alternative, we identified CD25, the IL-2 receptor alpha subunit (IL-2Rα), as a more favorable target for systemic radioimmunotherapy of HL (16–22). The scientific rationale is that, with the exception of Treg cells, CD25 is not expressed by normal resting lymphoid cells, but it is expressed on both a minority of Reed–Sternberg cells and, critically, on T cells rosetting around Reed–Sternberg cells in HL (6, 23, 24). ^{90}Y, an energetic β particle emitter with a mean tissue path length of 5 mm and a maximal path length of 11 mm, acts through "crossfire" throughout tumor masses, providing a strategy for killing tumor cells at a distance of several cell diameters, including Reed–Sternberg cells that lack CD25 expression provided that T cells in their vicinity

Significance

Despite advances, a significant proportion of patients with Hodgkin's lymphoma (HL) will not respond or will relapse. We demonstrated that up to seven infusions of ^{90}Y-daclizumab, an anti-CD25–directed monoclonal antibody, provided responses in 50% of patients with relapsed HL. The daclizumab was directed primarily not at tumor cells themselves but toward nonmalignant T cells rosetting around the Reed–Sternberg cells. ^{90}Y provided strong β emissions that killed antigen-nonexpressing tumor cells at a distance by a crossfire effect. Furthermore, the strong β irradiation killed normal cells in the tumor microenvironment that nurture the malignant cells in the lymphomatous mass. Therefore ^{90}Y-daclizumab infusions provide meaningful therapy for select HL patients.

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express the target antigen (16, 23, 24). In the current phase II trial we treated 46 patients with recurrent or refractory HL with ^{90}Y-daclizumab every 6–10 wk for up to seven doses, depending on hematological recovery. The activity of ^{90}Y used in the present trial was determined on the basis of three previous phase I/II dose-escalation trials of ^{90}Y–anti-CD25 performed in patients with lymphoproliferative disorders (16).

Results

Patient Characteristics. Thirty consecutive patients with refractory or relapsed HL were studied between April 2003 and October 2007; an additional 16 consecutive patients were studied between November 2009 and June 2014. The patients had a median age of 35 y (range 22–77 y); 29 were men, and 17 were women (Table S1). Patients had received a median of five prior chemotherapy regimens (range, 1–21). Twenty-five had undertone autologous stem-cell transplantation alone, seven patients had both an autologous and an allogeneic transplant, and 14 did not have a transplant. Using immunohistochemical analysis, in only 10 biopsies (in 44 patients) did >10% of the HL neoplastic cells express CD25 (Fig. 1*A*), whereas the associated rosetting T cells expressed CD25 in all the 44 cases examined (Fig. 1*B*). Patients were treated with an initial dose of 15 mCi ^{90}Y-daclizumab, with the exception that an initial dose of 10 mCi was used for patients who had received a prior stem cell transplant (Table S2). Patients without progressive disease and with hematological recovery received up to seven cycles of 15 mCi ^{90}Y-labeled daclizumab. There was a 6-wk interval between cycles. A total of 189 cycles of treatment were administered to 46 patients with a median aggregate radiation dose of 39 mCi (range, 10–90 mCi).

^{111}In-Labeled Daclizumab Imaging. Simultaneous with the administration of therapeutic ^{90}Y-daclizumab, ^{111}In-labeled daclizumab was administered to identify biodistribution and tumor targeting (Fig. 2). All patients, including those whose Reed–Sternberg cells did not express CD25, had positive localization of ^{111}In-daclizumab at disease sites. The estimated tissue radiation dose per 15 mCi of ^{90}Y-daclizumab was 173 cGy to bone marrow, 263 cGy to liver, 1,062 cGy to spleen, and 33 cGy to the whole body (Table S3). The estimated tumor dose with 15 mCi ranged from 210 to 365 cGy.

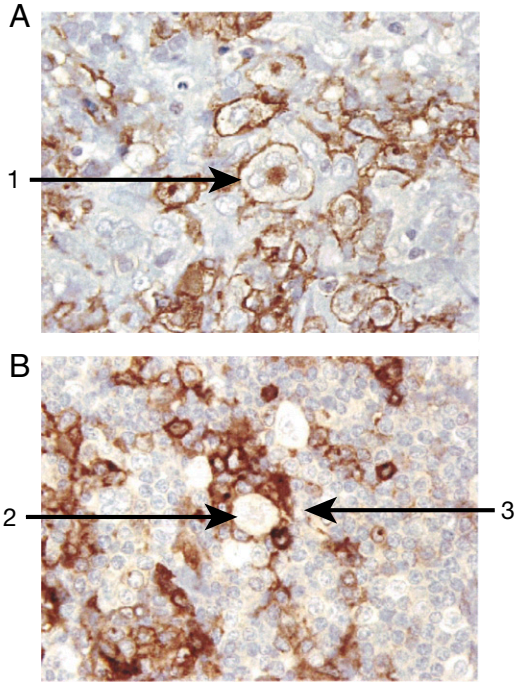

Fig. 1. Histochemical analysis of CD25 expression was performed in patients with HL. (*A*) Ten patients manifested CD25^{+} expression by both the Reed–Sternberg HL cells and the associated rosetting polyclonal T cells (1). (*B*) Thirty-four patients manifested no expression of CD25 on the Reed–Sternberg cells (2) but expression of CD25 on the polyclonal T cells rosetting around the Reed–Sternberg cells (3).

Toxicity. There were no acute infusion reactions. Isolated patients had the following grade 3 or greater nonhematopoietic toxicities: grade 3 hypoalbuminemia, serum glutamic pyruvic transaminase (SGPT; alanine aminotransferase) elevation, hyperglycemia, hypocalcemia, lipase elevation, and grade 3 or 4 pneumonitis/pulmonary infiltrates. Thrombocytopenia and neutropenia were predominant toxicities initiating at weeks 4–5 after ^{90}Y-daclizumab infusion, with nadirs usually occurring during weeks 5–7 (Table S4). Thrombocytopenia became more profound with the cumulative toxicity of multiple dosing. In seven patients, hematocytopenia, especially thrombocytopenia, persisted for more than 10 wk after treatment, requiring removal from the study. In all patients hematological values recovered to baseline values.

Six of the original 30 patients observed over a 7- to 11-y period following ^{90}Y-daclizumab administration developed myelodysplastic syndrome (MDS) (Table S2). These six patients had had a mean of 6.2 prior therapies. Five had received an autologous hematopoietic stem cell transplant, and one had received an allogeneic transplant as well. Four had prior radiation to the lesions. These six patients received a mean of 41 mCi ^{90}Y-daclizumab in a mean of 3.2 cycles of therapy. The mean time from diagnosis of HL to diagnosis of MDS was 99 mo, and the median time from the initiation of ^{90}Y-daclizumab to the diagnosis of MDS was 7 mo (range, 6–63 mo). Three of these patients received chemotherapy between the last dose of ^{90}Y-daclizumab and the diagnosis of MDS. In five of the six patients there was a disorder of chromosome 7, with other associated chromosomal abnormalities. The sixth patient had a translocation ± interstitial deletion of chromosome 6 and chromosome 9. A retrospective review revealed that one patient had cytogenetic aberrations on chromosomes 5 and 7 following chemotherapy before any systemic radioimmunotherapy with ^{90}Y. The other five patients had not had a bone-marrow karyotype analysis before ^{90}Y-daclizumab administration. On the basis of this observation the clinical trial protocol was amended to require pretreatment marrow cytogenetic studies before the initiation of systemic radioimmunotherapy and the exclusion of patients with chromosomal aberrations. Two patients found to have such aberrations at postchemotherapy evaluation were excluded from the study. None of the 16 patients entered with this criterion developed MDS over a mean 42-mo period of observation.

Immune Response to Daclizumab. Five patients, including three responding patients, developed nonsymptomatic human anti-human antibody (HAHA) titers to daclizumab after one or more treatments.

Clinical Responses. Of 46 patients with refractory or relapsed HL, as evaluated by Response Evaluation Criteria in Solid Tumors, who were treated with ^{90}Y-daclizumab, 14 achieved a CR, nine had a PR, 14 had stable disease, and nine progressed (Table S2). Among 40 patients with classical HL there were eight PRs and 12 CRs; 13 had stable disease, and seven progressed. Among the six patients with the nodular lymphocyte-predominant (NLP) form of HL there were two CRs and one PR; one patient had stable disease, and two patients progressed. Tumor responses were evaluated by ^{111}In-daclizumab single photon-emission computed tomography (SPECT) imaging (Fig. 2*A*), fluorodeoxyglucose (FDG) PET scans (Fig. 2*B*), and computed tomography (CT) scans (Fig. 3). The CRs were documented after two to five treatments. PRs or CRs were observed in 4 of the 10 patients (40%) with classical HL whose Reed–Sternberg cells expressed CD25 and in

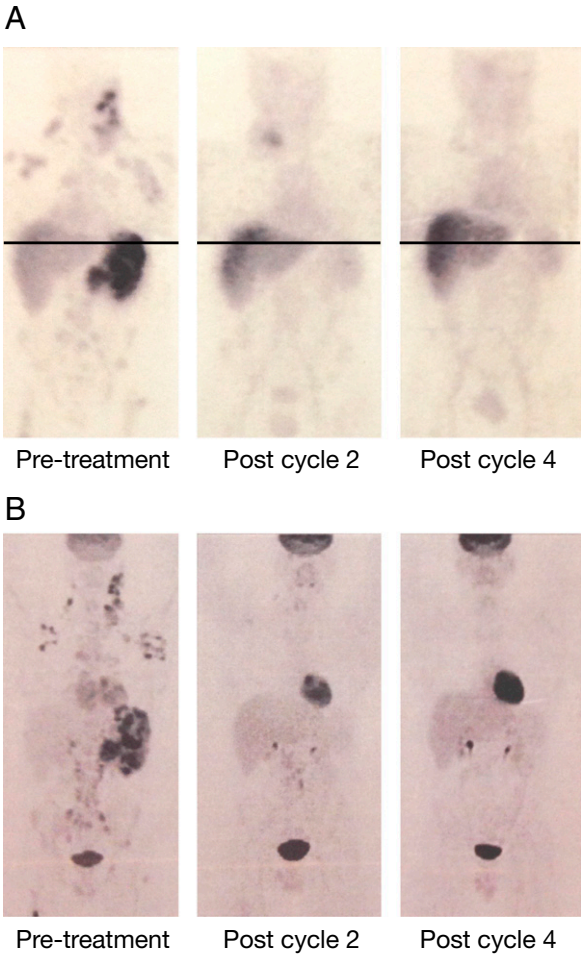

Fig. 2. Clinical response of a patient with HL was demonstrated with ^{111}In-daclizumab and FDG-PET. (*A*) ^{111}In-daclizumab maximal intensity projection images (MIP) SPECT imaging studies of patient 1. (*Left*) At the time of the initial treatment with ^{90}Y-daclizumab, there is localization in lymph nodes, bone, and spleen. (*Center*) After two treatments a decrease is seen in spleen, bone, and nodal uptake (the new uptake in the right base of the neck is related to central line placement). (*Right*) There is resolution of all abnormal uptake after four cycles. Findings with ^{111}In-daclizumab were congruent with FDG findings. Note that the chest and abdomen/pelvis views were obtained in two separate acquisitions with slight overlap and then spliced together manually for display purposes. (*B*) Corresponding FDG-PET scans. (*Left*) Scan before treatment showing involvement in lymph nodes, spleen, and bone. (*Center*) At the time of the second treatment most of disease has resolved, with the exception of some nodes below the diaphragm. (*Right*) The images at the time of fourth treatment show complete resolution of disease with a progression-free survival of 400 d.

19 of 36 patients (53%) whose malignant cells were CD25$^-$ but whose associated infiltrating T cells expressed CD25 (Fig. 1 and Tables S1 and S2). Freedom from progression ranged from 28 to 788 d with a median response duration of 151 d (range, 28–720 d). The median CR duration was 328 d, not counting the period in a PR (Figs. S1 and S2).

Correlative Studies

Impact of Therapy on the Number of Circulating Treg Cells. The number of circulating Treg cells in the 12 patients of the final group of 16 patients for whom adequate material was available before and after therapy was assessed by the number of CD3$^+$CD25$^+$ (7G7$^+$) FoxP3$^+$ cells before and after ^{90}Y-daclizumab therapy. When assessed by the nonparametric Wilcoxon matched-pair signed-rank test on days 5–7 after administration of ^{90}Y-daclizumab, there was a significant ($P = 0.0044$) reduction in the number of circulating Treg cells in each of the 12 cases examined, compared with the number pretherapy for the same patient. The reduction in Treg cells probably represents crossfire from radiolabeled antibody circulating in the blood, marrow, or spleen rather than depletion of Treg cells that is specific to antibody binding, because free Treg cells in the circulation would not be effectively exposed to crossfire of the antibody targeting them directly.

Immunohistochemistry to Define Apoptosis by Enumerating Cells Expressing Cleaved Caspase-3. The apoptosis induced by ^{90}Y-daclizumab therapy was defined by enumerating the percentage of cells expressing cleaved caspase-3 before and 4–10 d after treatment. The absolute number of cleaved caspase-3$^+$ cells was counted at 40× magnification over five high-power fields. There was more evidence of apoptosis in the Reed–Sternberg cells in patients biopsied less than 1 wk after treatment than in patients biopsied 7–10 d after treatment (Fig. S3).

Use of Phosphorylated H2AX as a Bioindicator of the Effects of the Exposure of HL Tissue to Radiation. One major outcome from radiation exposure is the formation of DNA double-strand breaks (DSBs). DSBs can be enumerated with a sensitive assay based on the immunohistochemical visualization of phosphorylated H2AX (γ-H2AX) (25–29). γ-H2AX immunostaining was enumerated in both malignant cells and surrounding nonmalignant cells in tumor biopsies before and 4–10 d after ^{90}Y-daclizumab infusions. Very modest increases in γ-H2AX expression were observed in malignant cells, which had 1 to more than 60 foci per cell, compared with the population of surrounding normal cells, which had less than one focus per cell on average (Fig. 4). Increased γ-H2AX incidence after ^{90}Y-daclizumab therapy, as compared with the pretherapy value, was observed in malignant cells in four patients. In two patients, however, the γ-H2AX signal was reduced in malignant cells after ^{90}Y-daclizumab therapy. In contrast to the

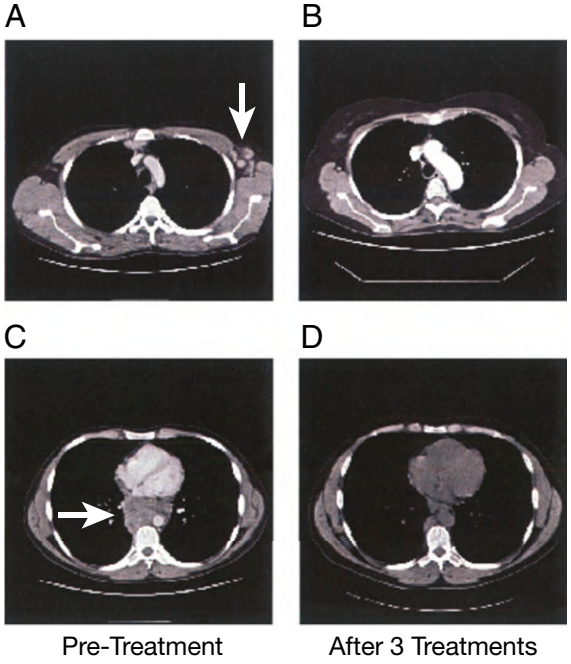

Fig. 3. CT scans demonstrating clinical response of a patient with HL. (*A* and *C*) CT scans of the thorax of a patient before treatment show enlarged nodal disease (arrows). (*B* and *D*) After three cycles of ^{90}Y-daclizumab therapy there was complete resolution of disease in three axillary nodes and almost complete resolution of posterior mediastinal disease.

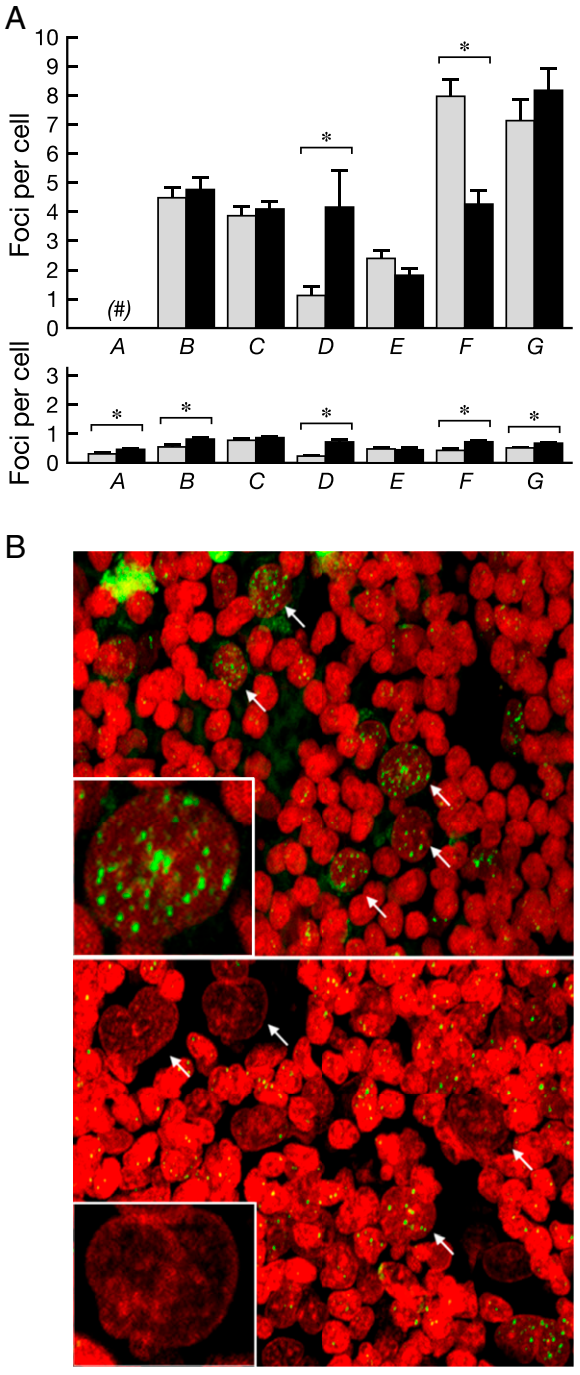

Fig. 4. Analysis of γ-H2AX in lymph node biopsies from patients undergoing ^{90}Y-daclizumab therapy. (*A*) γ-H2AX incidence in malignant cells (*Upper*) and surrounding nonmalignant cells (*Lower*) is shown in six and seven patients, respectively, and is presented as the average number of foci per cell ± SE. Gray bars indicate incidence before therapy; black bars indicate incidence following therapy. Asterisks indicate a statistically significant difference ($P < 0.05$) in malignant or in surrounding nonmalignant cells before and after treatment. (*B*) Representative confocal images of biopsies from a patient before (*Upper*) and after (*Lower*) ^{90}Y-daclizumab therapy. (*Insets*) Malignant cells at higher magnification. Note the high variation in the incidence of γ-H2AX foci among malignant cells. Green, γ-H2AX; red, DNA stained with propidium iodide. Arrows point to malignant cells.

modest effects on malignant cells, ^{90}Y-induced DNA damage was more evident in nonmalignant cells in the tumor microenvironment, as reflected by the increased expression of γ-H2AX in six of the seven patients examined; in five of these six patients the increase in γ-H2AX expression following ^{90}Y-daclizumab administration reached statistical significance.

Discussion

mAbs are among the most rapidly expanding classes of therapeutics for the treatment of cancer (21). Although not approved in this setting, rituximab (chimeric anti-CD20) is the only commercially available unmodified mAb routinely demonstrating antitumor activity in HL (6, 30). Arming of mAbs by linking them to cellular toxins, drug conjugates such as brentuximab vedotin (11), or radionuclides to target these agents specifically to tumors may provide valuable augmentation of antitumor activity. Indeed the US Food and Drug Administration has approved brentuximab vedotin for the treatment of relapsed HL after failure of autologous stem cell transplantation or after the failure of at least two prior multiagent chemotherapeutic regimens (7). Thirty-two percent of patients with HL had CRs, with a median response duration of 6.7 mo. Nevertheless there is a need for therapeutic agents in patients with refractory or relapsed HL following brentuximab vedotin. One advantage of the use of radiolabeled mAb conjugates for therapy is that, with appropriate choice of radionuclide, radiolabeled mAbs can kill cells at a distance of several cell diameters and thereby may kill antigen-negative tumor cells adjacent to antigen-expressing cells (6, 16, 31–33).

Various mAbs with different antigenic targets have been used to deliver targeted radioimmunotherapy (6, 12–16, 21, 31–33). The current study used daclizumab (humanized anti-Tac, i.e., anti-CD25; Zenapax) armed with the energetic β-particle emitter ^{90}Y. Daclizumab targets the 55-kDa IL-2Rα (CD25) subunit that is constitutively expressed on Treg cells but not on other resting normal cells (18, 34). In contrast, CD25 is overexpressed on certain lymphoid malignancies, on activated T cells involved in autoimmune disorders, and in allograft rejection (16–22, 34). Increased CD25 expression has been demonstrated in anaplastic large-cell lymphoma, adult T-cell leukemia (ATL)/lymphoma, chronic lymphocytic leukemia, cutaneous T-cell lymphoma, hairy cell leukemia, some B-cell non-Hodgkin's lymphomas, and HL (16–24, 35). Unmodified murine anti-CD25 was evaluated in a trial involving patients with ATL, a malignancy of mature $CD4^+CD25^+$ immunosuppressing T lymphocytes (17, 34). Seven of 19 treated patients responded to the antibody (17). In a subsequent study, 9 of 16 evaluable patients with ATL responded to ^{90}Y-labeled murine anti-CD25 (16, 34).

In the present study, 46 patients with relapsed or refractory HL received 10–15 mCi of ^{90}Y-daclizumab every 6–10 wk for up to seven doses. Toxicity in 40 of 46 cases was primarily limited to thrombocytopenia and granulocytopenia. In addition, at a median follow-up of 9 y, 6 of the initial 30 patients who had not had bone-marrow karyotype analyses before ^{90}Y therapy developed MDS, but none of the 16 patients who had pretherapy cytogenetic evaluations developed MDS. The overall incidence of MDS for all 46 patients was 13%. The median time from the initiation of ^{90}Y-daclizumab to the diagnosis of MDS was 37 mo. In one case, retrospective review revealed that the patient had cytogenetic aberrations on chromosomes 5 and 7 following chemotherapy but before systemic radioimmunotherapy with ^{90}Y-daclizumab. Therefore, clinical trial entry criteria were changed to mandate a bone-marrow karyotype analysis before ^{90}Y-daclizumab therapy and the exclusion of patients with aberrations. None of the 16 patients entered with this criterion developed MDS.

Therapy-associated MDS is a serious and well-recognized late complication that typically occurs after extensive exposure to alkylating agents and/or inhibitors of topoisomerase II in the treatment of lymphoid malignancy. The incidence of treatment-related MDS (tMDS) after therapy with the radiolabeled mAbs

^{90}Y-ibritumomab tiuxetan and ^{131}I-tositumomab has been analyzed in two large series (36, 37). Nineteen of 746 patients with non-Hodgkin's lymphoma treated with ibritumomab tiuxetan developed tMDS or treatment-related acute myeloid leukemia (tAML) 1.9 y (range, 0.4–6.3 y) after radioimmunotherapy, for an incidence of 2.5% (36). Bennett and coworkers (37) reported tMDS/tAML in 35 (3.5%) of 995 patients, 40% of whom had documented preexisting MDS before radioimmunotherapy. No case of tMDS/tAML was reported in any of the 76 patients receiving ^{131}I-tositumomab as initial therapy (38).

In our patients a mean of 9.9% (range, 5.8–21.7%) of the bone-marrow cells expressed CD25 before therapy with ^{90}Y-daclizumab. The red marrow received 172.5 cGy of radiation per 15 mCi dose of ^{90}Y-daclizumab (Table S1). The six patients who developed MDS received a mean of 3.2 doses. As noted above, one of the patients was found to have had aberrations of chromosomes 5 and 7 after chemotherapy but before the initiation of the systemic radioimmunotherapy. The present study was too small to draw meaningful conclusions about the relative roles played by the multiple courses (mean, 6.2) of chemotherapy that patients who developed MDS received before ^{90}Y-daclizumab treatment and the role of the systemic radioimmunotherapy in the pathogenesis of MDS in the patients in this study. Nevertheless this serious complication represents a concern, and on the basis of this observation any subsequent clinical trial should require pretreatment cytogenetic studies of bone-marrow specimens before the initiation of systemic radioimmunotherapy.

In contrast to previous results with immunotoxins and with select alternative antigenic targets identified by radiolabeled antibodies, the response rate to ^{90}Y-daclizumab therapy when used as a single agent was impressive. Among the 46 patients treated with multiple doses of 10–15 mCi ^{90}Y-daclizumab, 14 achieved a complete remission, and 9 manifested a PR. In a separate study of unconjugated antibody using saturating amounts of daclizumab in 22 patients with Hodgkin's disease, there were no PRs or CRs, and there were only five minor responses that did not meet the PR criteria of ≥50% reduction. Thus, the radionuclide ^{90}Y appears to be critical in the responses observed. One factor that may be involved in the improved response is that, in contrast to most previous systemic radioimmunotherapy trials, we used repeated dosing that permitted an increase in the total dose of radiation delivered to the tumor. This approach can be compared with ^{90}Y-ibritumomab that was capped at a maximum total dose of 32 mCi. However, a comparison of a single large dose with multiple lower doses would be of value. None of the 14 patients who achieved a complete remission did so following a single dose of ^{90}Y-daclizumab; at least two to five administrations were required. A number of additional factors may underlie this greater level of efficacy as compared with immunotherapeutic agents that target other HL antigens. In particular, one limitation in targeting the antigen CD30 is that it is expressed predominantly on HL Reed–Sternberg cells that represent less than 1% of the lymphoid cells in the tumor mass. Thus, relatively few CD30 targets are available to bind the antibody. In contrast, CD25 is expressed by some Reed–Sternberg cells and is overexpressed on the polyclonal rosetting T cells associated with Reed–Sternberg cells (23, 24). This expression greatly increases the number of targets for binding the radiolabeled antibody, thereby increasing the amount of radiation delivered locally to the tumor. Another pivotal advantage is that ^{90}Y has a high β-energy emission maximum, approximately five times greater than that of ^{131}I, and delivers a significantly higher radiation dose to the tumor and within 5–11 mm of the site of decay. Therefore, ^{90}Y-targeted mAbs can kill antigen-negative tumor cells through a crossfire effect from neighboring CD25-expressing T cells that have been targeted by the mAb. This ability to kill non–CD25-expressing malignant cells is pivotally important in the efficacy of ^{90}Y-daclizumab in the majority of patients whose Reed–Sternberg cells did not express CD25 but whose tumor-associated T cells did express this antigen. Another mechanism supported by the γ-H2AX studies that could underlie the increased efficacy observed with the present strategy may be an effect of the β irradiation on normal cells in the tumor microenvironment that nurture the malignant cells in the lymphomatous mass.

Although 14 patients manifested a CR, the lack of cures indicates that monotherapy with this agent is not optimal. In our plans for patients who have not received an autologous bone-marrow transplant, we have initiated a clinical trial that includes the administration of escalating doses of ^{90}Y-daclizumab followed by high-dose chemotherapy using carmustine, etoposide, cytarabine, and melphalan (BEAM) in association with an autologous hematological stem cell transplant using cells harvested from the patient before the administration of ^{90}Y-daclizumab (39). This use of an autologous bone-marrow transplant should permit an escalation of ^{90}Y-daclizumab doses, because the hematological stem cell transplant should ameliorate the otherwise dose-limiting hematological toxicity of the radiolabeled anti-CD25 mAb.

Materials and Methods

This study was approved by the Institutional Review Board of the National Cancer Institute, Protocol 95-C-110. This trial is registered at NCT00001575. All patients gave written informed consent.

Study Population. Forty-six patients with histologically confirmed HL and expression of CD25 (IL-2Rα) on at least 10% of Reed–Sternberg cells in classical HL or on tumor-infiltrating T cells were entered into the study. (See *SI Materials and Methods* for further details.)

^{90}Y-^{111}In–Labeled Daclizumab. Daclizumab (Hoffmann LaRoche, Nutley, NJ) was conjugated with 2-p-isothiocyanatobenzyl-transcyclohexyldiethylenetriamine penta-acetic acid (CHX-A) and was radiolabeled with ^{90}Y for therapy and with ^{111}In for imaging as described previously (16). (See *SI Materials and Methods* for further details.)

Study Design and Treatment. This was a single-institution, nonrandomized, open-label phase II trial of up to seven infusions of ^{90}Y-daclizumab in patients with refractory or relapsed HL. Patients without a prior stem cell transplant received 15 mCi ^{90}Y-daclizumab with 5 mg of unlabeled daclizumab as the initial dose; patients with a prior transplant received an initial dose of 10 mCI. (See *SI Materials and Methods* for further details.)

Use of γ-H2AX as a Bioindicator for the Exposure of HL Tissue to Radiation. γ-H2AX was used as a bioindicator of the effects on HL tissue of exposure to radiation using procedures described previously (25, 26). (See *SI Materials and Methods* for further details.)

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