



William Wolk. *Mist in the Valley*. Oil on canvas, 66" × 36".

Radioimmunotherapy is a strategy designed to increase the efficacy of native monoclonal antibodies, decrease the toxicity of therapy, and improve the long-term outcome for patients with leukemia.

Radioimmunotherapy for Acute Leukemia

John M. Burke, MD, Joseph G. Jurcic, MD, David A. Scheinberg, MD, PhD

Background: *The use of monoclonal antibodies to deliver radioactive isotopes directly to tumor cells has become a promising strategy to enhance the antitumor effects of native monoclonal antibodies. In this article, we summarize the role of radioimmunotherapy in the treatment of leukemia.*

Methods: *The authors reviewed the published clinical trials of radioimmunotherapy in acute leukemia.*

Results: *Radioimmunoconjugates that emit β -particles, such as ^{131}I -anti-CD33, ^{90}Y -anti-CD33, ^{131}I -anti-CD45, and ^{188}Re -anti-CD66c, deliver significant doses of radiation to the bone marrow and may be particularly effective when used as part of a conditioning regimen for hematopoietic stem cell transplantation. Radioimmunoconjugates that emit short-ranged α -particles, such as ^{213}Bi -anti-CD33, are better suited for the treatment of low-volume or residual disease.*

Conclusions: *Radiolabeled antibodies can be administered safely to patients with advanced leukemias and have significant antileukemic activity. Radiolabeled antibodies can potentially intensify the antileukemic effects of conditioning regimens when used in conjunction with hematopoietic stem cell transplantation. Whether or not radiolabeled antibodies improve the outcome of patients with leukemia remains to be demonstrated by randomized studies.*

Introduction

Despite advances in therapy, only about 20%-30% of patients with acute myelogenous leukemia (AML)

and 30%-40% of adults with acute lymphoblastic leukemia (ALL) achieve long-term disease-free survival.^{1,2} The most common cause of treatment failure is relapse. In addition, the toxicity of chemotherapy and complications of hematopoietic stem cell transplantation contribute significantly to mortality rates.

From the Department of Medicine (JMB) and the Leukemia Service (JGJ, DAS) at the Memorial Sloan-Kettering Cancer Center, New York, New York.

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Address reprint requests to John M. Burke, MD, Department of Medicine, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021. E-mail: burkej@mskcc.org

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Treatment with monoclonal antibodies (MAbs) has the potential to improve efficacy and decrease toxicity by targeting therapy to specific cell types and sites of disease. Native MAbs can be used to focus an inflammatory response against a tumor cell. The binding of MAbs to a target cell can result in complement

activation, thereby initiating a number of biologically important effects that disrupt the integrity of the cell membrane. Cells with antibody and complement on their surfaces may also be engulfed, or opsonized, by macrophages. Another important mechanism for tumor cell killing by native MAbs is antibody-dependent cell-mediated cytotoxicity (ADCC), in which an effector cell expressing an Fc receptor binds to a cell-bound MAb and is triggered to kill the target cell. Monocytes, macrophages, natural killer cells, and neutrophils can affect antibody-dependent cell-mediated cytotoxicity.

Chimeric and humanized antibodies have been constructed to overcome the weak antitumor activity and the immunogenicity of many murine MAbs. These antibodies retain the binding specificity of the original rodent antibody determined by the variable region but can potentially activate the human immune system through their human constant region. Clinical trials have demonstrated that both rituximab, a chimeric anti-CD20 MAb, and Campath-1H, a humanized anti-CD52 MAb, have activity against chronic lymphocytic leukemia.^{3,4} However, the activity of native antibodies in acute leukemias is more limited. The humanized anti-CD33 MAb HuM195 has been shown to eliminate minimal residual disease detectable by reverse transcription-polymerase chain reaction (RT-PCR) in patients with acute promyelocytic leukemia (APL)⁵ and to produce rare complete remissions in patient with

relapsed or refractory AML, but only in patients with low leukemic burdens.^{6,8}

In order to increase the antitumor effects of native antibodies, drugs and bacterial toxins have been conjugated to MAbs. For example, gemtuzumab ozogamicin consists of a humanized anti-CD33 antibody linked to calicheamicin, a potent tumor antibiotic. Gemtuzumab ozogamicin has produced remissions in 30% of carefully selected patients with relapsed AML.⁹ BL22 is a recombinant immunotoxin consisting of the variable domain of an anti-CD22 antibody fused to a fragment of *Pseudomonas exotoxin*. In a recent trial, BL22 resulted in an 81% response rate in patients with hairy cell leukemia refractory to cladribine.¹⁰ Table 1 lists the labeled and unlabeled antibodies that have been used to treat leukemias in recent trials.

In an alternative approach, antibodies can be used to target radioisotopes directly to sites of disease in order to increase the antitumor effects of native MAbs. This article reviews the role of radioimmunotherapy with both α -particles and β -particles in the treatment of leukemia.

Isotope Selection

When selecting a radioisotope for clinical use, the characteristics of the isotope must be considered,

Table 1. — Unlabeled and Labeled Antibodies Used to Treat Leukemia

Antibody (ref)	Antigen	Disease	Comments
Rituximab ³	CD20	CLL	Mediates ADCC, CMC; interrupts signaling pathway
Campath-1H ⁴	CD52	CLL, PLL	Mediates ADCC, CMC
HuM195 ⁵⁻⁸	CD33	AML, MDS, APL	Mediates ADCC, CMC
Gemtuzumab ozogamicin ⁹	CD33	AML	Delivers calicheamicin
BL22 ¹⁰	CD22	HCL	Delivers <i>Pseudomonas exotoxin</i>
¹³¹ I-M195 ¹¹⁻¹³	CD33	AML, MDS, myeloblastic CML	Delivers β -particle emitter
¹³¹ I-HuM195 ¹³	CD33	AML, MDS, myeloblastic CML	Delivers β -particle emitter
⁹⁰ Y-HuM195 ¹⁴	CD33	AML, CML	Delivers β -particle emitter
²¹³ Bi-HuM195 ¹⁵	CD33	AML, CML	Delivers β -particle emitter
¹³¹ I-p67 ¹⁶	CD33	AML	Delivers β -particle emitter
¹³¹ I-BC8 ¹⁷⁻¹⁹	CD45	AML, ALL, MDS	Delivers β -particle emitter
¹⁸⁸ Re-BW 250/183 ²⁰⁻²²	CD66	AML, ALL, CML	Delivers β -particle emitter
⁹⁰ Y-anti-Tac ²³	CD25	ATL	Delivers β -particle emitter

CLL = chronic lymphocytic leukemia	PLL = prolymphocytic leukemia
AML = acute myelogenous leukemia	MDS = myelodysplastic syndrome
APL = acute promyelocytic leukemia	HCL = hairy cell leukemia
CML = chronic myelogenous leukemia	ALL = acute lymphoblastic leukemia
ATL = adult T-cell leukemia	ADCC = antibody-dependent cellular cytotoxicity
CMC = complement-mediated cytotoxicity	

Table 2. — Characteristics of Several Isotopes Used for Clinical Radioimmunotherapy

Isotope	Particle(s) Emitted	Half-life	Particulate Energy (keV)	Mean Range of α - or β -Particle Emission (mm)
Iodine-131	β, γ	8.0 d	970	0.8
Rhenium-188	β, γ	17 h	2,120	2.4
Yttrium-90	β	64 h	2,280	2.7
Bismuth-213	α	46 min	5,982	0.05-0.08
Actinium-225	α	10.0 d	5,935	0.05-0.08

including its half-life and the type of particle(s) it emits. The physical properties of several commonly used isotopes for the clinical radioimmunotherapy of leukemia are summarized in Table 2.

β -Particles have a relatively long range (0.8-5 mm) and low linear energy transfer (approximately 0.2 keV/ μ m). This long range results in the delivery of radiation not only to the target cell but also to surrounding cells. Thus, cross-firing β -particles create a field effect, potentially irradiating antigen-negative tumor cells. Normal bystander cells, however, may be killed as well. The physical properties of β -particle-emitting radioimmunoconjugates make them useful in treating bulky disease and in selectively irradiating the entire bone marrow before hematopoietic stem cell transplantation. β -particle emitters, however, are less well-suited to the killing of single cells and to the treatment of small-volume, minimal residual, or micro-metastatic disease.

Most clinical studies have used ^{131}I , a long-lived β -particle emitter. The emissions from ^{131}I allow dosimetry studies to be performed easily, but treatment at high doses requires patient isolation and can result in radiation exposure to hospital staff. More recently, the use of radiometals, such as ^{90}Y and ^{188}Re , has been investigated. ^{90}Y is a pure β -emitter; its lack of γ -emissions allows outpatient administration of high doses. Therapy with ^{90}Y , however, poses several difficulties. Dissociation of ^{90}Y from the MAb complex in vivo can result in deposition of the isotope in bone. Unlike ^{131}I , which binds directly to tyrosine residues, ^{90}Y must be linked to the antibody by a chemical chelator. Furthermore, due to the absence of γ -emissions, biodistribution and dosimetry studies require administration of MAb trace-labeled with a second isotope, typically ^{111}In , whose biodistribution is not identical to ^{90}Y .

α -Particles are helium nuclei emitted from the decay of radioisotopes. There are approximately 100 radioisotopes that decay with α -particle emissions. Compared with β -particles, α -particles have a shorter range (50-80 μ m) and a higher linear energy transfer

(approximately 100 keV/ μ m).²⁴ As few as one or two α -particles traversing the nucleus can destroy a target cell. Therefore, because of the short range of α -particles, radioimmunotherapy with α -emitters should result in less nonspecific toxicity to normal bystander cells and in more efficient single-cell killing than β -emitting constructs. This potential for specific antitumor effects makes targeted

α -particle therapy an attractive approach for the treatment of cytoreduced or minimal residual disease.

Radioimmunotherapy With β -Particle Emitters

Radiolabeled Anti-CD33 Antibodies

CD33 is a 67-kD cell surface glycoprotein found on most myeloid leukemia cells and on committed myelomonocytic and erythroid progenitor cells. It is not found on lymphoid or nonhematopoietic cells.^{25,26} Three anti-CD33 MABs have been used in the radioimmunotherapy of myeloid leukemias: M195, HuM195, and p67. M195 is a murine monoclonal IgG2a antibody that is derived from a mouse immunized with live human leukemic myeloblasts.²⁷ HuM195 is a humanized antibody constructed by grafting the complementarity-determining region of M195 onto the constant region and variable framework of a human IgG1 antibody.²⁸ HuM195 differs from the murine M195 in two important ways. First, HuM195 can mediate the killing of leukemia cells in vitro by human peripheral blood mononuclear cells, whereas M195 cannot.^{29,30} Second, while significant numbers of patients treated with murine M195 develop human antimouse antibodies that adversely affect the pharmacokinetics of the antibody and preclude re-treatment with it, patients treated with HuM195 do not develop significant immune responses.^{6,7,11,12} The third anti-CD33 MAB formerly in clinical use for radioimmunotherapy is p67, a murine IgG1 antibody.

^{131}I -M195 and ^{131}I -HuM195 — In a phase I trial at Memorial Sloan-Kettering Cancer Center (MSKCC), 24 patients with relapsed or refractory myeloid leukemias were treated with escalating doses of ^{131}I -M195.¹¹ Twenty-two of the 24 patients had reductions in their leukemic burden. Gamma camera imaging showed targeting to areas of leukemic involvement, including the marrow of the vertebrae, pelvis, and long bones, as well as the liver and spleen. The isotope was retained at these sites for at least 3 days. Profound myelosuppres-

sion occurred at ^{131}I doses of 135 mCi/m^2 or greater, necessitating bone marrow transplantation in 8 patients. Three patients received autologous grafts, and 5 received allogeneic grafts after additional conditioning with busulfan and cyclophosphamide. The maximum tolerated dose of ^{131}I -M195 was not reached. Thirty-seven percent of patients developed human anti-mouse antibodies.

Subsequently, myeloablative doses of ^{131}I -M195 and ^{131}I -HuM195 (120 to 380 mCi) were studied in combination with busulfan (16 mg/kg) and cyclophosphamide (90 or 120 mg/kg) as a preparative regimen before allogeneic bone marrow transplantation. Thirty patients received the treatment on one of three protocols. Sixteen patients had refractory or relapsed AML, and 14 had relapsed, accelerated, or myeloblastic phase CML. Nineteen patients received ^{131}I -M195, and 11 received ^{131}I -HuM195. Twenty-eight of the 30 patients achieved complete remission. Three of the 16 patients with AML remain in remission between 4.5 and 8 years posttransplant. No significant toxicities were attributable to the addition of the radiolabeled antibody to the conditioning regimen, and engraftment was not delayed.¹² Taken together, these studies indicate that ^{131}I -M195 and ^{131}I -HuM195 have activity in myeloid leukemias and can be used safely in conjunction with standard chemotherapeutic agents as a conditioning regimen for hematopoietic stem cell transplantation. This approach potentially allows for intensification of antileukemic therapy before transplant.

The ability of nonmyeloablative doses of ^{131}I -M195 to eliminate minimal residual disease in patients with relapsed APL after induction with all-trans retinoic acid was studied. Six of 7 patients had minimal residual disease detectable by RT-PCR after induction with retinoic acid. The patients then received two doses of ^{131}I -M195 (50 or 70 mCi/m^2). Two of the 6 patients transiently became RT-PCR negative. The median disease-free survival was 8 months. This compared favorably to the 3-month median disease-free survival seen in patients with relapsed APL treated solely with all-trans retinoic acid. While ^{131}I -M195 had activity against minimal residual disease, this therapy was limited by myelosuppression in all patients and by the formation of human anti-mouse antibodies in 5 patients.^{12,13}

^{131}I -p67 — In a phase I study at the Fred Hutchinson Cancer Research Center (FHRC), ^{131}I -p67 was investigated in patients with advanced AML. Nine patients received a tracer dose of ^{131}I -p67, and the biodistribution and estimated radiation absorbed dose to various organs were determined. The half-life of ^{131}I -p67 in the marrow was 9 to 41 hours. This short half-life presumably resulted from internalization of the ^{131}I -

p67-CD33 complex with subsequent cleavage of ^{131}I from the antibody and excretion from the marrow space. Of the 9 patients, only 4 had “favorable biodistribution,” defined as a higher dose of radiation delivered to the marrow and spleen than to other organs. These 4 patients then received therapeutic doses of ^{131}I -p67 (110 to 330 mCi) together with cyclophosphamide (120 mg/kg) and total body irradiation (12 Gy) as conditioning regimen for allogeneic bone marrow transplantation. Although the therapy was well tolerated, 3 of the 4 patients eventually relapsed.^{16,31} Because of the short half-life of ^{131}I -p67 in the marrow and the unfavorable biodistribution in many patients, the investigators have since focused on the ^{131}I -anti-CD45 radioimmunoconjugate discussed below.³²

^{90}Y -HuM195 — Compared with other β -particle emitters, ^{131}I has several disadvantages. First, because of long-ranged γ emissions, patients must be hospitalized and isolated. Second, the long physical half-life of ^{131}I (8.1 days) delays the time from treatment to stem cell infusion in patients undergoing transplantation. Third, when IgG is labeled with high doses of ^{131}I , the ability of the antibody to bind to the target antigen is dramatically reduced. This occurs because approximately one third of the tyrosine residues, to which ^{131}I binds, are in the hypervariable regions of M195 and HuM195.³³ Therefore, multiple infusions of ^{131}I -M195 or ^{131}I -HuM195 are needed to deliver adequate radiation doses to the marrow for ablation.

^{90}Y offers several advantages over ^{131}I for myeloablation. After internalization of antigen-antibody complexes into target cells, radiometals such as ^{90}Y are better retained within these cells. Furthermore, because ^{90}Y is a pure β -emitter, large doses can be given safely in the outpatient setting with fewer consequences for medical personnel or patients' families. In a phase I trial at MSKCC, ^{90}Y -HuM195 was studied in patients with relapsed or refractory AML. Nineteen patients were treated with escalating doses of ^{90}Y -HuM195 (0.1 to 0.3 mCi/kg). Transient low-grade liver function abnormalities occurred in 11 patients. Myelosuppression lasted 9 to 62 days, and the maximum tolerated dose without stem cell rescue was 0.275 mCi/kg. Biodistribution and dosimetry studies were performed by co-administering ^{111}In -HuM195. Up to 56 Gy and 75 Gy were delivered to the marrow and spleen, respectively. Thirteen patients had reductions in bone marrow blasts, and 1 patient achieved a complete remission lasting 5 months. All patients treated with 0.3 mCi/kg had hypocellular bone marrow biopsies performed 2 or 4 weeks after treatment, without evidence of leukemia. These results suggest that ^{90}Y -HuM195 will be useful as conditioning before stem cell transplantation.¹⁴ Clinical trials investigating this agent as

part of preparative regimens for autologous and non-myeloablative allogeneic stem cell transplantation are now underway.

Radiolabeled Anti-CD45 Antibodies

CD45 is a tyrosine phosphatase expressed on virtually all leukocytes, including myeloid and lymphoid precursors in bone marrow and mature lymphocytes in lymph nodes. It is also expressed on most myeloid and lymphoid leukemic cells, but not on mature erythrocytes or platelets. Unlike CD33, it does not internalize after antibody binding. BC8 is a murine IgG1 anti-CD45 antibody.^{17,18} In a phase I trial at FHCRC, 44 patients with advanced acute leukemia or myelodysplasia received a biodistribution dose of ¹³¹I-BC8. Thirty-seven of the 44 had favorable biodistribution of the radiolabeled antibody. Thirty-four of these patients then received a therapeutic dose of ¹³¹I-BC8 followed by the conditioning regimen of cyclophosphamide (120 mg/kg) plus total body irradiation (12 Gy) before allogeneic or autologous transplant. An estimated radiation dose to the liver of 10.5 Gy was the maximum tolerated dose that could be administered with cyclophosphamide and total body irradiation. Of the 25 patients with AML or MDS, 7 survived disease-free at a median follow up of 65 months after transplantation. Of the 9 patients with ALL, 3 survived disease-free at 19, 54, and 66 months. The estimated maximum tolerated supplemental dose of radiation added by ¹³¹I-BC8 was 24 Gy to the bone marrow and 50 Gy to the spleen.¹⁷

Subsequently, a phase I/II trial of ¹³¹I-BC8 together with busulfan and cyclophosphamide prior to matched related allogeneic transplantation was begun in patients with AML in first remission. Ninety percent of patients had a favorable biodistribution of the radiolabeled antibody. These patients were then treated with therapeutic doses of ¹³¹I-BC8, delivering 3.5 Gy (4 patients) or 5.25 Gy (all subsequent patients) to the liver, half the maximum tolerated dose defined in the phase I study. Toxicities attributable to the ¹³¹I-BC8 were minimal. In an encouraging preliminary report, 18 of 24 patients treated with therapeutic doses were alive and disease-free at a median of 42 months after transplant.¹⁷⁻¹⁹ These studies indicate that ¹³¹I-BC8 can be administered safely to patients and can increase the dose of radiation delivered to the marrow when given as part of a conditioning regimen before hematopoietic stem cell transplantation.

Radiolabeled Anti-CD66c Antibodies

CD66c, also known as nonspecific cross-reacting antigen (NCA), is a glycoprotein expressed on myeloid

cells but not on leukemia cells. BW 250/183 is a murine monoclonal IgG1 antibody directed at CD66c.²⁰ ¹⁸⁸Re is a radiometal with a 17-hour half-life. It emits both β and γ radiation, which allows biodistribution and dosimetry studies to be performed easily.

A phase I dosimetry trial showed that administration of ¹⁸⁸Re-BW 250/183 resulted in a favorable biodistribution in 11 of 12 patients, with significant amounts of radiation delivered to the marrow.^{20,21} In a subsequent trial at the Ulm University Hospital in Germany,²² 36 patients with high-risk AML or myelodysplastic syndrome were treated with ¹⁸⁸Re-BW 250/183 prior to hematopoietic cell transplantation. After treatment with radiolabeled antibody, patients received one of three preparative regimens: total body irradiation (12 Gy) plus cyclophosphamide (120 mg/kg), busulfan (12.8 mg/kg) plus cyclophosphamide (120 mg/kg), or total body irradiation (12 Gy) plus thiotepa (10 mg/kg) and cyclophosphamide (120 mg/kg). Antithymocyte globulin was used to prevent graft rejection in patients receiving grafts from unrelated or mismatched related donors. Thirty-one patients received allogeneic grafts (mostly T-cell-depleted), 1 received a syngeneic graft, and 4 received autologous grafts. Favorable biodistribution of ¹⁸⁸Re-BW 250/183 occurred in all patients. The mean therapeutic dose of radiolabeled antibody administered was 11.1 GBq (300 mCi). The median dose delivered to the bone marrow was 14.9 Gy (range 8.1 to 28 Gy). Besides the toxicity normally associated with the conventional preparative regimens, no additional toxicity attributable to the radiolabeled antibody occurred. However, 6 patients developed nephrotoxicity between 6 and 12 months after the transplant. The authors note that nephrotoxicity after bone marrow transplantation may be an effect of radiation. Engraftment occurred in all patients and was not delayed. Disease-free survival was 45% at the median follow-up of 18 months. Disease-free survival was higher in patients undergoing transplantation while in remission (67%) than in those undergoing transplantation while not in remission (31%). Nine of 35 evaluable patients relapsed. Eight patients died from transplant-related toxicity.²² This study suggests that ¹⁸⁸Re-BW 250/183, similar to ⁹⁰YHuM195 and ¹³¹I-BC8, may deliver significant doses of radiation to the marrow without excessive toxicity.

Radiolabeled Anti-CD25 Antibodies

The interleukin-2 receptor (IL-2R) consists of at least three IL-2 binding subunits: IL-2R α (also known as CD25 or Tac), IL-2R β , and IL-2R γ . Normal lymphocytes do not express IL-2R α . However, in patients with human T-cell leukemia virus I (HTLV-I)-associated adult

T-cell leukemia, virtually all of the leukemic cells express 10,000 to 35,000 IL-2R α receptors per cell. Anti-Tac is a murine MAb that binds to IL-2R α and prevents its interaction with IL-2. In a phase I/II trial, 18 patients with adult T-cell leukemia were treated with ^{90}Y -anti-Tac. Nine patients were treated in a phase I dose escalation trial (5 to 15 mCi), and the remaining 9 patients were treated with a uniform dose of 10 mCi. Patients who had a remission were eligible for additional cycles of treatment. Of 16 evaluable patients, 7 had partial remissions (mean duration, 9.2 months), and 2 had complete remissions. Of the 2 patients with complete remissions, 1 patient developed myelodysplasia and died of secondary AML 3 years after receiving ^{90}Y -anti-Tac. At autopsy, the patient had persistent adult T-cell leukemia present in the skin. The second patient with a complete remission remained without evidence of disease for more than 3 years after the initiation of therapy. Toxicities of ^{90}Y -anti-Tac included myelosuppression, transient hepatic toxicity, and transient proteinuria. One patient died of unexplained cardiac asystole 23 days after the administration of ^{90}Y -anti-Tac. Six patients developed human antimouse antibodies.²³

Radioimmunotherapy With α -Particle Emitters

Preclinical Studies

The high linear energy transfer and short particle path length of α decays offer the potential for selective killing of tumor cells. The specificity and efficacy of targeted α -particle radioimmunotherapy with ^{212}Bi , ^{213}Bi , and ^{211}At have been reported in several experimental models.³⁴⁻³⁶ In one of the first reports suggesting the feasibility of this approach, ^{212}Bi conjugated to a tumor-specific MAb 103A was used against murine erythroleukemia. Targeting of the construct to neoplastic spleens was seen within 1 hour after injection. When ^{212}Bi -103A was injected on day 13 after inoculation with leukemia cells, reductions in splenomegaly and the absence of liver metastases were noted. When administered on day 8, no histological evidence of erythroleukemia developed.³⁷ Similarly, administration of ^{212}Bi -anti-Tac after inoculation of nude mice bearing CD25-expressing lymphoma cells led to prolonged tumor-free survival and prevented the development of tumors in some animals. Treatment of established tumors, however, failed to produce responses. The explanation for the failure of ^{212}Bi -anti-Tac to produce responses in established bulky tumors is that most of the ^{212}Bi had decayed by the time that adequate amounts of radiolabeled antibody were taken up into the tumor cells.³⁸

^{213}Bi has a half-life of 45.6 minutes and emits an α -particle of 8 MeV. Additionally, a 440 keV photon emission accompanies 26.5% of ^{213}Bi decays, allowing detailed biodistribution and dosimetry studies to be performed. The isotope has been prepared from a $^{225}\text{Ac}/^{213}\text{Bi}$ generator and conjugated to HuM195 using the bifunctional chelating agent 2-(4-isothiocyanatobenzyl) diethylenetriamine pentaacetic acid (SCN-CHX-A-DTPA).³⁹ Intravenous injections of up to 10 mCi/kg of ^{213}Bi -HuM195 were safe in mice. The application of bismuth-labeled HuM195 in vitro resulted in dose-dependent and specific activity-dependent killing of CD33 positive HL60 cells. Approximately 50% of target cells were killed when only two bismuth atoms were bound to the cell surface.⁴⁰

^{213}Bi -HuM195

A phase I dose escalation trial was conducted at MSKCC to determine the toxicity, biodistribution, dosimetry, and biological activity of ^{213}Bi -HuM195.¹⁵ Eighteen patients with relapsed or refractory AML or chronic myelomonocytic leukemia were treated with 0.28 to 1 mCi/kg of ^{213}Bi -HuM195. Treatment was well tolerated, and dose-limiting toxicity was not observed. Transient grade 1 or 2 liver function abnormalities occurred in 6 patients. Myelosuppression lasting 8 to 34 days was seen in all patients. Gamma camera imaging showed localization of ^{213}Bi to expected areas of leukemic involvement, including the bone marrow, liver, and spleen, within 5 to 10 minutes after injection. The absorbed dose ratios between these sites and the whole body were 1,000-fold greater than those seen with β -emitting constructs in this antigen system.⁴¹ Thirteen of 15 evaluable patients had reductions in peripheral blood leukemia cells, and 14 of the 18 patients had decreases in the percentage of bone marrow blasts. No complete remissions were observed. Because of the nature of α -particle radiation, complete remission at 30 days after treatment would have required the individual targeting and killing of 99.9% of the leukemia cells. Given that the patients treated on this study had tumor burdens of up to 10^{12} cells, each with an average CD33 density of 10,000 per cell, roughly 10^{16} leukemic binding sites were available to HuM195. Since approximately 1 in 2,700 molecules of HuM195 carried the radiolabel at the specific activities injected, it was difficult to deliver one to two ^{213}Bi atoms to every leukemia cell, even if optimal antibody targeting were assumed. Nevertheless, this trial is the first proof-of concept for systemic targeted α -particle immunotherapy in humans and provides the rationale for the continued investigation of this approach in a variety of cancers where minimal residual disease or micrometastatic disease may be present.

More recently, ^{225}Ac has been conjugated to a variety of MAbs using the bifunctional chelate SCN-DOTA. ^{225}Ac has a 10-day half-life and decays by α emission through three atoms, each of which also emits an α -particle. In vitro, ^{225}Ac coupled to internalizing MAbs specifically killed leukemia, lymphoma, breast, ovarian, neuroblastoma, and prostate cancer cells at doses 1,000 times less than ^{213}Bi -containing radioimmunoconjugates. In xenograft models of disseminated human lymphoma and solid prostate carcinoma, single doses at nanocurie levels of tumor-specific constructs prolonged survival and cured a substantial fraction of animals without toxicity.⁴² Therefore, in this strategy, ^{225}Ac -SCN-DOTA serves as an atomic nano-generator that delivers a cascade of four α -particles to the inside of a cancer cell by an internalizing antibody. A phase I trial of ^{225}Ac -HuM195 in advanced myeloid leukemias is planned.

Conclusions

Radioimmunotherapy for leukemia is a promising strategy designed to increase the efficacy of native MAbs, decrease the toxicity of therapy by targeting radiation to specific cell types or organ systems, and ultimately improve the long-term outcome for patients with leukemia. Radioimmunotherapy with β -particle emitters may be most effective for the treatment of bulky disease or as part of a conditioning regimen for hematopoietic stem cell transplantation, whereas radioimmunotherapy with α -particle emitters may be better suited for the treatment of small-volume or minimal residual disease. The phase I and phase II studies described above have demonstrated that radiolabeled antibodies have activity in refractory leukemias, can be administered safely, and can increase the dose of radiation delivered to the marrow when given as part of a conditioning regimen before hematopoietic stem cell transplantation. Whether or not radiolabeled antibodies improve outcome compared with standard chemotherapy agents or conditioning regimens remains to be demonstrated by randomized phase III clinical trials. Future research must also define optimal combinations of MAb and radioisotope in various clinical settings.

References

1. Cassileth PA, Harrington DP, Appelbaum FR, et al. Chemotherapy compared with autologous or allogeneic bone marrow transplantation in the management of acute myeloid leukemia in first remission. *N Engl J Med.* 1998;339:1649-1656.
2. Pui CH, Evans WE. Acute lymphoblastic leukemia. *N Engl J Med.* 1998;339:605-615.
3. O'Brien SM, Kantarjian H, Thomas DA, et al. Rituximab dose-escalation trial in chronic lymphocytic leukemia. *J Clin Oncol.* 2001;19:2165-2170.

4. Osterborg A, Dyer MJ, Bunjes D, et al. Phase II multicenter study of human CD52 antibody in previously treated chronic lymphocytic leukemia. European Study Group of CAMPATH-1H Treatment in Chronic Lymphocytic Leukemia. *J Clin Oncol.* 1997;15:1567-1574.
5. Jurcic JG, DeBlasio T, Dumont L, et al. Molecular remission induction with retinoic acid and anti-CD33 monoclonal antibody HuM195 in acute promyelocytic leukemia. *Clin Cancer Res.* 2000;6:372-380.
6. Caron PC, Jurcic JG, Scott AM, et al. A phase 1B trial of humanized monoclonal antibody M195 (anti-CD33) in myeloid leukemia: specific targeting without immunogenicity. *Blood.* 1994;83:1760-1768.
7. Caron PC, Dumont L, Scheinberg DA. Supersaturating infusion of humanized anti-CD33 monoclonal antibody HuM195 in myelogenous leukemia. *Clin Cancer Res.* 1998;4:1421-1428.
8. Feldman E, Kalaycio M, Schulman P, et al. Humanized monoclonal anti-CD33 antibody HuM195 in the treatment of relapsed/refractory acute myelogenous leukemia (AML): preliminary report of a phase II study. *Proc Annu Meet Am Soc Clin Oncol.* 1999;12. Abstract.
9. Sievers EL, Larson RA, Stadtmauer EA, et al. Efficacy and safety of gemtuzumab ozogamicin in patients with CD33-positive acute myeloid leukemia in first relapse. *J Clin Oncol.* 2001;19:3244-3254.
10. Kreitman RJ, Wilson WH, Bergeron K, et al. Efficacy of the anti-CD22 recombinant immunotoxin BL22 in chemotherapy-resistant hairy-cell leukemia. *N Engl J Med.* 2001;345:241-247.
11. Schwartz MA, Lovett DR, Redner A, et al. Dose-escalation trial of M195 labeled with iodine 131 for cytoreduction and marrow ablation in relapsed or refractory myeloid leukemias. *J Clin Oncol.* 1993;11:294-303.
12. Jurcic JG, Caron PC, Nikula TK, et al. Radiolabeled anti-CD33 monoclonal antibody M195 for myeloid leukemias. *Cancer Res.* 1995;55:5908s-5910s.
13. Jurcic JG, Caron PC, Miller WH Jr, et al. Sequential targeted therapy for relapsed acute promyelocytic leukemia with all-trans retinoic acid and anti-CD33 monoclonal antibody M195. *Leukemia.* 1995;9:244-248.
14. Jurcic JG, Divgi CCR, McDevitt MR, et al. Potential for myeloablation with yttrium-90-HuM195 (anti-CD33) in myeloid leukemia. *Proc Annu Meet Am Soc Clin Oncol.* 2000;19:24. Abstract.
15. Jurcic JG, McDevitt MR, Sgouros G, et al. Phase I trial of targeted alpha-particle therapy for myeloid leukemias with bismuth-213-HuM195 (anti-CD33). *Proc Annu Meet Am Soc Clin Oncol.* 1999;18:22. Abstract.
16. Appelbaum FR, Matthews DC, Eary JF, et al. The use of radiolabeled anti-CD33 antibody to augment marrow irradiation prior to marrow transplantation for acute myelogenous leukemia. *Transplantation.* 1992;54:829-833.
17. Matthews DC, Appelbaum FR, Eary JF, et al. Phase I study of (131)I-anti-CD45 antibody plus cyclophosphamide and total body irradiation for advanced acute leukemia and myelodysplastic syndrome. *Blood.* 1999;94:1237-1247.
18. Matthews DC, Appelbaum FR, Eary JF, et al. Radiolabeled anti-CD45 monoclonal antibodies target lymphohematopoietic tissue in the macaque. *Blood.* 1991;78:1864-1874.
19. Matthews DC, Appelbaum FR, Eary JF, et al. 131I-anti-CD45 antibody plus busulfan/cyclophosphamide in matched related transplants for AML in first remission. *Blood.* 1996;88:556. Abstract.
20. Seitz U, Neumaier B, Glatting G, et al. Preparation and evaluation of the rhenium-188-labelled anti-NCA antigen monoclonal antibody BW 250/183 for radioimmunotherapy of leukaemia. *Eur J Nucl Med.* 1999;26:1265-1273.
21. Kotzerke J, Glatting G, Seitz U, et al. Radioimmunotherapy for the intensification of conditioning before stem cell transplantation: differences in dosimetry and biokinetics of 188Re- and 99mTc-labeled anti-NCA-95 MAbs. *J Nucl Med.* 2000;41:531-537.
22. Bunjes D, Buchmann I, Duncker C, et al. Rhenium 188-labeled anti-CD66 (a, b, c, e) monoclonal antibody to intensify the conditioning regimen prior to stem cell transplantation for patients with high-risk acute myeloid leukemia or myelodysplastic syndrome: results of a phase III study. *Blood.* 2001;98:565-572.
23. Waldmann TA, White JD, Carrasquillo JA, et al. Radioimmunotherapy of interleukin-2R alpha-expressing adult T-cell leukemia

with Yttrium-90-labeled anti-Tac. *Blood*. 1995;86:4063-4075.

24. McDevitt MR, Sgouros G, Finn RD, et al. Radioimmunotherapy with alpha-emitting nuclides. *Eur J Nucl Med*. 1998;25:1341-1351.

25. Andrews RG, Torok-Storb B, Bernstein ID. Myeloid-associated differentiation antigens on stem cells and their progeny identified by monoclonal antibodies. *Blood*. 1983;62:124-132.

26. Griffin JD, Linch D, Sabbath K, etc. A monoclonal antibody reactive with normal and leukemic human myeloid progenitor cells. *Leuk Res*. 1984;8:521-534.

27. Tanimoto M, Scheinberg DA, Cordon-Cardo C, et al. Restricted expression of an early myeloid and monocytic cell surface antigen defined by monoclonal antibody M195. *Leukemia*. 1989;3:339-348.

28. Co MS, Avdalovic NM, Caron PC, et al. Chimeric and humanized antibodies with specificity for the CD33 antigen. *J Immunol*. 1992;148:1149-1154.

29. Scheinberg DA, Tanimoto M, McKenzie S, et al. Monoclonal antibody M195: a diagnostic marker for acute myelogenous leukemia. *Leukemia*. 1989;3:440-445.

30. Caron PC, Co MS, Bull MK, et al. Biological and immunological features of humanized M195 (anti-CD33) monoclonal antibodies. *Cancer Res*. 1992;52:6761-6767.

31. Ruffner KL, Matthews DC. Current uses of monoclonal antibodies in the treatment of acute leukemia. *Semin Oncol*. 2000;27:531-539.

32. Matthews DC. Immunotherapy in acute myelogenous leukemia and myelodysplastic syndrome. *Leukemia*. 1998;12(suppl 1):S33-S36.

33. Nikula TK, Bocchia M, Curcio MJ, et al. Impact of the high tyrosine fraction in complementarity determining regions: measured and predicted effects of radioiodination on IgG immunoreactivity. *Mol Immunol*. 1995;32:865-872.

34. Zalutsky MR, McLendon RE, Garg PK, et al. Radioimmunotherapy of neoplastic meningitis in rats using an alpha-particle-emitting immunoconjugate. *Cancer Res*. 1994;54:4719-4725.

35. Kennel SJ, Boll R, Stabin M, et al. Radioimmunotherapy of micrometastases in lung with vascular targeted ²¹³Bi. *Br J Cancer*. 1999;80:175-184.

36. McDevitt MR, Barendsward E, Ma D, et al. An alpha-particle emitting antibody (²¹³Bi]J591) for radioimmunotherapy of prostate cancer. *Cancer Res*. 2000;60:6095-6100.

37. Huneke RB, Pippin CG, Squire RA, et al. Effective alpha-particle-mediated radioimmunotherapy of murine leukemia. *Cancer Res*. 1992;52:5818-5820.

38. Hartmann E, Horak EM, Garmestani K, et al. Radioimmunotherapy of nude mice bearing a human interleukin 2 receptor alpha-expressing lymphoma utilizing the alpha-emitting radionuclide-conjugated monoclonal antibody ²¹²Bi-anti-Tac. *Cancer Res*. 1994;54:4362-4370.

39. McDevitt MR, Finn RD, Ma D, et al. Preparation of alpha-emitting ²¹³Bi-labeled antibody constructs for clinical use. *J Nucl Med*. 1999;40:1722-1727.

40. Nikula TK, McDevitt MR, Finn RD, et al. Alpha-emitting bismuth cyclohexylbenzyl DTPA constructs of recombinant humanized anti-CD33 antibodies: pharmacokinetics, bioactivity, toxicity and chemistry. *J Nucl Med*. 1999;40:166-176.

41. Sgouros G, Ballangrud AM, Jurcic JG, et al. Pharmacokinetics and dosimetry of an alpha-particle emitter labeled antibody: ²¹³Bi-HuM195 (anti-CD33) in patients with leukemia. *J Nucl Med*. 1999;40:1935-1946.

42. McDevitt MR, Ma D, Lai LT, et al. Tumor therapy with targeted atomic nanogenerators. *Science*. 2001;294:1537-1540.