

Radiolabeled Anti-CD33 Monoclonal Antibody M195 for Myeloid Leukemias¹

Joseph G. Jurcic,² Philip C. Caron,³ Tuomo K. Nikula, Esperanza B. Papadopoulos, Ronald D. Finn, Otto A. Gansow, Wilson H. Miller, Jr., Maurits W. Geerlings, Raymond P. Warrell, Jr., Steven M. Larson, and David A. Scheinberg⁴

Leukemia Service [J. G. J., P. C. C., D. A. S.], Developmental Chemotherapy Service [W. H. M., R. P. W.], and Bone Marrow Transplant Service [E. B. P.], Department of Medicine, and Nuclear Medicine Service, Department of Radiology [T. K. N., R. D. F., S. M. L.], Memorial Sloan-Kettering Cancer Center, New York, New York 10021; Chemistry Section, Radiation Oncology Branch, National Cancer Institute, Bethesda, Maryland 20892 [O. A. G.]; and Pharmactinium, Inc., Wilmington, Delaware [M. W. G.]

Abstract

M195, a mouse monoclonal antibody reactive with the early myeloid antigen CD33, has been shown to target leukemia cells in patients and to reduce large leukemic burdens when labeled with ¹³¹I. A complementarity-determining region-grafted, humanized version (HuM195) has demonstrated similar targeting of leukemia cells without immunogenicity. We have studied two applications of therapy with ¹³¹I-M195. First, to intensify therapy prior to bone marrow transplantation (BMT), we combined ¹³¹I-M195 with busulfan and cyclophosphamide. Fifteen patients received first BMT for relapsed or refractory acute myelogenous leukemia or accelerated or blastic chronic myelogenous leukemia; four received second BMT for relapsed chronic or accelerated chronic myelogenous leukemia. Doses of ¹³¹I-M195 ranged from 120 to 230 mCi/m². Few toxicities could be attributed to ¹³¹I-M195 therapy, and all patients engrafted. Eighteen patients achieved complete remission. Among those patients receiving first BMT, three have remained in unmaintained remission for 18+ to 29+ months. Six patients relapsed, including one with isolated central nervous system disease 32 months after BMT. Ten patients died in complete remission of transplant-related complications. Second, we studied whether ¹³¹I-M195 could reduce minimal residual disease and prolong remission and survival durations safely in patients with relapsed acute promyelocytic leukemia after they attained remission with all-*trans*-retinoic acid. Seven patients were treated with either 50 or 70 mCi/m² ¹³¹I-M195. Toxicity was limited to myelosuppression. As a measure of minimal residual disease, we monitored PML/RAR- α mRNA by reverse transcription PCR. Six patients had positive reverse transcription PCR assays prior to receiving ¹³¹I-M195; two converted transiently to negative. Median disease-free survival and overall survival of the seven patients were 8 (range, 3–14.5) months and 28 (range, 5.5–43+) months, respectively. This regimen compares favorably with others for relapsed acute promyelocytic leukemia. In an effort to avoid nonspecific cytotoxicity associated with ¹³¹I in future trials for minimal residual disease, we have conjugated short-range, α particle-emitting radioisotopes to HuM195 using a bifunctional chelate, 2-(*p*-isothiocyanatobenzyl)-cyclohexyldiethylenetriaminepentaacetic acid, with high efficiency and specific activities. ²¹²Bi-HuM195 has demonstrated dose- and specific activity-dependent killing of HL60 cells *in vitro*. Injection of ²¹³Bi-HuM195 into healthy BALB/c mice produced no effects on weight or viability.

Introduction

M195 is a mouse IgG2a monoclonal antibody reactive with the myeloid surface glycoprotein CD33. This antigen is found on most myeloid leukemic blasts and leukemic progenitors in addition to committed normal myelomonocytic and erythroid progenitor cells. It may be expressed in low numbers on early hematopoietic stem cells but is not found on nonhematopoietic tissues (1, 2). Previous trials

have demonstrated that small doses of M195 can target leukemia cells saturably within 1 h after injection and are retained within the marrow for up to 48 h after administration (3). When labeled therapeutically with ¹³¹I, M195 has produced significant reductions in peripheral blood blast counts and the number of bone marrow blasts in patients with myeloid leukemias (4).

Because of the lack of intrinsic cytotoxicity of murine M195 and its immunogenicity, which limits repeated dosing, HuM195,⁵ a complementarity determining region-grafted human IgG1 version of M195, has been developed. HuM195 has displayed an increased binding avidity over the murine antibody due to the elimination of a glycosylation site in the heavy-chain, variable region (5) and can mediate antibody-dependent cellular cytotoxicity against leukemic target cells *in vitro* using human peripheral blood mononuclear cells (6). In a Phase I trial, HuM195 demonstrated pharmacokinetics similar to murine M195, but repeated dosing did not produce immune responses. These findings suggest that HuM195 is a suitable agent to carry radionuclides, and that repeated administration is possible due to its apparent lack of immunogenicity (7).

We have studied two therapeutic applications of ¹³¹I-M195: (a) for myeloablation before BMT; and (b) for the treatment of minimal residual disease in the postremission setting. In this article, we report the initial results of a trial in which ¹³¹I-M195 is used as part of a conditioning regimen before BMT, update the results of a trial in which this agent is given as "consolidation" therapy after remission induction, and describe the construction of Bi-HuM195 conjugates, which may have a role in treating residual leukemia.

¹³¹I-M195 Prior to BMT

Twenty to 25% of patients with relapsed or refractory myeloid leukemias can be salvaged with allogeneic BMT (8). In an effort to increase this number, we have incorporated ¹³¹I-M195 into a transplant-preparative regimen. Escalating doses of ¹³¹I-M195 were given in two to four fractions, 48–72 h apart, as described previously (1, 3, 4, 9). After at least 4 days from the completion of ¹³¹I-M195 therapy, patients were given a total dose of 16 mg/kg busulfan p.o. in four divided daily doses over 4 days. Cyclophosphamide, at total doses of 120 mg/kg for first BMT and 90 mg/kg for second BMT, was administered i.v. on the next 2 days. Unmodified, human leukocyte antigen-compatible bone marrow was infused 48 h after the second dose of cyclophosphamide. This schedule allows four to five half-lives of ¹³¹I-M195 to elapse from the last dose of antibody to the infusion of bone marrow, leaving only minimal isotope present at the time of BMT. Cyclosporine and corticosteroids were given as prophylaxis against GVHD.

Nineteen patients (median age, 38 years) were treated. Fifteen patients underwent first BMT for relapsed ($n = 2$) or refractory ($n = 8$) acute myelogenous leukemia or accelerated ($n = 2$) and myeloblastic ($n = 3$) chronic myelogenous leukemia. Four patients received

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² To whom requests for reprints should be addressed, at Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021.

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⁴ A Lucille P. Markey Scholar.

⁵ The abbreviations used are: HuM195, humanized M195; BMT, bone marrow transplantation; RA, retinoic acid; APL, acute promyelocytic leukemia; GVHD, graft-versus-host disease.

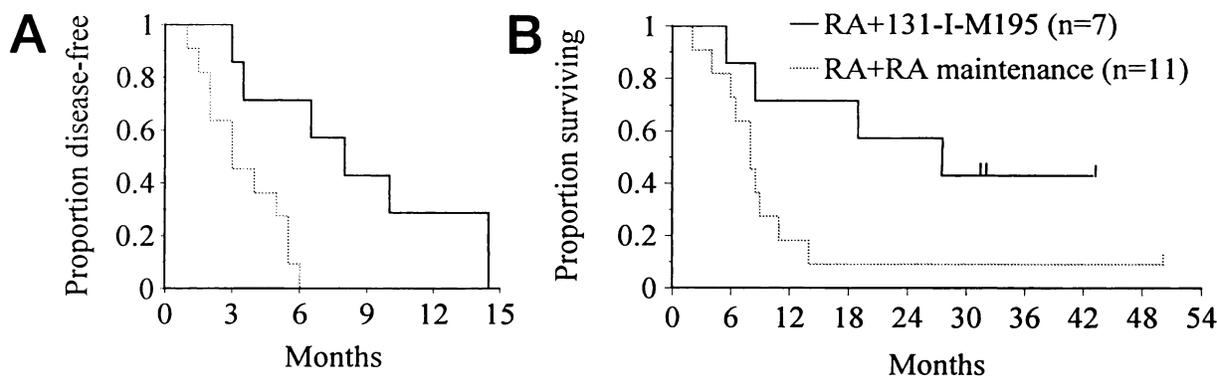


Fig. 1. Kaplan-Meier plots of the disease-free survival (A) and overall survival (B) of 7 patients with relapsed APL treated with all-*trans*-RA induction therapy followed by a single dose of postremission ^{131}I -M195 and of 11 patients with relapsed APL treated with all-*trans*-RA induction therapy followed by maintenance therapy with the drug. These patients were treated in sequentially performed trials. Tick marks indicate the time of last follow-up.

second BMT for either relapsed, chronic-phase ($n = 1$), or accelerated ($n = 3$) chronic myelogenous leukemia.

Doses of ^{131}I -M195 ranged from 120 to 230 mCi/m² (240–370 mCi). Extramedullary toxicity attributable to ^{131}I -M195 was limited to urticaria in one patient. All patients engrafted with a median time to neutrophil recovery (absolute neutrophil count, >500/ μl) of 14 (range, 10–23) days and a median time to platelet recovery (>20,000/ μl) of 27 (range, 12–85) days. Seven patients developed acute GVHD (two with grade I, three with grade II, and two with grade IV); two developed chronic GVHD. Despite the administration of myeloablative chemotherapy after ^{131}I -M195, Human antimouse antibodies were detected in 6 of 16 patients evaluated using a previously described ELISA (9).

Among the 15 patients who received first BMT, 14 achieved documented complete remission. Three patients remain in unmaintained complete remission at 18+, 26+, and 29+ months after BMT. Six patients relapsed from 3 to 32 months following transplant. The patient who relapsed after 32 months developed disease limited to the central nervous system. Six patients died in complete remission of GVHD or infection. The four patients who underwent second BMT died in complete remission of transplant-related complications 1–8 months after treatment. This approach potentially enables the intensification of antileukemic therapy prior to BMT without increased toxicity or impairment of engraftment.

^{131}I -M195 for Minimal Residual Disease

We have studied the effects of ^{131}I -M195 on minimal residual disease in patients with relapsed APL (10), because all-*trans*-RA can produce remissions in most patients (11–13), and because minimal residual disease can be monitored using a reverse transcription PCR assay that detects the PML/RAR- α mRNA associated with $t(15;17)$ (14–16).

Seven patients (median age, 53 years) with relapsed APL in second remission after all-*trans*-RA induction were treated with either 50 ($n = 5$) or 70 ($n = 2$) mCi/m² ^{131}I -M195. Toxicity was limited to myelosuppression. Six patients developed granulocyte counts <500/ μl , and all had platelet counts <20,000/ μl . No episodes of febrile neutropenia were seen, and neither growth factor nor autologous stem cell support was required. The maximum tolerated dose of ^{131}I -M195 in this postremission setting, resulting in neutropenic periods of less than 14 days, approximated 50 mCi/m². Human antimouse antibodies were seen in five of the seven patients. Six patients had detectable PML/RAR- α mRNA after all-*trans*-RA therapy using a previously described assay (14, 16); two had transiently negative reverse tran-

scription PCR determinations 5 and 13 weeks, respectively, following ^{131}I -M195.

The median disease-free survival of the seven patients was 8 (range, 3–14.5) months (Fig. 1A). Three patients have remained alive after additional antileukemic therapy from 31+ to 43+ months. The median overall survival was 28 (range, 5.5–43+) months (Fig. 1B). Patients' outcomes in this trial compare favorably with those of patients treated using other approaches, including induction therapy with all-*trans*-RA, followed by maintenance therapy with this drug in the immediately preceding trial (Fig. 1; Ref. 13), maintenance chemotherapy (12), consolidation chemotherapy (17), and BMT.⁶ These data support further study of antibody-based therapy for minimal residual disease in acute leukemia.

α Particle-emitting Constructs of HuM195

In an attempt to avoid nonspecific cytotoxic effects of ^{131}I on normal hematopoietic cells, α particle-emitting radionuclides have been conjugated to HuM195. Bismuth has two potentially useful isotopes for radioimmunotherapy, ^{212}Bi and ^{213}Bi . Both ^{212}Bi and ^{213}Bi generator systems yield high-purity isotopes capable of chelation to antibodies.

^{111}In , ^{206}Bi , ^{212}Bi , and ^{213}Bi have been conjugated to HuM195 using the bifunctional chelate 2-(*p*-isothiocyanatobenzyl)-cyclohexyldiethylenetriaminepentaacetic acid with efficiencies of >90% and specific activities ranging from 20 to 45 mCi/mg. The immunoreactivity of HuM195-2-(*p*-isothiocyanatobenzyl)-cyclohexyldiethylenetriaminepentaacetic acid has ranged from 70 to 90%. Chelated HuM195 is internalized into target cells within 1 h after binding. ^{212}Bi -HuM195 shows dose- and specific activity-dependent killing of HL60 target leukemia cells *in vitro*. ^{111}In - and ^{206}Bi -HuM195 conjugates were injected into healthy BALB/c mice without selective accumulation of either isotope in any organ. ^{213}Bi -HuM195 was injected into healthy BALB/c mice over 10 weeks in doses ranging from 37 to 222 megabecquerels/kg, without any effect on weight or viability.

Pharmacokinetic modeling of both M195 (18) and HuM195, as well as previous studies examining the cellular kinetics and dosimetry of α -particle radioimmunotherapy (19), have been used to predict the effects of ^{213}Bi -HuM195 in humans. Based on these data, the doses of ^{213}Bi -HuM195 given to mice have been at least four to ten times greater than those planned for administration to humans.

⁶J. G. Jurcic, D. A. Scheinberg, and C. Little, unpublished observations.

Future Directions

The immunological activity, lack of immunogenicity, and increased binding avidity of HuM195 offer advantages over use of the murine antibody. A trial using ^{131}I -labeled HuM195 for myeloablation is underway. Seven patients have been treated, with results similar to those of the ^{131}I -M195 trial described above. The use of other β particle-emitting radiometals such as ^{90}Y or ^{188}Re , which may be better retained within the marrow, could improve delivery of the isotope to leukemic cells further.

In the setting of minimal residual disease, in which specific, single cell killing is required, the use of an α particle-emitting construct may be more suitable. Additionally, several trials examining the role of unconjugated HuM195 on minimal residual disease, in which normal effector cell populations are present, have been initiated in patients with APL and in patients older than 60 years of age with acute myelogenous leukemia. *In vitro* studies have shown that interleukin-2 can potentiate the antileukemic activity of HuM195 (20), and clinical trials combining these agents are planned.

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