Molecular Remission Induction with Retinoic Acid and Anti-CD33 Monoclonal Antibody HuM195 in Acute Promyelocytic Leukemia

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ABSTRACT
Despite achieving complete remission with retinoic acid (RA), most patients with acute promyelocytic leukemia (APL) have minimal residual disease detectable by reverse transcription-PCR (RT-PCR) amplification. HuM195, a humanized monoclonal antibody reactive with the cell surface antigen CD33, specifically targets and kills myeloid leukemia cells. We studied whether HuM195 could eliminate minimal residual disease in patients with APL by using RT-PCR. After attaining clinical complete remission with RA and/or chemotherapy, patients received HuM195 twice weekly for 3 weeks. Patients in first remission were given consolidation chemotherapy, generally with three cycles of idarubicin and cytarabine. Patients in second or greater remission did not receive chemotherapy. All patients received six monthly courses of maintenance with two doses of HuM195. Twenty-five of 27 patients treated in first remission had positive RT-PCR determinations before HuM195 treatment. Of the 22 patients evaluable for conversion of positive RT-PCR assays, 11 (50%) became RT-PCR negative after treatment with HuM195. Twenty-five of 27 patients with newly diagnosed APL (93%) remain in clinical complete remission for 7 to 58 months, with median follow-up of 29 months. Seven patients in second or third remission and one patient in molecular relapse were also treated. Only one of these patients became RT-PCR negative after treatment with HuM195. These data suggest that HuM195 has activity against minimal residual disease in APL, particularly in newly diagnosed patients.

INTRODUCTION
APL is characterized by a specific translocation that fuses the PML gene on chromosome 15 with a RAR gene (RAR-α) on chromosome 17 (1–3). Detection of PML/RAR-α fusion mRNA by RT-PCR amplification is useful in establishing the diagnosis of APL, predicting response to therapy, and predicting relapse (4–12). All-trans-RA or 9-cis-RA induces remission in up to 95% of patients, most of whom have minimal residual disease detectable by RT-PCR before receiving additional therapy (13–16). Therefore, serial monitoring of bone marrow using RT-PCR enables the effect of postremission therapy to be assessed in patients who are in clinical complete remission but remain at risk for relapse.

M195 is a mouse monoclonal antibody that binds CD33, a cell surface glycoprotein found on most myeloid leukemias and clonalogenic leukemia progenitors (17, 18). Although CD33 is expressed on committed myelomonocytic and erythroid progenitor cells, it is not found on mature granulocytes or nonhematopoietic tissues (19, 20). Early trials showed that M195 rapidly targets leukemia cells in patients and that 131I-labeled M195 can eliminate large leukemic burdens (21–23). Lower doses of 131I-labeled M195 had activity against minimal residual disease in patients with APL in second remission (24). However, this therapy was limited by myelosuppression due to the nonspecific cytotoxicity of 131I and by formation of human antimouse antibodies that prevented repeated dosing.

Humanized M195 (HuM195), constructed by grafting complementarity-determining regions of murine M195 into a human IgG1 framework and backbone, mediates leukemia cell killing in vitro by human peripheral blood mononuclear cells (25, 26). It displayed rapid targeting of leukemia cells and a pharmacology similar to that of murine M195 in a Phase I trial, but without significant immunogenicity, thereby allowing the administration of multiple doses (27). Treatment with supersaturating doses of native HuM195 produced a complete remission

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3 The abbreviations used are: APL, acute promyelocytic leukemia; RA, retinoic acid; RT-PCR, reverse transcription-PCR; RAR, retinoic acid receptor; HAHA, human antihuman antibody.

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in 1 of 10 patients with advanced myeloid leukemias and reduced the percentage of bone marrow blasts in another 3 patients (28). We studied the ability of HuM195 to eliminate minimal residual disease detectable by RT-PCR in patients with APL.

MATERIALS AND METHODS

Eligibility. Patients with APL were eligible for treatment with HuM195 after achieving a clinically documented complete remission by conventional criteria (29) after induction with RA and/or chemotherapy. Initially, newly diagnosed patients were enrolled on study within 2 weeks after attaining a clinical complete remission, before the administration of consolidation chemotherapy. After the first 16 patients, the protocol was modified to permit up to two courses of consolidation chemotherapy before entering the study. Patients in clinical complete remission but with evidence of disease detectable by RT-PCR were also eligible.

Treatment. On entering the study, patients received 3 mg/m² HuM195 by i.v. infusion over a 40–60-min period twice weekly for six doses. Acetaminophen and diphenhydramine were given before each treatment to prevent infusion-related toxicity. Patients enrolled after induction with RA alone received three cycles of consolidation chemotherapy. The first cycle consisted of 200 mg/m²/day cytarabine by continuous i.v. infusion for 5 days and 12 mg/m² idarubicin i.v. for 3 days. Subsequent courses were repeated every 6–8 weeks and consisted of cytarabine for 4 days and idarubicin for 2 days at the same doses. Patients entering the study after induction with concomitant RA and chemotherapy received only the second and third chemotherapy courses as described above. Patients enrolled after the administration of consolidation therapy received additional cycles of chemotherapy so that a total of three courses were given. After the completion of chemotherapy, six monthly courses of maintenance with two doses of HuM195 given 3–4 days apart were administered.

Patients in second or greater remission or in molecular relapse were treated with six doses of HuM195 over 3 weeks as described above. After this therapy, appropriate candidates proceeded to bone marrow or peripheral blood progenitor cell transplantation. The remaining patients received maintenance with HuM195 as described above. These patients did not receive additional consolidation chemotherapy because they relapsed after standard treatment. One patient in second remission was treated on an earlier protocol in which maintenance with HuM195 was continued for 1 year. Patients were treated on protocols approved by the Institutional Review Board and the Food and Drug Administration after informed consent was obtained.

RT-PCR Analysis. Serial bone marrow aspirates were analyzed using previously described RT-PCR techniques to detect PML/RAR-α rearrangements with a sensitivity of approximately 1 in 10⁴ cells (5, 9). We examined samples before patients began treatment with HuM195, after initial antibody therapy, after each consolidation chemotherapy course, and periodically thereafter.

Plasma HuM195 Levels and HAHA Response. Plasma levels of HuM195 were measured before and 5 min after each HuM195 infusion by an ELISA. This assay uses a version of the “double-antibody sandwich” technique with a high-affinity mouse anti-idiotypic antibody to M195 (30). We used a previously described double antigen ELISA to detect HAHA (27). Specificity of HAHA was assessed by competition of the patient’s serum with a panel of monoclonal antibodies sharing various regions of homology with HuM195 and unrelated controls.

Historical Controls and Statistical Analysis. We compared newly diagnosed patients entered on this study with a group of historical control patients treated at Memorial Hospital immediately before the initiation of the current trial. This group consisted of 50 consecutively diagnosed patients with APL who attained clinical complete remission with all-trans-RA (n = 49) or 9-cis-RA (n = 1) and then received consolidation chemotherapy, generally with three courses of cytarabine and idarubicin, as described above (16, 31, 32). The median follow-up duration is 62 months (range, 8–104+ months). The χ² test was used to compare molecular remission rates between groups. To ensure that these groups were comparable, we included patients who received induction solely with RA for this analysis. The Kaplan-Meier method was used to determine the probability of disease-free survival. Comparison of disease-free survival duration between groups was performed using the log-rank test. For uniformity among groups, this analysis was limited to patients induced with RA followed by either two or three cycles of consolidation chemotherapy.

RESULTS

Patient Characteristics. Thirty-five patients (median age, 45 years) were enrolled between October 1994 and March 1999 (Table 1). Among the 27 patients treated in first remission, 21 patients received induction with either all-trans-RA (n = 18) or 9-cis-RA (n = 3) alone. Five patients received all-trans-RA concomitantly with chemotherapy as induction. One patient (patient 24) received chemotherapy alone as an induction. Only two patients (patients 24 and 26) were given consolidation chemotherapy before entering the study. The characteristics of the historical control group are compared with the newly diagnosed patients treated on the current study in Table 2. These groups are comparable in all respects except that patients treated on the current study received fewer cycles of consolidation therapy than patients in the historical group. This is a result of a greater number of patients receiving induction with concomitant RA and chemotherapy in the current study.

Among the seven patients treated in second or third remission, five received induction with all-trans-RA (n = 2) or 9-cis-RA (n = 3), and two received concomitant all-trans-RA and chemotherapy. Patient 15 was treated in molecular relapse 35 months after completing therapy with all-trans-RA followed by three courses of chemotherapy.

Molecular Remission Induction. Among the 27 patients treated in first remission, 25 had minimal residual disease detectable by RT-PCR before receiving HuM195. One of these patients (patient 24) received consolidation chemotherapy before enrolling on study. Patients were considered evaluable for response only if adequate RNA samples were obtained for RT-PCR analysis. After six doses of HuM195, 11 of the 22
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*CR1, first complete remission; CR2, second complete remission; CR3, third complete remission; NA, not applicable; ATRA, all-trans-RA; IDR, idarubicin; DNR, daunorubicin; Ara-C, cytarabine; HiDAC, high-dose cytarabine; MTX, methotrexate; 6TG, 6-thioguanine.

*Patient removed from study; received chemotherapy for persistent positive RT-PCR assay.

*Patient removed from study; underwent autologous bone marrow transplant.

*Patient received only two cycles of consolidation due to severe chemotherapy-related toxicity.

*Patient removed from study; received consolidation with ATRA/IDR.

*Patient removed from study; unable to receive IDR due to cardiomyopathy.
remaining evaluable patients became RT-PCR negative with either one (n = 9) or two (n = 1) courses of consolidation chemotherapy.

Among the eight patients with relapsed APL, only one patient treated in second remission (patient 2) became RT-PCR negative after HuM195 treatment without any additional therapy (Fig. 1B). HuM195 maintenance was continued for 1 year. After 18 months, this patient tested positive for the PML/RAR-α rearrangement but remained in clinical remission. She failed to respond to additional HuM195 and relapsed clinically 1 month later.

Comparison of Molecular Remission Induction Rates with and without HuM195. To compare the molecular remission rates of patients treated with or without HuM195, we limited the analysis to patients induced with RA alone. In the current study, 21 patients received HuM195 after RA induction followed by consolidation chemotherapy. Among the historical control group, 43 patients were induced into remission with RA and then maintained on the drug for 1 month before receiving consolidation therapy. Patients were evaluable for response only if adequate RNA samples were obtained for analysis.

At the time of clinical complete remission, 1 of the 21 patients (5%) in the current study and 3 of the 40 evaluable patients (8%) in the historical group were RT-PCR negative. One month after achieving clinical remission with RA, 8 of 18 evaluable patients (44%) in the current trial were RT-PCR negative after receiving HuM195 but before the administration of chemotherapy (Fig. 2A). In previous trials, 7 of 34 patients (21%) who continued RA therapy alone for 1 month after attaining clinical remission tested negative (P = 0.07; Fig. 2B).

Remission and Survival Duration. Twenty-five of 27 patients (93%) with newly diagnosed APL remain in remission with a median follow-up duration of 29 months (range, 7+–58+ months) from the time of clinically documented complete remission. Two patients (patients 21 and 22) relapsed after 16 and 20 months, respectively. The estimated probability of remaining disease free at 29 months for all newly diagnosed patients treated with RA, HuM195, and chemotherapy is 89%. We compared the probability of disease-free survival for the 21 patients in the current study, who received induction with RA followed by postremission HuM195 and then two or three cycles of consolidation therapy, with that of 47 patients in the historical control group, who were induced with RA and then received two or three cycles of consolidation chemotherapy. The estimated disease-free survival after 29 months for this subset of study patients is 86%, and the estimated disease-free survival is 76% for the patients in the historical control group (P = 0.22; Fig. 3).

Twenty-six of the 27 patients (96%) treated on the current study are alive, with a median follow-up duration of 30 months (range, 8+–60+ months) from the time of diagnosis. The estimated probability of survival at 30 months is 93%. One patient died with relapsed APL 26 months after diagnosis.

Among the eight patients treated in second or third remission or in molecular relapse, five patients relapsed clinically after 4–27 months. Four of these patients achieved remission with additional therapy and remain disease free. Patient 11 died with relapsed APL after 15 months. The remaining three patients underwent autologous bone marrow transplantation after they failed to achieve molecular remission with six infusions of HuM195. The disease-free and overall survival times of this group range from 4–32+ months and 6–56+ months, respectively, with a median follow-up duration of 31 months. A previous series of relapsed patients treated without HuM195 had a median disease-free survival duration of 3–11 months (24).

Adverse Effects. HuM195 infusions were generally associated with few or no side effects (Table 3). The most common adverse reactions included low-grade fever, nausea, and chills and were usually seen within 2 h after completion of the infusion and only after the first dose. These reactions were effectively treated with acetaminophen, diphenhydramine, and meperidine. Therapy was administered entirely in the outpatient setting. Mild neutropenia (usually grade 1) and thrombocytopenia (grade 1) lasting for 3–7 days occurred in six patients, likely because of CD33 expression by normal hematopoietic progenitor cells. No episodes of neutropenic fever were observed. Five patients also developed transient postural hypotension requiring the administration of i.v. fluids.

HAHA Response. Patient 2 developed an immune response to HuM195 9 weeks after beginning therapy. A compe-
tition assay to assess the specificity of HAHA showed suppression of the signal with only murine M195 and HuM195, indicating a pure anti-idiotypic response. HAHA levels increased over 10 weeks to an anti-idiotypic equivalent concentration of 37.617.2 ng/ml. Because of the low level of immune response, serum levels of HuM195 were unaffected, and this patient was able to complete 1 year of maintenance therapy. Peak serum levels of HuM195 were 1.3 ± 0.4 μg/ml before the development of HAHA and 1.1 ± 0.2 μg/ml after a HAHA response was detected.

**DISCUSSION**

Elimination of minimal residual disease detectable by RT-PCR for the PML/RAR-α fusion transcript appears necessary for patients with APL to achieve long-term remissions (8–12,

![Fig. 1 Longitudinal RT-PCR results from bone marrow aspirates of 27 patients with newly diagnosed APL treated with postremission HuM195 and cytotoxic therapy (A) and eight patients in second or third remission or molecular relapse treated with HuM195 (B). Each circle represents a RT-PCR assay performed at the indicated time after achieving clinical complete remission. Only samples with adequate RNA quality for amplification of control RNA are included. •, a positive result; ○, a negative result. □, treatment with HuM195; ■, treatment with chemotherapy. Arrows, the time of clinical relapse.](image)
in 61% of patients before the administration of consolidation therapy.

At least 90% of newly diagnosed patients with APL will become RT-PCR negative after receiving RA induction followed by multiple courses of consolidation chemotherapy (8, 9, 11). In this study, all 19 evaluable patients (100%) tested RT-PCR negative after RA induction, HuM195 treatment, and one course of consolidation chemotherapy. This is comparable to the 90% of patients treated on earlier studies who were RT-PCR negative after RA induction and three intensive consolidation courses with combination chemotherapy. Similarly, Diverio et al. (12) reported that 96% of patients achieved a molecular remission after induction with all-trans-RA and idarubicin followed by three cycles of consolidation chemotherapy. These data suggest that postremission HuM195 could potentially reduce the number of consolidation chemotherapy courses required to achieve long-term remissions and thereby decrease therapy-related morbidity.

After treatment with RA and chemotherapy, approximately 75% of patients with newly diagnosed APL will achieve long-term remissions (31, 32, 34–36). Among patients treated with all-trans-RA induction followed by two cycles of consolidation chemotherapy, Tallman et al. (37) report the estimated probability of disease-free survival after 1, 2, and 3 years to be 87%, 70%, and 67%, respectively. Mandelli et al. (34) report 1- and 2-year disease-free survival rates of 83% and 79% for patients induced with combined all-trans-RA and idarubicin followed by three cycles of chemotherapy. Fenaux et al. (38) noted an event-free survival rate of 77% at 2 years for patients treated with all-trans-RA induction followed by two courses of consolidation chemotherapy and 84% for patients treated with concomitant all-trans-RA and chemotherapy as induction followed by two cycles of consolidation. Among patients treated in earlier studies with RA induction and consolidation chemotherapy at our institution, the estimated probabilities of disease-free survival after 1, 2, and 3 years were 86%, 80%, and 76%, respectively. In the current study for all newly-diagnosed patients treated with RA, HuM195, and chemotherapy, the 1-, 2-, and 3-year disease-free survival rates were 100%, 89%, and 89%, respectively. Although not statistically significant, these results suggest that the addition of HuM195 to postremission chemotherapy may extend disease-free survival times. Larger patient numbers, a longer follow-up duration, and prospective randomized trials will be necessary to prove a statistically significant benefit to this approach.

In general, biological therapies designed to act on minimal disease require large randomized trials to evaluate their effects. Because APL is characterized by a detectable molecular marker whose presence directly correlates with relapse, this disease serves as a model in which one can assess the activity of immunotherapy in a relatively brief period of time using RT-PCR techniques. In this study, 50% of the patients with newly diagnosed APL who had positive RT-PCR determinations before receiving HuM195 became negative after antibody therapy alone. These data suggest that HuM195 has activity against minimal residual disease in APL and that its use overcomes the nonspecific myelosuppression and immunogenicity seen in earlier studies with 131I-labeled M195 (22–24). Moreover, these results support the investigation of postremission therapy with
HuM195 in other subtypes of acute myelogenous leukemia and myeloid leukemias in general, where molecular markers of residual disease may not be available.

The effect of maintenance therapy with HuM195 after the completion of chemotherapy is difficult to assess without a randomized trial because the molecular marker for residual disease is almost always undetectable after completion of consolidation chemotherapy. Nevertheless, Fenaux et al. (38) and Tallman et al. (37) have reported that maintenance with RA and/or chemotherapy prolongs remission duration in patients with newly diagnosed APL. These studies provide the rationale for the study of HuM195 in the postchemotherapy setting.

Only one molecular remission was observed among eight patients (13%) with APL in second or greater remission, suggesting that the optimal time for treatment with HuM195 may be in newly diagnosed disease. No differences in antigen expression were noted among the relapsed patients. The lower molecular remission rate in relapsed patients may be explained in part by a quantitative difference in the level of residual APL in patients with newly diagnosed and relapsed disease after remission induction. Additionally, resistance to immunologically mediated mechanisms of cytotoxicity may account for the difference in molecular remission rates. In vitro studies showed that multidrug resistance cell lines created by continuous selection with vincristine or by retroviral infection were resistant to complement-mediated cytotoxicity by HuM195. This resistance was found to be related to an elevated intracellular pH observed in the multidrug resistance cell lines (39).

This study suggests that HuM195 has activity against minimal residual disease in APL. Multiple doses of HuM195 can be administered safely to patients after remission induction and appear to eliminate minimal residual disease detectable by RT-PCR in some patients. The use of postremission HuM195 after induction with RA could potentially reduce the number of consolidation chemotherapy courses required for long-term remissions. Combinations of HuM195 with other active agents, such as arsenic trioxide (40), may further decrease or eliminate the need for consolidation chemotherapy in APL. This study provides the rationale for monoclonal antibody-based therapy for residual or reduced disease in various malignancies.

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Molecular Remission Induction with Retinoic Acid and Anti-CD33 Monoclonal Antibody HuM195 in Acute Promyelocytic Leukemia


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