Sequential Cytarabine and α-Particle Immunotherapy with Bismuth-213–Lintuzumab (HuM195) for Acute Myeloid Leukemia

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Abstract

Purpose: Lintuzumab (HuM195), a humanized anti-CD33 antibody, targets myeloid leukemia cells and has modest single-agent activity against acute myeloid leukemia (AML). To increase the potency of the antibody without the nonspecific cytotoxicity associated with β-emitters, the α-particle-emitting radionuclide bismuth-213 (213Bi) was conjugated to lintuzumab. This phase I/II trial was conducted to determine the maximum tolerated dose (MTD) and antileukemic effects of 213Bi-lintuzumab, the first targeted α-emitter, after partially cytoreductive chemotherapy.

Experimental Design: Thirty-one patients with newly diagnosed (n = 13) or relapsed/refractory (n = 18) AML (median age, 67 years; range, 37-80) were treated with cytarabine (200 mg/m2/d) for 5 days followed by 213Bi-lintuzumab (18.5-46.25 MBq/kg).

Results: The MTD of 213Bi-lintuzumab was 37 MBq/kg; myelosuppression lasting >35 days was dose limiting. Extramedullary toxicities were primarily limited to grade ≤2 events, including infusion-related reactions. Transient grade 3/4 liver function abnormalities were seen in five patients (16%). Treatment-related deaths occurred in 2 of 21 (10%) patients who received the MTD. Significant reductions in marrow blasts were seen at all dose levels. The median response duration was 6 months (range, 2-12). Biodistribution and pharmacokinetic studies suggested that saturation of available CD33 sites by 213Bi-lintuzumab was achieved after partial cytoreduction with cytarabine.

Conclusions: Sequential administration of cytarabine and 213Bi-lintuzumab is tolerable and can produce remissions in patients with AML. Clin Cancer Res; 16(21); 5303–11. ©2010 AACR.

Although standard induction therapy with cytarabine and an anthracycline produces complete remissions (CR) in 50% to 70% of patients with acute myeloid leukemia (AML), long-term survival is seen in only 20% to 40% of patients (1). Following relapse, additional chemotherapy produces remissions in only 20% to 25% of patients. Although allogeneic hematopoietic stem cell transplantation (HSCT) can produce long-term remissions in ~30% of patients with relapsed AML, most patients are not appropriate candidates due to age, comorbidities, or lack of a suitable donor (2). The prognosis for older patients is even worse, with a 5-year survival rate of 5% for patients ≥65 years of age (3). Therefore, new therapies are needed to improve overall survival and reduce therapy-related toxicity.

Early studies showed that β-particle-emitting anti-CD33 constructs containing iodine-131 or yttrium-90 could eliminate large leukemic burdens but produced prolonged myelosuppression requiring HSCT (4, 5). The unique physical and radiobiological properties of α-particles, however, may provide more efficient tumor cell killing and reduce the nonspecific cytotoxic effects seen with β-emitters. Compared with β-particles, α-particles have a shorter range (50-80 versus 800-10,000 μm) and a higher linear energy transfer (100 versus 0.2 keV/μm; ref. 6). As few as one or two α-particles can kill a target cell. Therefore, the potential for specific antitumor effects makes α-particle immunotherapy an attractive approach for the treatment of cytoreduced or minimal disease.

Lintuzumab (HuM195) is a humanized monoclonal antibody that targets CD33, a 67-kDa cell surface glycoprotein expressed on most myeloid leukemia cells. It is also found on committed myelomonocytic and erythroid progenitors but not on pluripotent stem cells, granulocytes, or nonhematopoietic tissues (7, 8). Lintuzumab induces...
Translational Relevance

This report offers proof of principle that targeted α-particle immunoconjugate therapy can produce remissions in patients with advanced myeloid leukemia. Because of the short range and high energy of α-emissions, this approach may be beneficial in the setting of small-volume or micrometastatic disease in a variety of tumors. This study provides the rationale for the further development of α-particle immunoconjugate using 213Bi and alternative radioisotopes in leukemia as well as other cancers, such as breast and prostate carcinoma, where micrometastatic disease is present.

Materials and Methods

213Bi-lintuzumab preparation

The bifunctional chelate 2-(4-isothiocyanoatobenzyl) diethylentriamine pentacetic acid (SCN-CHX-A-DTPA) was conjugated to lintuzumab (Protein Design Labs, Inc.) by TSI Washington, with a ligand-to-protein ratio of 4.5 (16–19). 225Ac, supplied by Actinium Pharmaceuticals, Inc., was obtained from Oak Ridge National Laboratory or the Institute for Transuranium Elements. Following construction of 225Ac/213Bi generators, 213Bi was eluted every 3 to 4 hours and conjugated to lintuzumab–SCN-CHX-A-DTPA using previously described methods (17, 20–23). Unconjugated antibody was added to adjust the specific activity to 555 to 740 MBq/mg to preserve the immunoreactivity of the radioconjugate. The final product was administered as an injection over 5 minutes.

Patient eligibility

Patients with previously untreated AML age >60 years or those who were unable to receive intensive chemotherapy due to comorbid conditions, such as cardiovascular disease, were eligible. Patients with relapsed or primary refractory AML were also included. More than 25% of the patients’ bone marrow blasts were required to express CD33. No antileukemic therapy was administered for 3 weeks before study entry except for hydroxyurea, which was discontinued before treatment. Concurrent use of either oral or intravenous antibiotics was allowed. Entry criteria included creatinine <2 mg/dL or creatinine clearance >60 mL/min, bilirubin ≤2 mg/dL, and alkaline phosphatase and aspartate aminotransferase ≤2.5 times normal. Patients could not have detectable antibodies to lintuzumab or active central nervous system involvement by leukemia. Patients were treated from April 2001 to June 2006 at Memorial Sloan-Kettering Cancer Center on a protocol approved by the Center’s institutional review board. All subjects gave written informed consent according to the Declaration of Helsinki.

Treatment

Patients were hospitalized and received cytarabine at a dose of 200 mg/m² daily by i.v. continuous infusion for 5 days. Within 8 days after completion of cytarabine, two to four injections of 213Bi-lintuzumab (518–1,262 MBq each) were given over 1 to 2 days. Because 213Bi yields 4.5. 225Ac/213Bi generator, we escalated radioactivity doses by increasing the number of injections. Four dose levels of 213Bi-lintuzumab were administered in the phase I portion of the trial: 18.5, 27.75, 37, and 46.25 MBq/kg. Additional patients were treated at the maximum tolerated dose (MTD) of 37 MBq/kg in the phase II portion of the trial. Total administered activities ranged from 1,195 to 4,755 MBq, and total antibody doses ranged from 2 to 6.3 mg. Given the low level of γ-emissions from 213Bi, radiation isolation for patients and precautions for staff were not required. Hematopoietic growth factor support was allowed if clinically indicated according to American Society of Clinical Oncology guidelines (24). Prophylactic antibiotic and antifungal therapy was not given routinely. Toxicity was assessed according to the Common Toxicity Criteria version 2.0 established by the National Cancer Institute. To measure antileukemic effects, we did bone marrow aspirations at baseline, before administration of 213Bi-lintuzumab, and then 4 and 8 weeks after the start of treatment. Four of the six responding patients received consolidation therapy, generally with single-agent cytarabine, at the discretion of the treating physician.
Biodistribution and pharmacokinetics
The 440-keV $\gamma$-emissions of $^{213}$Bi allowed biodistribution studies to be done as previously described (15, 25). Patients underwent continuous $\gamma$-camera imaging for 60 minutes beginning immediately after the first and last injections of $^{213}$Bi-lintuzumab using a dual-head Vertex $\gamma$-camera (ADAC Laboratories). We calculated activity in the liver as the geometric mean of the counts/minutes in the anterior and posterior images. Activity in the spine was converted to marrow %ID by scaling a nominal estimate of the red marrow mass in the vertebrae according to body weight (25). Additional pharmacokinetic data were obtained through parametric rate images by fitting a linear expression to the counts in each pixel/minute over the 60-minute imaging period as previously described (26).

Response definitions
CR was defined as <5% bone marrow blasts with a neutrophil count of >1,000/μL, a platelet count of >100,000/μL, and no extramedullary disease. CR with incomplete platelet recovery (CRp) was defined similarly, except that the platelet count was <100,000/μL in the setting of transfusion independence. Partial remission (PR) was defined as a >50% decrease in bone marrow blasts with all of the hematologic values for CR (27).

Statistical analysis
Three to six patients were treated at each dose level in the phase I portion of the study. The MTD was defined as the highest dose for which the incidence of dose-limiting toxicity (DLT) is ≤33%. DLTs included (a) any grade 4 non-hematologic toxicity, (b) grade 3 abnormalities of liver function or serum creatinine, and (c) grade 4 leukopenia lasting ≥35 days in patients with baseline leukocyte counts of >1,000/μL. The primary end point in the phase II portion of the trial was response (CR + CRp + PR). Secondary end points included disease-free survival and overall survival. A two-stage design was used in which the probabilities of type I and II errors were 0.05 and 0.2, respectively. This design yielded at least 80% probability of a positive result if the true response rate was at least 20%.

We correlated toxicity and antileukemic effects with various clinical parameters. Reductions in marrow blasts following cytarabine alone and in combination with $^{213}$Bi-lintuzumab were compared using the two-sided $t$ test. We compared clinical parameters, such as number of prior treatment regimens, baseline percentage of marrow blasts, and level of CD33 expression, between those patients with untreated AML/relapsed AML receiving first salvage treatment and those patients with primary refractory AML/multiply treated relapsed disease using the two-sided $t$ test. We estimated the probability of overall survival using the Kaplan-Meier method.

Results

Patient characteristics
Thirty-one patients (median age, 67 years; range, 37-80) were treated with sequential cytarabine and $^{213}$Bi-lintuzumab. Thirteen patients had untreated AML (5 with de novo AML; 8 with secondary AML). Among these previously untreated patients, six (46%) had Charlson comorbidity scores of >1, an established adverse prognostic factor for response to standard induction chemotherapy (28, 29). Fifteen patients had relapsed AML (7 of whom received prior salvage treatment), and 3 patients had primary refractory AML. According to the Cancer and Leukemia Group B risk classification system (30), 22 patients (71%) had intermediate-risk karyotypes, whereas 9 (29%) had poor-risk cytogenetic abnormalities. Fifteen patients were treated in the phase I

<table>
<thead>
<tr>
<th>Table 1. Patient characteristics</th>
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<tr>
<td>Characteristic</td>
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<tr>
<td>Median age (range), y</td>
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<tr>
<td>Sex</td>
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<tr>
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<tr>
<td>Female</td>
</tr>
<tr>
<td>Disease status</td>
</tr>
<tr>
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<tr>
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<td>Secondary</td>
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<td>1</td>
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<td>2</td>
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<tr>
<td>Primary refractory</td>
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<tr>
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<td>Untreated</td>
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<tr>
<td>Previously treated</td>
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<td>(range), mo</td>
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<td>Median age (range), y</td>
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<td>Cytogenetics</td>
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<tr>
<td>Intermediate-risk</td>
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<tr>
<td>Poor-risk</td>
</tr>
<tr>
<td>Median CD33 expression</td>
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<tr>
<td>(range), %</td>
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</table>

*Includes two patients who underwent allogeneic HSCT.
portion of the trial: 3 patients each received 18.5 and 27.75 MBq/kg, 5 received 37 MBq/kg, and 4 were treated with 46.25 MBq/kg. An additional 16 patients were enrolled in the phase II portion of the study.

**Adverse effects**

As expected, myelosuppression was the most common toxicity. Among 10 patients who began therapy with an absolute neutrophil count of ≥1,000/μL, 9 (90%) had grade 3 (n = 1) or grade 4 (n = 8) neutropenia. Among 15 patients who were platelet transfusion independent before treatment, all developed grade 3 (n = 4) or grade 4 (n = 11) thrombocytopenia. The median time from the start of cytarabine until recovery from grade 4 leukopenia was 29 days (range, 2-59). In the six patients who responded, the median time to neutrophil recovery ≥1,000/μL was 30 days (range, 25-57), and the median time to platelet transfusion independence was 34 days (range, 23-87). The duration of myelosuppression was unrelated to the administered activity (P = 0.221) or the level of CD33 expression (P = 0.084) due in part to the effect of cytarabine. During the phase I portion of the trial, dose-limiting myelosuppression (defined as grade 4 leukopenia lasting ≥35 days) was seen in two of four patients treated with 46.25 MBq/kg. Therefore, we determined the MTD of 213Bi-lintuzumab following cytarabine to be 37 MBq/kg.

Myelosuppression-associated febrile episodes occurred in most patients. Twenty (65%) had documented bacterial infections, predominantly catheter-related coagulase-negative *Staphylococcus* bacteremia. Nineteen patients (61%) developed presumed fungal pneumonia, and 1 had candidemia. Eight patients (26%) had neutropenic fever without an identifiable source of infection. Peri-induction mortality related to infectious complications occurred in 2 of 21 patients (10%) receiving 37 MBq/kg and in 1 of 4 patients (25%) treated with 46.25 MBq/kg.

Liver function abnormalities were the most common extramedullary toxicity (Table 2). Across all dose levels, 21 of 31 patients (68%) developed transient elevations of bilirubin, alkaline phosphatase, or transaminases; however, grade 3 or 4 abnormalities were seen in only 5 patients (16%). No patients had evidence of sinusoidal obstructive syndrome. The median time to the onset of liver function abnormalities was 7 days (range, 3-30), and the median duration was 6 days (range, 1-27). Increases in serum creatinine occurred in 11 patients (35%), but only 1 developed a grade 3 elevation while receiving concomitant liposomal amphotericin and aminoglycosides. Nine patients (29%) had infusion-related reactions following administration of 213Bi-lintuzumab, typically characterized by fever, chills, and rigors, including 1 patient with grade 3 bronchospasm. Additionally, 1 patient developed a grade 4 gastrointestinal hemorrhage.

**Table 2. Extramedullary toxicity**

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Dose level</th>
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<tbody>
<tr>
<td></td>
<td>18.5 MBq/kg (n = 3)</td>
</tr>
<tr>
<td>Bilirubin</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>0</td>
</tr>
<tr>
<td>Grade 4</td>
<td>0</td>
</tr>
<tr>
<td>Alkaline phosphatase (grade 3)</td>
<td>0</td>
</tr>
<tr>
<td>AST/ALT (grade 3)</td>
<td>0</td>
</tr>
<tr>
<td>Creatinine (grade 3)</td>
<td>0</td>
</tr>
<tr>
<td>Infusion-related reaction (grade 3)</td>
<td>0</td>
</tr>
<tr>
<td>GI bleeding (grade 4)</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; GI, gastrointestinal.

**Antileukemic activity and responses**

Patients underwent bone marrow aspirations at baseline, after receiving cytarabine but before administration of 213Bi-lintuzumab, and finally 4 or 8 weeks after the start of therapy. Not all patients had evaluable specimens at each time point. Reductions in bone marrow blasts were seen at all dose levels (Fig. 1). Of the 31 patients, 26 had interpretable bone marrow aspirations 4 or 8 weeks after the start of therapy. Among these 26 patients, 20 (77%) had a >20% decrease in marrow blasts. The mean reduction ± SD was 47 ± 71% (range, −223 to 99). The reduction of marrow blasts was not related to level of CD33 expression (P = 0.083) or the administered activity (P = 0.439), likely due to variability in disease burden and leukemia subtypes, and in resistance to chemotherapy or radiation.

A bone marrow aspiration done following the completion of cytarabine but before administration of 213Bi-lintuzumab served to evaluate the relative contribution of each agent to the overall activity of the regimen. Typically, the marrow was assessed on day 6 or 7 of treatment before the full antileukemic effects of cytarabine may be apparent. Nevertheless, among 25 patients whose bone marrow was evaluable at this time point, all...
had detectable disease with blast counts ranging from 7% to 96%. Only 11 (40%) had a >20% reduction in marrow blasts, and the mean decrease was 10 ± 55% (range, −116 to 88).

A total of 21 patients had assessable bone marrow evaluations before treatment, after cytarabine but before 213Bi-lintuzumab, and 4 or 8 weeks after the start of treatment. Following cytarabine alone, 13 patients (62%) had reductions in marrow blasts with a mean decrease of 10 ± 58% (range, −116 to 88). From the postcytarabine time point to recovery following treatment with 213Bi-lintuzumab, 16 patients (76%) had reductions in marrow blasts, including 6 (29%) who showed progression with cytarabine alone. Five patients (24%) had increases in marrow blasts compared with the postcytarabine evaluation. The mean decrease in blasts during this interval was 41 ± 57% (range, −90 to 98). These data suggest that 213Bi-lintuzumab enhanced the antileukemic effect of cytarabine in most patients.

Clinical responses were seen in 6 of the 25 patients (24%; 95% confidence interval, 11-44) who received doses of ≥37 MBq/kg (Table 3). All responders had poor-risk features, including age ≥70 years or secondary AML; however, none of the 6 patients receiving <37 MBq had a clinical response. Among the 11 patients with untreated AML who received doses greater than or equal to the MTD, 2 achieved CR (18%), 1 achieved CRp (9%), and 2 achieved PR (18%). Among the seven patients with AML in first relapse who had not received prior salvage therapy, one (14%) attained a CRp. None of the seven patients with primary refractory AML or multiply treated relapsed disease responded, indicating that effective cytoreduction was necessary to achieve remission after administration of 213Bi-lintuzumab. Except for the number of prior regimens (P < 0.005), no clinical differences, such as baseline marrow blast percentage (P = 0.779) or level of CD33 expression (P = 0.258), between the group with untreated AML

Table 3. Characteristics of responding patients

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age/sex</th>
<th>Diagnosis</th>
<th>Cytogenetics</th>
<th>Prior therapy</th>
<th>Dose level (MBq/kg)</th>
<th>Response (duration, mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>74/M</td>
<td>2° AML after MDS</td>
<td>Complex</td>
<td>5-Aza (MDS)</td>
<td>37</td>
<td>PR (4)</td>
</tr>
<tr>
<td>10</td>
<td>79/M</td>
<td>2° AML, h/o APL</td>
<td>der(7), t(17)</td>
<td>ATO, SAHA (AML); ATRA, IDR/Ara-C × 3 (APL)</td>
<td>37</td>
<td>CR (9)</td>
</tr>
<tr>
<td>11</td>
<td>80/M</td>
<td>AML, M2 de novo</td>
<td>8</td>
<td>None</td>
<td>37</td>
<td>CR (12)</td>
</tr>
<tr>
<td>14</td>
<td>74/M</td>
<td>2° AML after MDS</td>
<td>Normal</td>
<td>None</td>
<td>46.25</td>
<td>PR (7)</td>
</tr>
<tr>
<td>15</td>
<td>70/F</td>
<td>AML, M2, relapsed</td>
<td>Normal</td>
<td>IDR/Ara-C × 3</td>
<td>46.25</td>
<td>CRp (5)</td>
</tr>
<tr>
<td>18</td>
<td>75/M</td>
<td>2° AML after MDS</td>
<td>del(8q)</td>
<td>Decitabine (MDS)</td>
<td>37</td>
<td>CRp (2)</td>
</tr>
</tbody>
</table>

Abbreviations: MDS, myelodysplastic syndrome; 5-Aza, azacitidine; ATO, arsenic trioxide; SAHA, vorinostat; ATRA, all-trans retinoic acid; IDR, idarubicin; Ara-C, cytarabine; APL, acute promyelocytic.

Clinical responses were seen in 6 of the 25 patients (24%; 95% confidence interval, 11-44) who received doses of ≥37 MBq/kg (Table 3). All responders had poor-risk features, including age ≥70 years or secondary AML; however, none of the 6 patients receiving <37 MBq had a clinical response. Among the 11 patients with untreated AML who received doses greater than or equal to the MTD, 2 achieved CR (18%), 1 achieved CRp (9%), and 2 achieved PR (18%). Among the seven patients with AML in first relapse who had not received prior salvage therapy, one (14%) attained a CRp. None of the seven patients with primary refractory AML or multiply treated relapsed disease responded, indicating that effective cytoreduction was necessary to achieve remission after administration of 213Bi-lintuzumab. Except for the number of prior regimens (P < 0.005), no clinical differences, such as baseline marrow blast percentage (P = 0.779) or level of CD33 expression (P = 0.258), between the group with untreated AML.
or receiving first salvage therapy and the group with refractory or multiply treated relapsed AML were apparent. The lack of responses in heavily pretreated patients indicates the need for effective reduction in disease burden before administration of α-particle immunotherapy to achieve remission.

The overall response rate (CR + CRp + PR) among the 21 patients receiving the MTD of 37 MBq/kg was 19% (95% confidence interval, 7-41). Two patients achieved CR, one had a CRp, and one had a PR. This group included 9 patients with previously untreated AML (3 de novo AML; 6 secondary AML), 10 with relapsed AML (6 first salvage; 4 previously treated), and 2 with primary refractory AML. Although all four responding patients were >70 years of age and three had secondary AML, none had received prior therapy for AML.

The median response duration was 6 months (range, 2-12). The median overall survival duration for all patients was 4.6 months (range, 1-30). Among the six responders, the median survival was 13.7 months (range, 5-30). The median survival for patients with untreated AML (n = 13) was 7.7 months (range 1-30), whereas for patients with previously treated AML (n = 18), the median survival was 3.1 months (range, 1-30; Fig. 2).

**Biodistribution and pharmacokinetics**

Four patients (one at each dose level) underwent detailed biodistribution and pharmacokinetic studies. Posterior γ-camera imaging showed rapid localization of isotope to areas of leukemic involvement, including the bone marrow of the vertebrae and pelvis, the liver, and the spleen in all patients (Fig. 3). Despite avidity for free bismuth, the...
kidsneys were not visualized, confirming the stability of $^{213}$Bi-lintuzumab in vivo. In contrast to the results seen in the initial phase I trial, in which $^{213}$Bi-lintuzumab was given without prior cytoreduction (15), cardiac blood pooling was seen after the last injection in one patient treated with 27.5 MBq/kg, indicating saturation of CD33 antigen sites within the bone marrow, liver, and spleen (Fig. 3A and B). Additional pharmacokinetic data were obtained by parametric rate imaging, in which an increase in the rate of isotope accumulation over the 1-hour imaging period is depicted by red-orange, and clearance is shown in blue-green. Reduced bone marrow uptake or clearance of $^{213}$Bi-lintuzumab was seen after multiple injections in all four patients who were studied, indicating saturation of antigen sites after partial cytoreduction with cytarabine (Fig. 3C and D). This is consistent with a reduction in the total number of target antigens by cytarabine and not due to loss of target leukemia cells because imaging was done at the time of injection and α-particle-mediated cytoreduction of the leukemia would not be expected for several days.

Uptake of $^{213}$Bi by the marrow and liver, accounting for 70% to 90% of the injected activity, occurred within 5 to 10 minutes after injection and was maintained throughout the 1-hour period of image collection. Marrow activity after the first and last injections of $^{213}$Bi-lintuzumab was constant in three patients and decreased in one patient after multiple injections (Fig. 3E). All four patients had decreases in liver uptake following multiple injections, indicating a "first-pass" binding effect with CD33 saturation of target cells (including leukemia cells and Kupffer cells) within the imaged liver space after several milligrams of antibody, as previously observed (Fig. 3F; ref. 15).

**Discussion**

In this study, we show that sequential administration of cytarabine and $^{213}$Bi-lintuzumab is tolerable and can produce remissions in some patients with AML. Although a relatively small group of heterogeneous patients was included in this trial, it provides proof of principle that targeted α-particle immunotherapy may be effective at reducing low-volume disease. These results suggest that further investigation of radioimmunotherapy with α-emitters after cytotherapy and in the postremission or adjuvant setting is warranted.

Except as conditioning for HSCT, radioimmunotherapy with long-range, low-energy β-emitters is useful only in treating bulky, radiosensitive cancers such as lymphoma because DLT usually results from nonspecific irradiation of normal tissues. Conversely, therapy with high-energy, short-range α-particles can provide far more potent and selective delivery of radiation to individual tumor cells, yielding enhanced antitumor activity with decreased toxicity. Despite these advantages, CRs with $^{213}$Bi-lintuzumab alone would require extraordinarily high injected activities. If we assume tumor burdens of $10^{12}$ cells with an average CD33 density of 10,000/cell, $10^{16}$ binding sites are available to lintuzumab. With the specific activities that are feasible, only 1 in 2,700 lintuzumab molecules carries the radiolabel. Therefore, it remains difficult to deliver one to two $^{213}$Bi atoms to every leukemia cell, particularly because of its 46-minute physical half-life. In the setting of small-volume disease, however, the short path length and high linear energy transfer of α-particles are ideal. In this study, cytotherapy provided by cytarabine allowed further reduction of residual leukemia by $^{213}$Bi-lintuzumab to produce remissions.

Although decreases in marrow blasts were seen at all dose levels, we observed a $^{213}$Bi dose-response relationship with remission occurring only at doses ≥37 MBq/kg. This suggests that cytarabine was not likely the sole cause of remissions. Moreover, serial bone marrow evaluations suggest that $^{213}$Bi-lintuzumab augmented the antileukemic activity of cytarabine alone. Although blasts were seen on all marrow samples after administration of cytarabine but before $^{213}$Bi-lintuzumab, these specimens were obtained before day 14 of therapy. Because of this early time point, it is impossible to determine whether any patient would have achieved a CR with cytarabine alone. The ability of sequential cytarabine and $^{213}$Bi-lintuzumab to induce remissions was seen only in patients where effective cytoreduction with cytarabine was possible. Six of 18 patients (33%) with untreated AML or untreated first relapse who received doses of ≥37 MBq/kg responded, whereas none of the 7 patients with primary refractory AML or multiply treated relapsed disease benefited. This group of patients would not be expected to have a significant reduction in leukemic burden after single-agent cytarabine, and, as noted in an initial phase I study, treatment with $^{213}$Bi-lintuzumab alone at similar activities did not produce CRs in patients with large disease volumes.

The response rate in this trial was higher than expected from a single course of standard-dose cytarabine alone (31). Bodey et al. (32) reported a 3% response rate after a single course of cytarabine at the dose and schedule used in this study. Similarly, in a report by Bickers et al. (33), no responses were seen after one course of cytarabine at doses of 200 mg/m$^2$/d for 5 days. The response rate reported in these early trials may be lower than expected today because of significant improvements in supportive care over the past 30 years. There are numerous nonrandomized trials in the literature that report a response rate of 10% to 20% with low-dose cytarabine in older patients with AML, but, in general, multiple cycles of therapy are necessary. The largest randomized trial of low-dose cytarabine compared with hydroxyurea confirmed a response rate of 18% but only after a median of three courses. One of 103 patients (1%) achieved CR after the first cycle of therapy (34).

The current study shows the effect of disease burden on antibody biodistribution. In the initial phase I study of single-agent $^{213}$Bi-lintuzumab, in which similar total antibody doses were used, the percentage of injected activity reaching the marrow after multiple doses increased in 38% of patients, whereas activity in the liver and spleen decreased in 75% and 56% of patients, respectively (15). This suggested that CD33 sites in the liver and spleen can...
act as antigen sinks and that, as they become saturated, more drug reaches the marrow with repeated injections. Similarly, high numbers of circulating CD33-positive blasts or cell-free CD33 can adversely affect biodistribution of the drug by rapidly binding antibody and preventing it from reaching target sites within the marrow. In contrast, after partial cytoreduction in this trial, marrow activity remained constant or decreased in all four patients who were studied. γ-Camera imaging revealed cardiac blood pooling after the last injection of 213Bi-lintuzumab in one patient, suggesting that saturation of all antigen sites was possible in patients with smaller disease burdens. Additionally, parametric rate imaging following the last injection of 213Bi-lintuzumab showed decreased uptake or clearance of drug when compared with the first injection in all patients. Taken together, these data indicate greater saturation of antigen sites by 213Bi-lintuzumab in target organs after partial cytoreduction than with 213Bi-lintuzumab alone.

The strategy of arming lintuzumab with an α-particle-emitting radionuclide was originally proposed to increase the modest immunologically mediated antileukemic effects of the antibody itself. Based on a pilot study in which 1 of 10 patients with relapsed or refractory AML achieved a CR lasting over 5 years (12) and a phase II study that confirmed a 6% response rate (13), the role of lintuzumab in cytoreduced disease was examined in a randomized phase III trial (35). Patients with relapsed or refractory AML received mitoxantrone, etoposide, and cytarabine alone or with lintuzumab. Although an improvement in response rate attributable to unconjugated antibody therapy did not reach statistical significance (36% versus 28%; \( P = 0.28 \)), no difference in adverse events or treatment-related mortality between the two groups was seen. A more recent phase I trial was conducted to determine whether higher concentrations of lintuzumab sustained over prolonged periods could result in greater therapeutic efficacy (14). In this study, 7 of 17 patients with AML responded. These results have led to an ongoing randomized phase II study of low-dose cytarabine with or without lintuzumab in older patients with untreated AML who are unable to tolerate standard induction chemotherapy.

Gemtuzumab ozogamicin (GO) represents an alternative antibody-based treatment to this radioimmunotherapeutic approach. GO agent is composed of a humanized anti-CD33 monoclonal antibody conjugated to a derivative of the potent antitumor antibiotic calicheamicin. When released from the immunoconjugate within the cytoplasm of a leukemic cell, calicheamicin induces DNA damage and subsequent apoptotic cell death. In a series of trials conducted in adults with AML in first relapse, a response rate (CR + CRp) of 26% was achieved (36).

Typically, significant myelosuppression is seen with GO, even as a single agent. The median time to neutrophil recovery \( \geq 1,500/\mu L \) in responding patients was 48 days (36). In contrast, resolution of grade 4 leukopenia occurred after a median of 22 days following administration of single-agent 213Bi-lintuzumab in an earlier phase I study (15). Following sequential therapy with cytarabine and 213Bi-lintuzumab, responding patients in the current trial had neutrophil recovery after a median of 30 days. Treatment with GO is also associated with significant liver function abnormalities. Grade 3 or 4 hyperbilirubinemia and transaminase elevations were reported in 29% and 18% of patients, respectively. Sinusoidal obstructive syndrome was seen in 5% of patients (36). Grade 3 or 4 liver function abnormalities, however, did not occur with single-agent 213Bi-lintuzumab (15). When given after cytarabine in the current study, only 16% of patients developed significant hyperbilirubinemia, and 3% had grade 3/4 transaminase elevations. Sinusoidal obstructive syndrome was not observed. The more favorable toxicity profile of 213Bi-lintuzumab suggests that integration of targeted α-particle immunotherapy into treatment strategies with standard chemotherapy may be more feasible than chemotherapy-GO combinations.

The ability of 213Bi-lintuzumab to produce remissions in some patients with poor-risk AML in this trial provides the rationale for the use of α-particle immunotherapy in the setting of small-volume leukemias and cancers, or micrometastatic disease. The major obstacles to the widespread use of radioimmunotherapy with 213Bi, however, are its short half-life and the requirement of an on-site 225Ac/213Bi generator. Therefore, we developed a second-generation construct in which the isotope generator is directly conjugated to a tumor-specific antibody. In this strategy, 225Ac \( (t_{1/2} = 10 \text{ days}) \) can serve as an in vivo generator of four α-particles at or within a cancer cell. Based on the activity of 225Ac-containing radioimmunoconjugates in several xenograft models (37), we are currently conducting a phase I trial of 225Ac-lintuzumab in advanced myeloid leukemia. Additional studies combining 225Ac-lintuzumab with cytoreductive chemotherapy are planned.

Disclosure of Potential Conflicts of Interest

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References


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