ABSTRACT

Lintuzumab, a humanized anti-CD33 antibody, targets myeloid leukemia cells and has modest activity against acute myeloid leukemia (AML). To increase the antibody’s potency yet avoid nonspecific cytotoxicity seen with β-emitting isotopes, lintuzumab was conjugated to the α-emitters bismuth-213 (213Bi) and actinium-225 (225Ac). The 46-minute half-life of 213Bi limits its widespread use. Therefore, 225Ac was also conjugated to various antibodies using DOTA-SCN. We conducted a phase I trial of 213Bi-lintuzumab and subsequently administered cytarabine with 213Bi-lintuzumab in a phase I/II study. The toxicity and biological activity of 225Ac-lintuzumab in patients with relapsed/refractory AML was determined in a phase I dose-escalation trial. An initial phase I trial demonstrated the feasibility, safety and antileukemic activity of 213Bi-lintuzumab. 213Bi-lintuzumab produced responses in 24% of AML patients receiving doses >37 MBq/kg after partial cytoreduction with cytarabine. 225Ac-labeled immunoconjugates killed in vitro at doses at least 1,000 times lower than 213Bi analogs. Eighteen patients with relapsed/refractory AML received 18.5 to 148 kBq/kg of 225Ac-lintuzumab in a phase I study. Dose-limiting toxicities were myelosuppression lasting >35 days in one patient and death due to sepsis in two patients. The maximum tolerated dose (MTD) was 111 KBq/kg. Bone marrow blast reductions were seen across all dose levels. Targeted α-particle immunotherapy with 213Bi- and 225Ac-lintuzumab is safe, has significant antileukemic effects, and can produce remissions after partial cytoreduction.

Keywords: Alpha particle, Immunotherapy, Acute myeloid leukemia.

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INTRODUCTION

Although standard induction therapy with cytarabine and an anthracycline produces complete remissions (CR) in 50 to 70% of patients with acute myeloid leukemia (AML), but long-term survival is seen in only 20 to 40% of patients.1 Following relapse, salvage chemotherapy produces remissions in only 20 to 25% of patients. While allogeneic hematopoietic cell transplantation (HCT) can result in long-term survival in approximately 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 elderly patients is even worse with a 5-year survival rate of 5% for patients more than 65 years of age.3 Therefore, new therapies are needed to improve survival and reduce therapy-related toxicity.

Early studies showed that anti-CD33 constructs containing β particle-emitting iodine-131 or yttrium-90 could eliminate large leukemic burdens but resulted in prolonged myelosuppression requiring HCT.4,5 The unique physical and radiobiological properties of α-particles may provide more efficient tumor cell killing and reduce the nonspecific cytotoxic effects seen with β-emitters. The α-particles have a shorter range (50-80 µm compared to 800-10,000 µm of β-particles) and a higher linear energy transfer (LET) (100 keV/µm compared to 0.2 keV/µm of β-particles).6 As few as one or two α-particles can kill a target cell. Therefore, the potential for more efficient and specific antitumor effects with less damage to surrounding normal tissues 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 antibody-dependent cell-mediated cytotoxicity and can fix human complement in vitro.9 Previous studies demonstrated that lintuzumab can target leukemia cells in patients without immunogenicity,10 eliminate minimal residual disease in acute promyelocytic leukemia,11 and produce occasional remissions in AML.12-14 To increase the potency of the antibody but avoid the nonspecific cytotoxicity seen with the β-emitters like iodine-131 (131I) and yttrium-90 (90Y), we conjugated lintuzumab to bismuth-213 (213Bi) and actinium-225 (225Ac), the α-emitters.

Alpha-Particle Immunotherapy with 213Bi-Lintuzumab Preclinical Studies

213Bi (t½ = 45.6 minutes) is a radiometal that emits α-particle with 8 MeV energy. Additionally, a 440 keV photon emission accompanies 26.5% of 213Bi decays, allowing detailed biodistribution and dosimetry studies to be performed. Bismuth-labeled lintuzumab in vitro resulted in dose- and specific activity-dependent killing of CD33+ HL60 cells. Approximately 50% of target cells were killed...
when only two bismuth atoms were bound to the cell surface.16

**Single-Agent Phase I Trial**

In our previous work, based on these preclinical data, 18 patients with relapsed and refractory AML (17 patients) or chronic myelomonocytic leukemia (one patient) were treated with $^{213}\text{Bi}$-lintuzumab.17 The drug was given as a 5-minute infusion, two to four times daily in 148 to 925 MBq fractions over 2 to 4 days. Because $^{213}\text{Bi}$ yields were limited by the activity of each $^{225}\text{Ac}^{213}\text{Bi}$ generator and because of constraints on the specific activity that could be achieved for any one injection, we escalated radioactivity doses by increasing the number of injections. Patients received a total of 3 to 7 injections, Five dose levels were studied: 10.36, 15.54, 20.72, 25.9 and 37 MBq/kg. Biodistribution and dosimetry studies were performed by obtaining γ camera images after the first and last dose of $^{213}\text{Bi}$-lintuzumab. Using a 20% photopeak window centered at 440 keV, thirty, 1-minute images beginning at the start of each injection followed by ten, 3-minute images were collected.18

No significant extramedullary toxicities were seen. Grade I and II liver function abnormalities were seen in four patients (22%). The onset was typically 5 to 14 days following treatment, and these abnormalities resolved within 3 to 14 days. All 17 evaluable patients developed myelosuppression with a median time to recovery of 22 days (range: 12-41 days). Nearly all the $^{213}\text{Bi}$-lintuzumab rapidly localized to and was retained in areas of leukemic involvement, including the bone marrow, liver and spleen. Despite avidity for free bismuth, the kidneys were not visualized. There was no significant catabolism or clearance of the drug, confirming the stability of the construct. The mean absorbed dose per amount of injected activity to the marrow, and therefore to CD33+ target cells, was 9.8 mSv/MBq (range: 2.6-29.4 mSv/MBq). 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 and patient population. Blood and plasma antibody concentrations displayed typical α distributions over the first 20 to 40 minutes, followed by slower β clearance over the remaining 3 hours of sample collection.17

Fourteen (93%) of 15 evaluable patients had reductions in circulating blasts, and 14 (78%) of 18 patients had reductions in the percentage of bone marrow blasts (Fig. 1). No patients achieved CR, likely due to large tumor burdens in heavily pretreated patients and to the relatively low specific activities (329-766 MBq/mg) of $^{213}\text{Bi}$-lintuzumab. Nevertheless, this study demonstrated the safety, feasibility and antileukemic effects of $^{213}\text{Bi}$-lintuzumab and was the first proof-of-concept for systemic targeted α-particle immunotherapy in humans.17

**Sequential Cytarabine and $^{213}\text{Bi}$-Lintuzumab**

It may be hypothesized that a 1-2 log reduction in tumor burden could increase the number of $^{213}\text{Bi}$ atoms delivered to leukemia cells and produce remissions. To determine the effects of $^{213}\text{Bi}$-lintuzumab against cytoreduced disease, the authors conducted a phase I/II trial in which patients first received a nonremittive dose of cytarabine to decrease the leukemic burden.19 Thirty-one patients with newly diagnosed (n = 13) or relapsed/refractory (n = 18) AML were treated. Patients received cytarabine at a dose of 200 mg/m² daily by intravenous continuous infusion for 5 days. Within 8 days after completion of cytarabine, 2 to 4 injections of $^{213}\text{Bi}$-lintuzumab (518–1, 262 MBq each) were given over 1 to 2 days. Four dose levels of $^{213}\text{Bi}$-lintuzumab were administered in the phase I portion of the trial: 18.5, 27.75, 37 and 46.25 MBq/kg. An additional 16 patients were treated at the maximum tolerated dose (MTD) in the phase II portion of the trial.

During the phase I portion, dose-limiting myelosuppression (defined as grade IV leukopenia lasting ≥ 35 days) was seen in two of four patients treated with 46.25 MBq/kg. The MTD of $^{213}\text{Bi}$-lintuzumab following cytarabine was found to be 37 MBq/kg. Extramedullary toxicities were mainly limited to grade I and II events, including infusion-related reactions in nine patients (29%). Transient grade III/IV liver function abnormalities were seen in five patients (16%). No patient had evidence of sinusoidal obstructive syndrome. Treatment-related deaths occurred in two of 21 patients (10%) who received the MTD.19
Significant reductions in marrow blasts were seen across all dose levels. Clinical responses were observed in six of the 25 patients (24%; 95% CI: 11-44) who received doses of $\geq 37$ MBq/kg [2 CR, 2 CR with incomplete platelet recovery (CRp), and 2 PR] (Table 1). All responders had poor-risk features, including age $\geq 70$ years or secondary AML; however, none of the six patients receiving less than 37 MBq had a clinical response. None of the seven patients either with primary refractory AML or multiple treated relapsed disease responded, indicating that effective cytoreduction was necessary to achieve remission after administration of $^{213}$Bi-lintuzumab. The median response duration was 6 months (range: 2-12) with the median survival of 13.7 months (range: 5-30 months) among responders.19

Four patients (one at each dose level) underwent detailed biodistribution and pharmacokinetic studies. In contrast to the results seen in the initial phase I trial where $^{213}$Bi-lintuzumab was given as a single agent,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. Moreover, reduced bone marrow uptake of $^{213}$Bi-lintuzumab was seen after multiple injections in all four patients, indicating saturation of antigen sites after partial cytoreduction with cytarabine. Although a relatively small group of heterogeneous patients were included in this trial, it showed that targeted $\alpha$-particle immunotherapy can be effective at reducing low-volume disease.

**Actinium-225-Lintuzumab: A Targeted Alpha-Particle Nanogenerator**

**Preclinical Studies**

The major obstacles to the widespread use of $^{213}$Bi are its short half-life and the requirement of an on-site $^{225}$Ac/$^{213}$Bi-generator. Therefore, the author developed a second generation construct in which the isotope generator is directly conjugated to a tumor-specific antibody. In this strategy, $^{225}$Ac ($t_{1/2} = 10$ days) can serve as an in vitro generator of four $\alpha$-particles at or within a cancer cell. The macrocyclic ligand 1,4,7,10-tetraazaacyclododecane tetraacetic acid (DOTA) and its derivatives have been used for labeling of antibodies with $^{225}$Ac. A two-step procedure was developed in which $^{225}$Ac is first conjugated to DOTA-SCN followed by labeling of this construct to antibody.20

$^{225}$Ac-labeled tumor-specific antibodies can kill multiple cell lines in vitro with LD$_{50}$ values 1,000 to 10,000 times less than those of analogous $^{213}$Bi constructs. These findings led to in vivo studies in nude mice bearing human prostate carcinoma and lymphoma xenografts. Single nanocure doses of $^{225}$Ac-labeled tumor-specific antibodies significantly improved survival over controls and cured a substantial fraction of animals.21

**Phase I Study of $^{225}$Ac-Lintuzumab**

Based on the activity of $^{225}$Ac-containing radioimmunoconjugates in the animal models, we conducted a phase I trial of $^{225}$Ac-lintuzumab in advanced AML.22 Eighteen patients with relapsed (n = 11) or refractory (n = 7) AML were treated with a single infusion of $^{225}$Ac-lintuzumab at doses of 18.5 (n = 3), 37 (n = 4), 74 (n = 3), 111 (n = 6) or 148 (n = 2) kBq/kg. Dose-limiting toxicities including myelosuppression lasting more than 35 days in one patient receiving 148 kBq/kg and death from sepsis in two patients receiving 111 and 148 kBq/kg occurred. The MTD was determined to be 111 kBq/kg. As expected myelosuppression was the most common toxicity. Median time to resolution of grade IV leukopenia was 27 days (range: 0-71 days). Significant extramedullary toxicities were limited to transient grade III liver function abnormalities in three patients. We analyzed plasma pharmacokinetics by gamma counting at energy windows for two daughters of $^{225}$Ac, francium-221 ($^{221}$Fr) and $^{213}$Bi. Two-phase elimination kinetics was seen with mean plasma $t_{1/2-\alpha}$ and $t_{1/2-\beta}$ of 1.9 and 38 hours, respectively, similar to other lintuzumab constructs containing long-lived radionuclides. This is in contrast to $^{213}$Bi-lintuzumab, where the half-life is determined primarily by the short-lived radionuclide. Peripheral blasts were eliminated in 10 of 16 evaluable patients (63%), but only at doses of $\geq 37$ kBq/kg. Bone marrow blast reductions were seen in 10 of 15 evaluable patients (67%) at 4 weeks, including eight patients (53%) who had blast reductions of more than 50%. Three patients receiving 37, 111 and 148 kBq/kg respectively achieved marrow blasts of 5% or less.

**SUMMARY**

Systemically administered targeted $\alpha$-particle immunotherapy is feasible and has significant antitumor activity.
The shorter range and higher linear energy transfer of α-particles compared with β-particles may allow for more efficient and selective killing of individual tumor cells. These physical properties suggest that radioimmunotherapy with α-emitters may be best suited for the treatment of small-volume disease, as borne out in these clinical trials. Although reductions in leukemic blasts were seen when both 213Bi- and 225Ac-lintuzumab were given as single agents in phase I trials, remissions were only seen after effective cytoreduction. The use of 225Ac can overcome the logistical difficulties associated with short-lived radionuclides such as 213Bi. Building on the encouraging results seen with 213Bi-lintuzumab for cytoreduced leukemia, we are now conducting a multicenter phase I/II trial of 225Ac-lintuzumab in combination with low-dose cytarabine for elderly patients with untreated AML. These studies provide the rationale for further investigation of targeted α-particle immunotherapy for minimal residual disease or small-volume disease in a variety of malignancies.

REFERENCES


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