Phase I trial of a novel human monoclonal antibody mAb216 in patients with relapsed or refractory B-cell acute lymphoblastic leukemia

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ABSTRACT

Background

This phase I trial was conducted to determine the safety and pharmacokinetics of monoclonal antibody 216, a human monoclonal Immunoglobulin M antibody targeting a linear B-cell lactosamine antigen, administered alone and in combination with vincristine in patients with relapsed or refractory B-cell acute lymphoblastic leukemia, and to preliminarily assess tumor targeting and efficacy.

Design and Methods

Three cohorts of patients received escalating doses of monoclonal antibody 216 administered as an intravenous infusion. In the case of poor response to the first dose of monoclonal antibody 216 alone, defined as less than 75% reduction in peripheral blood blast count, a second dose of the antibody with vincristine was given between days 4 and 7. Responses were assessed weekly until day 35. Serum concentration of monoclonal antibody 216 was measured before and after infusion. Monoclonal antibody 216 targeting was determined with an anti-idiotypic antibody to monoclonal antibody 216 and preliminary efficacy was analyzed by changes in peripheral blood blasts.

Results

Thirteen patients were enrolled. One episode of grade 3 epistaxis was the only dose-limiting toxicity observed. All patients showed a poor response to the first monoclonal antibody 216 infusion with a decrease in peripheral blasts from 6-65% in 9 patients. In 8 patients, addition of vincristine to monoclonal antibody 216 resulted in an average reduction of the peripheral blasts of 81%. One patient without peripheral blasts achieved a hypoplastic marrow without evidence of leukemia after one infusion of monoclonal antibody 216 and monoclonal antibody 216/vincristine each. Monoclonal antibody 216 was detected on peripheral blasts in all patients.

Conclusions

Treatment with monoclonal antibody 216 in combination with vincristine is feasible and well tolerated in patients with relapsed or refractory B-cell acute lymphoblastic leukemia. Binding of monoclonal antibody 216 to leukemic blasts was efficient, and favorable early responses were observed. *(clinicaltrials.gov identifier 00313053/00313079)*

Key words: antibody 216, relapsed, refractory, B-cell acute lymphoblastic leukemia.

Introduction

Over recent decades, there has been a steady improvement in treatment outcome of patients with ALL. This progress is due to factors including intensification of chemotherapy and risk-adapted therapy, as well as better supportive care.[1] As a result, cure rates now approach 90% and 40% for pediatric and adult ALL, respectively.[2] While the risk of relapse is much lower in the pediatric population, both pediatric and adult patients face significantly inferior outcomes if the disease recurs. Less than one third of children and few adults with relapsed ALL survive, despite the use of aggressive salvage regimens and stem cell transplantation.[3,4] Novel therapies are, therefore, needed that have both activity against ALL and a toxicity profile distinct from conventional chemotherapy. Targeted therapy using monoclonal antibodies such as rituximab and trastuzumab has become an integral part of standard treatment for certain malignancies.[5,6] For ALL, there is preclinical and early clinical experience with a variety of monoclonal antibodies including rituximab, alemtuzumab, epratuzumab and gemtuzumab,[7-10] suggesting that the use of monoclonal antibodies alone or in combination with standard chemotherapy is a viable treatment option.

Human mAb216 is a naturally occurring human monoclonal IgM antibody. The variable heavy chain of mAb216 is derived from the VH4-34 gene encoding cold agglutinin-IgM antibodies against human fetal or adult red blood cells (RBC).[11,12] A subset of anti-fetal RBC antibodies also binds and kills human B lymphocytes.[13-15] The epitope on human B cells is a linear lactosamine determinant similar to the fetal RBC i-antigen that is not expressed on more than 90% of CD34-positive cells in normal bone marrow and is rarely expressed on normal adult RBC. A branching transferase enzyme converts i to I-antigen following birth. Rare individuals do not branch their i-antigen due to inheritance of an autosomal recessive mutation in the transferase gene.[16,17]

In a library of 30 VH4-34 encoded antibodies, mAb216 was found to have high binding and cytotoxicity against normal B lymphocytes, B-cell lymphomas and B-progenitor lymphoblasts.[18-20] Binding of mAb216 to its linear lactosamine ligand leads to disruption of the plasma membrane and formation of large membrane pores resulting in cell lysis (*Online Supplementary Figure S1*).[14,18] This non-classical apoptosis occurs in the absence of complement fixation but *in vitro* cytotoxicity is increased in assays using human complement.[20] *In vitro* binding and cytotoxicity studies with B-cell ALL bone marrow specimens demonstrated that mAb216 binds and kills blasts from patients with B-progenitor ALL.[20] Using flow cytometry, the cytotoxic effect of mAb216 and chemotherapeutic drugs (daunomycin 4ng/mL, L asparginase 0.8 U/mL and vincristine 2ng/mL) used to treat B-progenitor ALL either alone or in combination was assessed in pre-B ALL cell lines. The combination of vincristine and mAb216 caused an enhanced degree of cytotoxicity when compared to the additive effect of each single agent alone.[20]

The development of mAb216 for clinical trial evaluation was governed by the Rapid Access to Intervention Development (RAID) program. Clinical grade antibody was manufactured by the Biologic Resource Branch (BRB) of the National Cancer Institute. Investigators from the NCI BRB, NCI Cancer Treatment Evaluation Program (CTEP) and Stanford worked with the FDA on the development, pre-clinical testing and clinical trial design for mAb216. Because this was the first natural occurring human mAb given to patients, the FDA only allowed two doses of mAb216 until *in vivo* toxicity could be evaluated. Rather than giving two doses of mAb216 alone, vincristine was added to the second dose of mAb216 because *in vitro* testing had shown enhancement of mAb216 cytoxicity in combination with vincristine, thereby providing a higher chance for response. The starting dose was 5% of the highest amount given to rabbits which did not cause toxicity.

The primary objective of this first-in-human phase I study was to determine the maximum-tolerated dose and dose-limiting toxicities of mAb216 as a single agent and in combination with vincristine in patients with relapsed or refractory B-cell ALL. The secondary objectives were to characterize the pharmacokinetic behavior of mAb216 and to preliminarily assess tumor targeting and clinical efficacy.

Design and Methods

Patient eligibility

Patients of any age with B-lineage ALL in first or later relapse or primary refractory disease occurring at any time after initial diagnosis were eligible provided their red blood cells did not bind mAb216. Due to a risk of hemolysis in the rare case of expression of the fetal i-antigen on adult red cells, patients' RBCs were tested for mAb216 binding by flow cytometry. Additional eligibility requirements included a Karnofsky score of at least 50%, life expectancy of at least eight weeks, and adequate renal, hepatic and cardiac function. Patients could not have received myelosuppressive chemotherapy within one week of study entry or biological agents including monoclonal antibodies at least two weeks before. Patients with CNS leukemia or isolated extramedullary relapse were excluded. The study was approved by the Institutional Review Board of Stanford University. The trial IND number is 11887; the trial registration number is NCT-00313053/00313079. Informed consent was obtained from patients aged 18 years and older or from parents/legal guardians of children under the age of 18, with child assent when appropriate.

Dosage and drug administration

MAb216 was supplied by the Rapid Access to Intervention Development (RAID) program of the National Cancer Institute (NCI) as a sterile lyophilized powder that was reconstituted and diluted to a final concentration of 1 mg/mL. After pre-medication with acetaminophen and diphenhydramine, mAb216 was administered as a slow intravenous infusion starting at a rate of 1 mg/kg/h, with gradual incremental increases in the infusion rate to a maximum of 200 mg/h, as tolerated. Corticosteroids and meperidine could be administered for infusion reactions, but were otherwise not part of routine pre-medication.

Criteria for assessment of response

For the early response evaluation on days 4-7, good response was defined as less than 25% leukemic blasts detected on bone marrow examination or a more than 75% reduction in peripheral blood blast count. Patients not fulfilling criteria for good response were considered to have poor response. For all subsequent weekly response evaluations, residual disease was defined as more than 5% leukemic blasts detectable on bone marrow examination or the presence of blasts in the peripheral blood.

Complete remission (CR) was defined as attainment of M1

bone marrow (less than 5% blasts) with no evidence of circulating blasts or extramedullary disease and with recovery of peripheral counts (absolute neutrophil count above $1\times10^9/L$ and platelet count above $100\times10^9/L$). Partial remission (PR) was defined as complete disappearance of circulating blasts, no evidence of extramedullary disease and achievement of M2 bone marrow status (equal or more than 5% but less than 25% blasts and adequate cellularity) with recovery of peripheral counts as above. Partial-remission cytolytic response (PRCR) was defined as complete disappearance of circulating blasts and achievement of at least 50% reduction from baseline in bone marrow blast count. Hematologic improvement (HI) was defined as a 75% or more decrease in the peripheral blast count when compared with baseline counts, regardless of bone marrow response. Progressive disease (PD) was defined as an increase of at least 25% in the absolute number of leukemic cells in the peripheral blood or bone marrow, or the development of extramedullary disease. Patients not fulfilling criteria for CR, PR, PRCL, HI or PD were considered to have stable disease (SD).

Trial design

Patients received two treatment courses of mAb216, with the same dose of antibody administered on days 0 and 7. Early response evaluation was performed on days 4-7 (Figure 1). Patients who demonstrated a good response to the first dose of antibody received the second dose in identical fashion as on day 0. Patients who had a poor response received the second dose of mAb216 in conjunction with vincristine 1.5 mg/m² up to 2 mg total dose followed by 3 weekly doses of vincristine. In the event that a patient clearly had a poor response to therapy by day 4, i.e. had an obvious rise in peripheral blast count, the patient could proceed with the second dose of mAb216 in conjunction with vincristine as early as day 4. The dose of mAb216 was escalated from 1.25 mg/kg to 5 mg/kg in three consecutive dose cohorts using a 3+2 dose escalation design. The quantity of material produced by the NCI did not permit dosing of 3 patients at 10 mg/kg as initially planned, therefore one extra patient was added to the 5mg/kg cohort.

Toxicities were graded according to the National Cancer Institute's Common Terminology Criteria (version 3.0). Dose-limiting non-hematologic toxicity was defined as any grade 3 or 4 adverse event attributable to mAb216 with the specific exclusion of grade 3 nausea and vomiting. The toxicity profile for vincristine is well characterized, and should be distinguishable from toxicities associated with an immunotherapeutic agent such as mAb216.

Dose-limiting hematologic toxicity was defined as absence of peripheral blood count recovery (absolute neutrophil count above $0.5\times10^9/L$ and platelet count above $20\times10^9/L$) of more than six weeks duration as documented by bone marrow aplasia, not marrow infiltration with leukemia cells. The highest dose level reached at which no more than one of 5 patients experienced a DLT was considered the maximum tolerated dose (MTD).

Response to mAb216 alone was determined on day 4-7 by peripheral blood count with differential or bone marrow aspirate as described above. Subsequent early response evaluations were performed weekly on days 14, 21 and 28 by bone marrow aspiration unless the patient's peripheral blood blast count was clearly indicative of residual disease. Patients without residual disease continued on prior therapy, which consisted of mAb216 alone on days 0 and 7 of subsequent cycles in case of response to mAb216 single agent therapy or weekly vincristine alone for 3 doses starting on day 14 in case of response to mAb216 combined with vincristine. Patients with residual disease could discontinue the trial to begin reinduction with alternative chemotherapy regimens as early as day 14. At the end of the treatment block on day 35, response was again assessed by bone marrow morphology using standard criteria for assessment of response unless the patient's peripheral blood blast count was clearly indicative of residual disease.

Plasma concentration studies

Blood samples for pharmacokinetic studies were collected before and at specific time points after completion of mAb216 infusion on days 0, 1, 2 and 3. Plasma concentrations of mAb216 were determined by enzyme-linked immunosorbent assay (ELISA) using the anti-idiotype rat mAb9G4 as the solid phase capture mAb. Since mAb216 is an IgM antibody with 10 binding sites, the anti-idiotype could be used for both capture and detection.
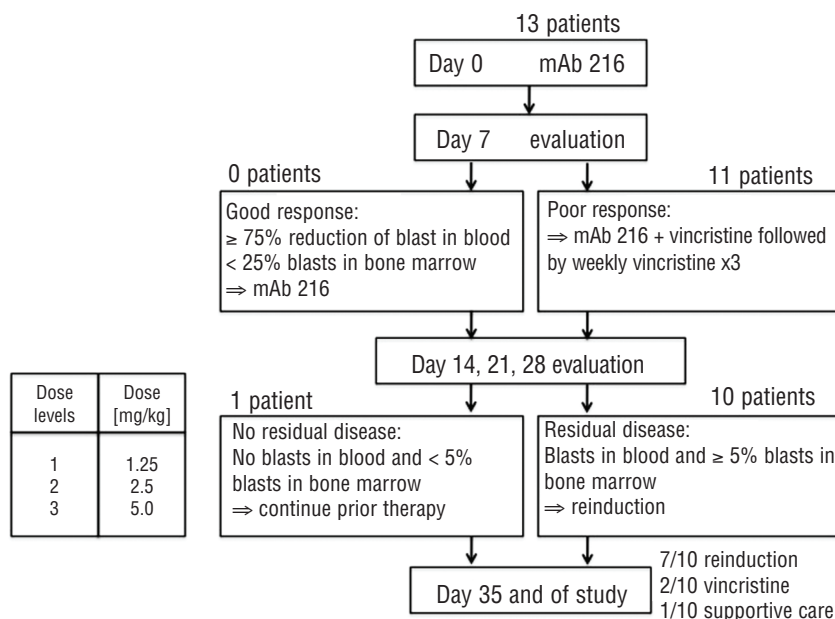

Figure 1. Trial schema.

Plasma was serially diluted (1:2) and binding detected with biotin-labeled 9G4 and horseradish peroxidase-streptavidin (HRPO-SA). Pharmacokinetic values were determined from the ELISA data using Prism software (GraphPad Software).

Flow cytometry assessment of mAb216 binding to patient RBCs and blasts

Bone marrow aspirate or peripheral blood samples were obtained before therapy to determine binding of mAb216 to leukemic blasts and red blood cells by flow cytometry. Buffy coat and red cells were separated by centrifugation and analyzed independently. Leukemic blasts were gated using a combination of fluorescence labeled antibodies against CD45, CD19 (Caltag/Invitrogen, Carlsbad, CA and BD Bioscience, San Jose, CA, USA) and side scatter signal. Mean channel fluorescence of mAb216 biotin/streptavidin PE on blasts and RBCs was quantified. Human IgM mAbMS2B6-biotin[21] was used as an isotype control to determine background fluorescence. Similarly, binding of mAb216 biotin/streptavidin PE to red blood cells was measured. Control RBCs obtained from normal healthy subjects were run in parallel. For a positive control, the pre-B cell line Nalm-6 was stained with mAb216 biotin or mAbMS2B6-biotin, followed by streptavidin PE in each experiment. Samples were analyzed on Facscan or LSRII flow cytometer (Becton Dickinson) using Cell Quest (BD) for data interface and Flojo (Treestar) software for analysis. Ratio of mean channel fluorescence of mAb216 to isotype control mAb was used to compare binding among patients.

Peripheral blood samples taken pre-infusion, end of infusion, one hour, 4 h and 24 h post infusion were stained with fluorescent labeled anti-CD antibodies and anti-idiotypic antibody 9G4-biotin in various combinations and analyzed as described above. Percentage of blasts at each time point was determined by electronic gating for CD45 low, side scatter low cells. Mean channel fluorescence of anti-idiotypic Ab 9G4-biotin/SA-PE on CD45 low, side scatter low blasts was determined to assess presence of mAb216 on blasts. Complete blood count (CBC) and serum chemistries were performed in the Stanford Clinical Laboratories.

Formation of human anti-mAb216 antibodies and immune complex formation

Patient IgG mediated immune response to mAb216 was measured on days 0, 7, 14 and 35 using an ELISA. Purified mAb216 and control human IgM mAbMS2B6 was bound to the solid phase. Patient sera were serially diluted and IgG antibody binding detected using HRPO-labeled anti-human IgG (Caltag/Invitrogen). Binding of post-infusion mAb216 sera was compared to pre-infusion sera and IgG binding to mAb216 compared to the control human IgM mAbMS2B6. Blood was assayed for detection of circulating immune complexes on days 0 and 7 using the C1Q assay performed at a reference laboratory.

Results

Patients' characteristics

From April 2005 to November 2008, 14 patients were screened for participation in the study. All screened patients showed binding of mAb216 to blasts, and none of the screened patients showed binding of mAb216 to red cells. One patient declined the trial. Accordingly, 13 patients, all Philadelphia chromosome translocation negative, were enrolled in the study. Twelve of these 13 were on study and fully assessable for toxicity with follow up of at least 14 days. One patient received 2.5 mg/kg mAb216 on day 0 and died on day 5 due to sepsis not attributed to mAb216. The median age of the reported cohort was 33 years (range 10-73 years). Eight patients had refractory disease, and 5 patients were in second or greater relapse. Most patients had received three or more prior lines of treatment including allogeneic stem cell transplantation in 4 cases (Table 1).

Toxicity

Overall, mAb216 was well tolerated with acceptable toxicity when given alone or in combination with vincristine. Grade 1 or 2 infusion reactions, characterized by urticaria and pruritus, were observed in 3 of 13 patients. These reactions resolved after the infusions were temporarily stopped and diphenhydramine and hydrocortisone were administered. All patients were able to resume and complete the infusions. One patient experienced a dose limiting-toxicity. The patient developed grade 3 epistaxis three days after the first infusion of 1.25 mg/kg of mAb216, and left the protocol. While this is a common event in a patient population with thrombocytopenia and can be easily managed, it was cautiously decided to categorize it as a DLT given that the patient was a child and it was the very first dose of mAb216 given. The first dose cohort was, therefore, expanded to 5 patients. There were no other grade 3 or greater toxicities (Table 2). At a dose of 2.5 mg/kg, one patient developed sepsis and died on day 5 prior to receiving the second dose of therapy. The patient had entered the study in second relapse following allogeneic stem cell transplantation. At the time of death, the patient had residual leukemia as demonstrated by the presence of peripheral blood blasts. The event was not attributed to mAb216. Because the patient was not fully assessable for toxicity, one additional patient was added to the 2.5 mg/kg dose cohort.

Response

In 10 patients with circulating blasts, the median absolute blast count in the peripheral blood decreased from 4,850/μL (range 1,300-50,000/μL) at study entry to 3,900/μL (range 450-38,600/μL) within 24 h after completion of the first infusion of mAb216 resulting in a median blast count reduction of 23.5% (range 0-65%) (Table 3). At the time of planned response assessment on days 4-7, all 13 patients had a poor response to the initial dose of mAb216 including 3 patients who had a rising blast count. Two patients received only one dose of mAb216; one patient due to grade 3 epistaxis constituting a DLT and one patient due to death related to sepsis. Owing to poor response to mAb216 alone, the remaining 11 patients received their second dose of mAb216 in conjunction with vincristine. Three of the 11 patients had a rise in their peripheral blast count after the initial mAb216 infusion and received mAb216 and vincristine on day 4, the other 8 patients received the combination therapy on day 7. In 8 patients with circulating blasts, the absolute blood blast count decreased from a median of 17,200/μL (range 460-110,000/μL) to 1,250/μL (range 0-11,300/μL) by day 14 of mAb216 and vincristine resulting in a median blast count reduction of 92.5% (range 40-100%) (Table 3). Two patients did not have peripheral blasts and one patient's peripheral blasts were only detectable by flow cytometry and remained unchanged. Response to chemoimmunotherapy with mAb216 and vincristine was determined weekly starting on day 14 up to the end of the treatment block on day 35. For patients with residual dis-

ease on day 14 or thereafter, alternative reinduction chemotherapy could be given. By day 14, none of the 11 patients had achieved a CR or PR. However, 8 of 11 patients were in the response category of "Hematological Improvement" (HI), used by Kantarjian *et al.*[22] (Table 2). One of these patients achieved a partial remission cytolyt-

ic response with complete absence of blasts in a hypoplastic bone marrow. While the protocol specified continuing treatment with vincristine only, the FDA allowed the patient to receive one additional dose of mAb216 in combination with vincristine. Unfortunately he subsequently relapsed after two weeks and died without receiving fur-

Table 1. Patients' characteristics.

Pt #	MAb dose (mg/kg)	Age (yrs) /Sex	Cytogenetics	Duration CR1 (mo)	Total prior tx #	Status	Prior therapy
1	1.25	10/M	1;19	29	7	4th relapse	POG9900 CR POG9906 CR Ifosfamide-etoposide NR High-dose cytarabine NR Cyclophosphamide-cytarabine-thioguanine CR Fludarabine-cytarabine-idarubicin- CR Allogeneic transplant in CR4
2	1.25	32/M	not done	5	5	refractory	Vincristine-prednisone-cyclophosphamide- Doxorubicin CR HyperCVAD-course 1 NR MEC CR HyperCVAD-course 2 NR Fludarabine, cytarabine NR
3	1.25	28/F	not done	7	5	refractory	Cytarabine-mitoxantrone-methotrexate CR MEC NR HyperCVAD CR Mylotarg NR Cytoxan, etoposide NR
4	1.25	14/M	der 14q32, t(1;19)	32	5	3rd relapse	POG9906 CR Dexamethasone-vincristine-asparginase CR Allogeneic transplant in CR2 Daunorubicin-asparaginase-prednisone-vincristine CR Allogeneic transplant in CR3
5	1.25	49/F	MLL-AF4	5	2	refractory	CALGB 9511 CR HyperCVAD NR
6	2.5	73/M	3 cells normal	0	3	refractory	Vincristine,daunorubicin, prednisone NR Cyclophosphamide, mercaptopurine, cytarabine, Vincristine NR Rituxan, vincristine, prednisone NR
7	2.5	27/M	normal	4	3	2nd relapse	CALGB 9511 CR hyperCVAD CR Allogeneic transplant in CR2
8	2.5	33/F	complex; hypodiploid	5	2	2nd relapse	CALGB 9511 CR Allogeneic transplant in relapse CR
9	2.5	21/M	1;19	5	5	refractory	CALGB 9511 CR hyperCVAD-course 1 NR Clofarabine NR HyperCVAD-course 2 NR MEC refractory
10	5	22/F	not done	4	3	refractory	CALGB 9511 CR HyperCVAD NR MEC NR
11	5	66/F	NA	41	2	refractory	CALGB 9511 CR Cytarabine, clofarabine NR
12	5	23/M	complex, del 6q, der9	4	4	refractory	HyperCVAD CR MEC CR Liposomal vincristine NR Cytarabine, clofarabine NR
13	5	37/F	not done	0	5	2nd relapse	HyperCVAD NR MEC NR Clofarabine CR Clofarabine NR Cytarabine, asparginase, dexamethasone CR

POG: pediatric Oncology Group; CALGB: Cancer and Leukemia Group B; CR: complete response; NR: no response; hyperCVAD (cyclophosphamide, vincristine, adriamycin, dexamethasone); MEC: (mitoxantrone, etoposide, cytarabine).

ther therapy. One additional patient chose not to undergo further therapy. The remaining 9 patients received reinduction with alternative chemotherapy regimens (n=7) or palliative chemotherapy (n=2) with weekly vincristine on day 15 or thereafter.

Plasma concentrations and half-life of mAb216

Plasma mAb216 concentrations measured at the end of the initial infusion by ELISA increased in a dose-dependent manner with median values of 2.3 μg/mL (range 1.1-3.6 μg/mL), 3.95 μg/mL (range 0.8-5.9 μg/mL), and 11.2 μg/ml (range 9.8-39 μg/mL) for mAb216 dose cohorts of 1.25 mg/kg, 2.5 mg/kg and 5 mg/kg, respectively. The median half-life of mAb216 was 1.99 h (range 0.78-9.36 h).

Formation of human anti-mAb216 antibodies and immune complex formation

None of the patients developed anti-mAb216 IgG anti-

bodies on days 0, 7, 14 or 35 as determined by ELISA or showed any evidence of immune complex formation when measured on days 0 and 7 by C1Q assay.

Table 2. Toxicity possibly or probably related to mAb216 and response to mAb216 and vincristine by dose level.

Dose levels

mg/kg	N. of patients	Toxicity ≥ Grade 3 and Possibly Attributed to MAb216	Best response
1.25	5	1 grade 3 epistaxis, pt.1	3 HI
2.5	4	None	3 HI
5	4	None	2 HI

HI: "Hematologic Improvement" is defined as ≥75% decrease in the peripheral blast count when compared with the baseline counts, regardless of bone marrow response on Day 15.[22]

Table 3. Blood counts before and after infusion of mAb216 and mAb216/vincristine.

Patient #	1	2	3	4	5	6	7	8	9	10	11	12	13
MAb 216 dosage mg/kg	1.25	1.25	1.25	1.25	1.25	2.5	2.5	2.5	2.5	5	5	5	5
MAb 216 binding to blasts Pre-infusion (ratio)[1]	6.2x	3.5x	2.7x	5.5x	5.4x	8.6x	9.2x	2.9x	21x	8.7x	9.8x	2x	2.2x

MAb216 only pre and post first infusion

	1	2	3	4	5	6	7	8	9	10	11	12	13
WBC K/μL	17	50	33.3	3.7	29.5	6.8	30.2	7.2	0.1	7.1	1.4	2.2	3.4
	15.6	39.6	23.8	2.5	33	6.0	25	6.1	0.1	6.1	1.3	2.6	3.7
Percent blasts	28	100	96	36	65	73	70	29	100(m)[3]	42	7	90	1.7
	23	97	86	18	55	71	70	26		21	7	80	1.6
Blast count K/μL[2]	4.7	50	31.9	1.3	19.2	5.0	21.0	2.1	0	3.0	0.098[4]	2	0.06[4]
	3.5	38.6	20.3	0.45	18.0	4.3	17.4	1.6	0	1.3	0.09[4]	2.1	0.06[4]
ANC K/μL	10.7	0	0	1.0	2.4	0.8	2.3	2.8	NA[5]	1.1	0.6	0.1	2.8
	9.8	0	0	1.3	3.6	0.6	3.7	3.5		1.9	0.68	0.45	3.1
ALC K/μL	1.0	0	0.1	0.9	5.1	0.8	5.4	1.7	NA[5]	2.8	0.6	0.1	0.5
	1.6	0	0.1	0.5	6.8	0.8	2.7	0.6		2.6	0.5	0	0.6

MAb216 and vincristine on Day 7 or earlier as noted pre and post second infusion

	1	2	3	4 (Day 4)	5	6 (Day 4)	7	8	9	10	11	12 (Day 4)	13
WBC K/μL		110	62.7	1.8	26.8	9.5	68		0.1	10.6	1.1	19.4	2.8
		27.5	36.9	1.8	13.4	3.9	31.6		0.1	2.9	0.8	13.2	2.9
Percent blasts		100	96	26	58	91	76		>90 (m)[3]	46	90 (m)[3]	97	2.1
		100	100	32	43	74	72			42		94	0
Blast count K/μL[6]	ND DLT	110	60.2	0.46	15.5	8.7	51.6	ND died D5	0	4.9	0	18.9	0.06[4]
		27.5	36.9	0.58	5.8	2.9	23		0	1.2	0	12.4	0
ANC K/μL		0	0.5	0.2	4.1	0.09	8.8		NA[5]	0,2	0.4	0	2.5
		0	0	0.3	4.0	0.25	4.9			0.6	0.5	0.1	2.4
ALC K/μL		0	0.6	1.1	5.0	0.2	1.8		NA[5]	4.4	0.5	0.5	0.2
		0	0	0.59	3.2	0.26	1.7			1.1	0.3	0.7	0.2
Day 14 WBC K/μL		8.9	4.8	1.0	1.8	1.8	2.4		0.1	3.5	0.8	11.7	3.9
Day 14 percent blasts		95	92	17	83 (m)[3]	11	25		(m)[3]	54	56 (m)[3]	97	82 (m)[3]
Day 14 blast count K/μL		8.5	4.4	0.17	0	0.2	0.6		0	1.9	0	11.3	0
Day 14 ANC K/μL		0	0	NA5	0.21	0.09	1		NA5	0.7	NA5	0	2.6
Day 14 ALC K/μL		0.32	0.3	NA5	1.2	1.4	0.7		NA5	0.8	NA5	0.4	1.0

[1]Mean channel fluorescence to MAb216 binding to patient's blasts divided by isotype control mAb. [2]Within 24 h of end of infusion. [3]In patients without peripheral blasts response was determined by bone marrow analysis (m). Pt. 9: Day 14, marrow hypocellular with one small area of blasts. [4]Blasts determined by percent on flow cytometry times WBC. [5]NA; clinical lab does not determine differential on WBC less than 1 K/μL. [6]At 24 h from end of infusion.

MAb216 binding to patient RBC and blasts

Prior to enrollment in the trial, patient RBC and blasts were tested for mAb216 binding *in vitro*. None of the patients showed binding of mAb216 to red blood cells. Binding of mAb216 to blasts, measured as a ratio of mean channel fluorescence of biotin-labeled mAb216 to isotype control, ranged from low (1.3) to high (31) (median 4.3). The efficiency of blast targeting *in vivo* by mAb216 was determined by measuring binding of anti-idiotypic antibody 9G4 to blast cells post mAb216 infusion. Presence of mAb216 was detected in all 13 patients (Figure 2). There was no significant correlation between degree of mAb216 binding *in vitro* prior to treatment using biotin-labeled mAb216 and response.

Figure 2. MAb216 binds to blasts *in vivo*. Mean channel fluorescence of blasts pre [black] and post [stippled] infusion of mAb216. Peripheral blood was stained with CD19APC, CD45FL and mAb 9G4 biotin followed by Streptavidin PE. Blasts were gated and the mean channel fluorescence of mAb 9G4 bound to mAb216 was calculated. MAb216 bound to blasts *in vivo* in all patients.

Discussion

Despite improvements in initial therapy for ALL, results in relapsed patients remain significantly inferior for both children and adults, as patients frequently do not tolerate or respond to reinduction therapy.[3,4,23] This is a first-in-human study of the human monoclonal IgM antibody 216 alone and in combination with vincristine for pediatric and adult patients with relapsed or refractory B-cell ALL. MAb216 administration was well tolerated, both as a single agent and when combined with vincristine. The most common single-agent toxicities observed were grade 1 and 2 infusion reactions that responded to administration of diphenhydramine and/or hydrocortisone and slowing of the infusion rate. In all cases, the full dose of mAb216 could be administered. Given the high-risk status of the population treated in phase I trials, the single death observed secondary to sepsis was not unexpected.[22,24-26]

Although efficacy was not a primary endpoint of this phase I study, the preliminary results obtained, including clearance of peripheral blood blasts with mAb216 and vincristine in 3 patients, and a hypoplastic marrow without evidence of leukemia in one patient are encouraging. Because this was the first clinical trial with a human derived monoclonal antibody, the FDA only permitted short-term administration and initial low dosage. Most clinical trials with mAbs use repeated weekly infusions.[27,28] With repeat dosing the efficacy of mAb216 is expected to increase. As is frequently seen in phase I trials, the degree of response to mAb216 did not correlate with dose level,[29,30] but the ability to assess a dose-response relationship was limited as only three dose cohorts were tested.

All patients enrolled in this trial were heavily pre-treated; the majority of patients had received at least three prior regimens and 4 had undergone allogeneic stem cell transplantation. Although we cannot entirely exclude the possibility that in some patients the decrease in blasts observed after the infusion of mAb216 and vincristine was due to vincristine, this appears unlikely because all patients had been previously treated with vincristine-containing regimens and the majority had been refractory.

None of the patients screened showed binding of mAb216 to red blood cells, and accordingly no cases of hemolysis were observed. As expected, the plasma level of mAb216 increased with increasing dose levels. The median half-life was 1.99 h, indicating that mAb216 was immediately bound to leukemic blasts. None of the patients developed anti-mAb216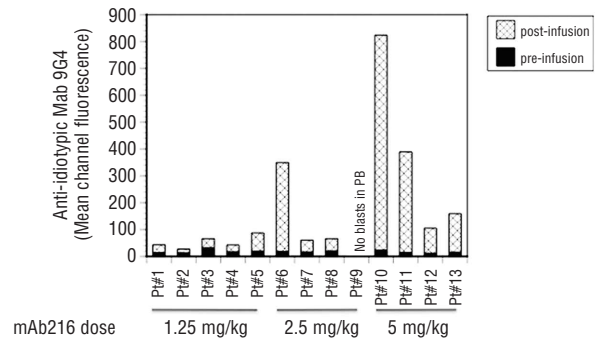 IgG antibodies or showed any evidence of immune complex formation during the study. Prior to treatment, binding of mAb216 to leukemic blasts *in vitro* was confirmed for all patients using biotin-labeled mAb216. After the administration of mAb216 alone, anti-idiotypic mAb 9G4 was used to confirm MAb216 binding to circulating blasts using flow cytometry. While there was no significant correlation between the degree of pre-treatment mAb216 binding to leukemic blasts *in vitro* and response, a trend suggesting more blast killing with higher mAb216 binding was observed. Additional factors potentially determining response include disease burden and mAb216 dose. *In vitro*, the ability of mAb216 to lyse B-cell ALL cell lines depends on the expression of the lactosamine antigen and the antibody concentration.[14,20]

In conclusion, this first-in-human trial with the naturally occurring human mAb216 in children and adults with relapsed or refractory B-cell ALL demonstrated that mAb216 is well tolerated as a single agent and in combination with vincristine. At the dosage of mAb given (mAb216 at 5mg/kg and less) the decrease in peripheral blasts indicates biological activity. The encouraging early responses seen after administration of mAb216 with vincristine suggest that the antibody may enhance the effect of cytotoxic chemotherapy. Given the limitations of using monoclonal antibodies as single agents in rapidly proliferative diseases, the goal of future trials is to use mAb216 in combination with chemotherapy to exploit synergy as demonstrated for rituximab and chemotherapy in B-lymphoblastic leukemia and Burkitt's leukemia.

MAb216 for this study was produced by the RAID program of the NCI, and the trial was performed under an investigator IND.

Authorship and Disclosures

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