VOLUME 27 · NUMBER 25 · SEPTEMBER 1 2009

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Phase I Dose Escalation, Pharmacokinetic and Pharmacodynamic Study of Naptumomab Estafenatox Alone in Patients With Advanced Cancer and With Docetaxel in Patients With Advanced Non–Small-Cell Lung Cancer

Hossein Borghaei, Katherine Alpaugh, Gunnar Hedlund, Göran Forsberg, Corey Langer, Andre Rogatko, Robert Hawkins, Svein Dueland, Ulrik Lassen, and Roger B. Cohen

From the Department of Medical Oncology, and the Protocol Supportive Laboratory, Fox Chase Cancer Center, Philadelphia, PA; Active Biotech, Lund, Sweden; Christie Research Centre, Manchester, United Kingdom; the Department of Clinical Cancer Research, Norwegian Radium Hospital, Oslo, Norway; and the Department of Oncology, Rikshospitalet University Hospital, Copenhagen, Denmark.

Submitted September 29, 2008; accepted February 26, 2009; published online ahead of print at www.jco.org on July 27, 2009.

Supported in part by Grant No. P30 CA006927 from the National Cancer Institute, Bethesda, MD.

Presented in part at 2007 American Association for Cancer Research National Cancer Institute European Organisation for the Research and Treatment of Cancer International Conference on Molecular Targets and Cancer Therapeutics, October 22-26, 2007, San Francisco, CA.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Corresponding author: Hossein Borghaei, DO, MS, Department of Medical Oncology, Fox Chase Cancer Center, 333 Cottman Ave, Philadelphia, PA 19111; hossein.borghaei@fccc.edu.

The Acknowledgment and Appendix are included in the full-text version of this article; they are available online at www.jco.org. They are not included in the PDF version (via Adobe® Reader®).

0732-183X/09/2725-4116/$20.00

DOI: 10.1200/JCO.2008.20.2515

ABSTRACT

Purpose
Two phase I studies were conducted of ABR-217620 alone or in combination with docetaxel. This is a recombinant fusion protein consisting of a mutated variant of the superantigen staphylococcal enterotoxin E (SEA/E-120) linked to fragment antigen binding moiety of a monoclonal antibody recognizing the tumor-associated antigen 5T4.

Patients and Methods
Patients with non–small-cell lung cancer (NSCLC), pancreatic cancer (PC), and renal cell cancer (RCC) received 5 daily boluses of ABR-217620 (3-month cycles) in escalating doses to determine the maximum-tolerated dose (MTD; ABR-217620 dose escalation monotherapy [MONO] study). Doses were selected based on individual patient anti–SEA/E-120 titers pretreatment. Patients with NSCLC received 4 daily, escalating doses of ABR-217620 followed by docetaxel in 21-day cycles (ABR-217620 dose escalation combination with docetaxel [COMBO] study).

Results
Thirty-nine patients were enrolled in the MONO study and 13 were enrolled in the COMBO study. The monotherapy MTD was 26 μg/kg (NSCLC and PC) and 15 μg/kg (RCC). Dose-limiting toxicities (DLTs) in the MONO study were fever, hypotension, acute liver toxicity, and vascular leak syndrome. In the COMBO study, the MTD was 22 μg/kg (neutropenic sepsis). Adverse events included grade 1 to 2 fever, hypotension, nausea, and chills. Treatment caused a systemic increase of inflammatory cytokines and selective expansion of SEA/E-120 reactive T-cells. Tumor biopsies demonstrated T-cell infiltration after therapy. Fourteen patients (36%) had stable disease (SD) on day 56 of the MONO study. Two patients (15%) in the COMBO study had partial responses, one in a patient with progressive disease on prior docetaxel, and five patients (38%) had SD on day 56.

Conclusion
ABR-217620 was well tolerated with evidence of immunological activity and antitumor activity.

J Clin Oncol 27:4116-4123. © 2009 by American Society of Clinical Oncology

INTRODUCTION

Monoclonal antibodies can be designed to deliver a wide variety of agents including chemotherapeutic drugs, toxins, radioisotopes, and cytokines.[1] Immunotoxins are antibodies or antibody fragments that are conjugated to a toxin to facilitate selective delivery of the toxin to the cell surface and subsequent internalization and release of the toxin into the cytoplasmic compartment.[2]

Immunotoxins have demonstrated significant antitumor effects in preclinical models and in clinical trials.[3-5]

ABR-217620 (5T4FabV18–staphylococcal enterotoxin E [SEA/E-120] or naptumomab estafenatox) is a novel immunotoxin with a distinct mechanism of action and consists of a recombinant fusion protein developed from ABR-214936,[6] consisting of a mutated variant of the superantigen (SAg) SEA/E-120[7] linked to a fragment antigen binding (Fab) moiety of a monoclonal antibody recognizing the tumor-associated oncofetal trophoblast glycoprotein antigen 5T4.[8-10] The proposed mechanism of action is Fab targeting of ABR-217620 to tumor where the SAg portion of the fusion protein elicits a potent tumoricidal cytotoxic T proof-of-mechanism cell response (Fig 1).[11]

Preclinical evaluation[7] suggests several advantages for ABR-217620 over the predecessor compound ABR-214936,[12,13] including reduced binding to preformed anti-SAg antibodies, lower toxicity,

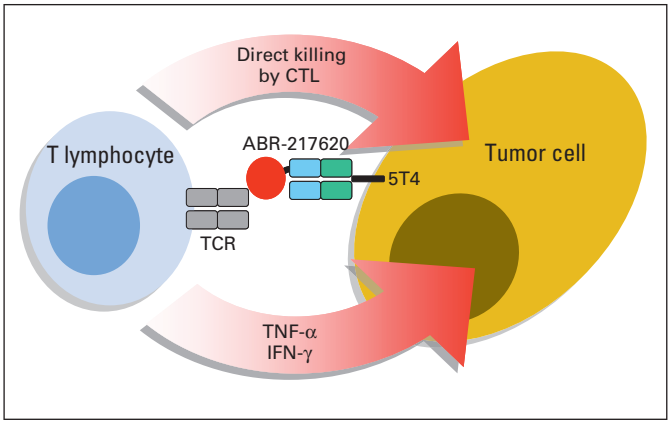

Fig 1. ABR-217620 proposed mechanism of action. The ABR-217620 fusion protein binds to the 5T4 tumor-associated antigen and activates a T lymphocyte through its T-cell receptor (TCR). The T cell produces cytokines (tumor necrosis factor [TNF] –α and interferon [IFN]-γ) and executes direct tumor killing if it is a cytotoxic T lymphocyte.

higher affinity for 5T4, and improved tumor cell killing. We evaluated the safety and efficacy of ABR-217620 alone in patients with advanced solid malignancies (ABR-217620 dose escalation monotherapy [MONO] study), and with docetaxel in patients with advanced non–small-cell lung cancer (NSCLC; ABR-217620 dose escalation combination with docetaxel [COMBO] study).

Docetaxel was chosen for the COMBO study based on data showing synergy in a preclinical model for ABR-217620[14] and its use as a standard second-line agent for the treatment of patients with recurrent NSCLC.

PATIENTS AND METHODS

Patient Selection

Eligible patients had histologically or cytologically confirmed refractory non–small-cell lung cancer (NSCLC), renal cell cancer (RCC) and pancreatic cancer (PC; MONO study) or NSCLC with progression on first-line platin-based therapy or had failed or declined other regimens (COMBO study). Tumor types for the trials were based on our own data showing expression of 5T4 in the majority (> 95%) of these tumor types. Other criteria included age ≥ 18 years; Eastern Cooperative Oncology Group performance status ≤ 1; prior radiation completed ≥ 3 weeks earlier; and adequate bone marrow (platelets ≥ 100×10^9/L, absolute neutrophil count > 1.5×10^9/L, hemoglobin ≥ 10 g/dL), hepatic function (MONO study: total bilirubin ≤ 2 times the upper limit of normal (ULN), AST ≤ 2.5 times ULN; COMBO study: consistent with docetaxel labeling and renal [serum creatinine ≤ than 1.5 times ULN] function). Exclusions included active infections, active cardiac disease, other active, invasive malignancies, allergy or hypersensitivity to kanamycin, or current use of any corticosteroids. All patients gave written informed consent under federal and institutional guidelines.

MONO Study Treatment

The MONO study was conducted at three sites in the United States and Europe. Patients received a daily bolus injection of ABR-217620 preceded by 1 L normal saline on 5 consecutive days (days 1 through 5). The schedule was based on preclinical experiments showing that cycles of daily IV injections (≥ 3) of immunotoxin resulted in the best antitumor effects.[15,16] Furthermore, clinical results with the predecessor ABR-214936 using a similar schedule showed promising efficacy.[13] Premedications included acetaminophen, indomethacin, or sulindac, and as needed medication for nausea, vomiting, and rigors. Starting dose was 0.5 μg/kg and the maximum dose given was 27.4 μg/kg. The starting dose in the MONO study, 0.5 μg/kg, was selected on the basis of the ABR-214936 experience and safety/toxicology evaluation of ABR-217620 in comparison to ABR-214936 in cynomolgus monkeys. The design of the dose escalation is the same as in the dose escalation with ABR-214936[12] with the exception that the hypothesized outcome was that ABR-217620 dosing would be insensitive to preformed anti-SAg antibodies.

Individual doses higher than 0.5 μg/kg were calculated using a Bayesian dose-escalation method taking into account individual pretreatment levels of anti-SAg antibodies. Tumor response was assessed on days 28 and 56 and every 56 days thereafter until progression. Patients with stable disease or better (Response Evaluation Criteria in Solid Tumors) and anti-SAg titer ≤ 1,000 pmol/L could receive a second and third 5-day treatment cycle. Each patient was observed for 5 to 7 days after treatment before enrollment of another patient in order to collect toxicity information for the Bayesian model.

Table 1. Patient Characteristics

Characteristic	MONO				COMBO: NSCLC
	NSCLC	PC	RCC	Total	
No. of patients	20	8	11	39	13
Sex, male/female	9/11	2/6	7/4	18/21	8/5
Median age, years	53	56.5	56	54	59
Range	39-69	37-69	39-75	37-75	38-74
Stage III/IV	6/11	1/5	3/4	10/20	2/11
Median No. prior therapies	2	1	1	1	1
Range	0-11	0-2	1-2	0-11	0-3
Histology, No.					
Well differentiated	2				6
Moderately differentiated	3				1
Poorly differentiated	10				4
Could not be assessed	1				
Unknown	4				2
ECOG PS, %					
0	25	63	73	46	62
1	70	37	27	51	38
Missing	5			3	

Abbreviations: MONO, ABR-217620 dose escalation monotherapy study; COMBO, ABR-217620 dose escalation combination with docetaxel study; NSCLC, non–small-cell lung cancer; PC, pancreatic cancer; RCC, renal cell cancer; ECOG PS, Eastern Cooperative Oncology Group performance status.

COMBO Study Treatment

The COMBO study was conducted at five study sites in the United States and Europe. Cohorts of three to seven patients received ABR-217620 in combination with a fixed dose of docetaxel (75 mg/m^2) and dexamethasone premedication. ABR-217620 doses were based on the MONO study that was run in parallel: 10.3 μg/kg (three patients), 16.5 μg/kg (three patients), and 22 μg/kg (seven patients). Patients received ABR-217620 on days 1 through 4 and days 22 through 25 and docetaxel on days 5, 26, and 47 comprising cycles 1 and 2. Patients with stable diseaese or better (RECIST) and anti-SAg levels $\leq$ 500 pmol/mL could receive a third and fourth treatment cycle. Intrapatient dose escalation was not permitted.

Safety Monitoring

For the MONO study, dose-limiting toxicity (DLT; National Cancer Institute Common Toxicity Criteria, version 2.0) was considered to be any toxicity possibly due to the study drug. This included any grade $\geq$ 3 toxicity leading to withdrawal of treatment, or any grade 3 fever, edema, hypotension or hepatic and hematologic toxicities, or grade 2 cardiovascular or allergic reactions.

For the COMBO study, DLT included any $\geq$ grade 3 toxicity occurring up to day 33 after treatment, except for known docetaxel adverse effects. For pharmacokinetics and pharmacodynamics description, see online-only Appendix.

Immunohistochemistry

In the COMBO study, tumor samples were obtained using an 18G needle under computed tomography or ultrasound guidance for histopathology on day 3 of ABR-217620 treatment in either cycle 1 or 2. Archival tissues were used in place of predose samples. Biopsies were optional and used a separate, institutional review board–approved consent form. Both patients had NSCLC and had recent archival tissue for comparison.

Samples were both fixed in 10% neutral buffered formalin for 24 hours at room temperature and snap frozen. Samples were stained with hematoxylin and eosin to confirm the diagnosis and biopsy quality and by immunohistochemistry for 5T4 expression (frozen samples) and T-lymphocyte (anti-CD3) infiltration (formalin samples). For a description of the statistics, pharmacokinetics, and pharmacodynamics, see online-only Appendix.

RESULTS

We performed two parallel phase I trials, a monotherapy study with ABR-217620 (MONO study) and a study in combination with docetaxel (COMBO study).

Table 2. DLTs: Most Common Adverse Events and No. of Patients With Adverse Events Displayed by Dose and Tumor Type

Dose (μg/kg)	No. of Patients	Tumor Type and DLT	Constipation	Diarrhea	Nausea	Vomiting	Chills	Fatigue	Pyrexia	Dyspnea	Hypotension	Neutropenia
MONO												
0.5	3	NSCLC	2	1	2	1	2	3	1	—	3	—
0.7	3	NSCLC	1	—	1	1	—	—	1	1	—	—
0.9	3	NSCLC	—	—	—	—	1	2	—	1*	—	—
1.2	1	NSCLC	—	—	—	—	—	—	—	—	—	—
1.6	1	NSCLC	—	—	1	—	—	—	—	—	—	—
2.1	1	NSCLC	—	—	1	—	—	—	1	—	—	—
2.8	1	NSCLC	—	—	1	—	1	1	1	—	—	—
3.7	1	NSCLC	1	—	1	—	—	—	1	—	1	—
4.9	2	NSCLC, PC	—	1	2	1	—	—	2	—	—	—
6.6	1	NSCLC	—	1	—	1	1	1	1	—	—	—
8.8	2	NSCLC, RCC	—	—	2	1	2	—	2	—	—	—
11.6	1	NSCLC	—	1	1	1	—	—	—	—	—	—
12.8	1	RCC	—	1	—	1	1	—	1	1	1	—
15.5	1	PC	—	—	1	1	1	—	1	—	—	—
15.6	1	RCC	1	1	1	1	—	—	1	—	—	—
17.0	1	PC	—	—	1	1	—	—	1	—	—	—
17.3	1	RCC	1	1	1	1	—	—	1	1	1	—
19.7	1	RCC	—	1	1	1	1	—	1	1	1	—
20.2	1	RCC^{DLT}	—	1	1	1	1	—	1	—	1†	—
20.6	2	PC, RCC	2	1	1	1	1	1	1	1	1	—
21.0	1	RCC	—	—	1	—	1	—	1	1	1	—
22.2	1	NSCLC	1	1	1	1	—	1*	—	—	—	—
23.2	1	RCC^{DLT}	—	1	1	1	—	—	1*	—	1	—
23.4	1	NSCLC	1	1	1	1	1	—	1	—	1	—
24.7	2	PC, RCC^{DLT}	1	1*	2	2	—	2	2	1	2*	—
25.7	1	PC	—	1	1	1	—	—	1	—	1	—
26.4	1	PC^{DLT}	—	—	1	1	—	—	—	—	1	—
27.4	2	PC^{DLT}, RCC^{DLT}	—	—	2	1	1	1	2*	1	1	—
COMBO												
10.3	3	NSCLC	—	2	2	1	2	1	2*	2	1	2‡
16.5	3	NSCLC	—	1	2	2	3	1	3	1	1	2§
22.0	7	NSCLC$^{1\times DLT}$	2	5	5	5	6	3	6*	4	3*	2‡

Abbreviations: DLT, dose-limiting toxicity; MONO, ABR-217620 dose escalation monotherapy study; COMBO, ABR-217620 dose escalation combination with docetaxel study; NSCLC, non–small-cell lung cancer; PC, pancreatic cancer; RCC, renal cell cancer.

*National Cancer Institute Common Toxicity Criteria (CTC) grade 3 patient.

†One CTC grade 4 patient.

‡Two CTC grade 4 patients.

§One CTC grade 3 patient and one CTC grade 4 patient.

MONO Study

Thirty-nine patients (20 with NSCLC, eight with PC, and 11 with RCC) were enrolled (Table 1) between April 2003 and November 2006. Thirty-two patients received one cycle, six patients received two cycles, and one patient received three cycles across multiple dose levels (range, 0.5 to 27.4 μg/kg). Median age of patients was 54 years (range, 37 to 75 years). Most were white ($>$ 90%) and had a favorable ECOG PS of 0 (46%) or 1 (51%). Four patients had received no prior chemo- or immunotherapy, 16 patients had received one regimen, and 19 patients had received $\geq$ two chemo- or immunotherapy regimens. Median number of prior chemo- or immunotherapy regimens was 1 (range, 0 to 11 prior regimens). Sixteen patients had received prior radiation therapy. Twenty patients had stage IV disease.

COMBO Study

Thirteen NSCLC patients were enrolled (Table 1) between October 2005 and September 2006. Median age was 59 years (range, 38 to 74 years). Eleven patients had stage IV disease. Median number of prior therapies was 1 (range, 0 to 3 prior therapies). Five patients had ECOG PS 1. Three patients each received ABR-217620 10.3 or 16.5 μg/kg. Seven received 22 μg/kg.

Toxicity

The adverse effects of ABR-217620 are presented in Table 2. There was one death in the MONO study due to progressive disease. In the COMBO study, two deaths occurred, one due to neutropenic sepsis (felt related to docetaxel) and another due to hemoptysis related to tumor necrosis. Both patients had a diagnosis of NSCLC and both were treated with 22 μg/kg of ABR-217620 and docetaxel.

The most common drug-related adverse events in the MONO study were fever, nausea, vomiting, diarrhea, chills, and hypotension. There was a dose-dependent, transient blood pressure decrease during cycle 1, with nadir usually 4 to 6 hours after ABR-217620 administration on day 1, together with a dose-dependent, transient increase in mean pulse and body temperature. Changes in laboratory parameters and vital signs in treatment cycle 1 were independent of individual pretreatment anti-SAg levels. In patients receiving a second cycle, the same effects were observed as in cycle 1, although consistently to a lesser extent.

The most common toxicities in the COMBO study were fever, hypotension, nausea, and chills, and the same changes in blood pressure, pulse, and temperature as in the MONO study. All of these effects were most evident during treatment cycle 1. Less toxicity occurred in cycle two, probably because of a combination of induced anti-SAg (in some patients) and lower capacity of SAg-activated T lymphocytes to produce cytokines (partial anergy).

Hematologic

In the MONO study, there were no treatment-related effects on hemoglobin or basophils. There was a dose-dependent decrease in WBC, lymphocyte, monocyte, and eosinophil levels 3 hours after drug administration in all patients on days 1 through 5, with return of WBC and eosinophils to baseline by the next day, and normalization of lymphocytes and monocytes on day 6. Neutrophil levels declined transiently 3 hours after drug administration on days 1 through 4 in patients with RCC and NSCLC at ABR-217620 doses higher than 15 μg/kg. Platelet count decreased in a dose-dependent manner on days 1 through 6, increasing by day 12 to above baseline with return to baseline on day 28. In the COMBO study, mean neutrophil counts decreased after each treatment as expected from docetaxel. Docetaxel was reduced (from 75 to 55 mg/m^2) for neutropenia in two patients.

Clinical Chemistry

In the MONO study, transient increases in liver enzymes (one grade 3) were seen in cycle 1 at higher than 15 μg/kg of ABR-217620. A dose-dependent transient decrease in albumin levels was noted at all dose levels. In the COMBO study, there was a transient increase in liver enzymes in all dose groups during treatment cycle 1 and to a lesser extent during treatment cycle 2.

DLTs

In the MONO study, DLTs of fever, hypotension, liver toxicity, and acute vascular leak syndrome occurred in six patients at higher than 20 μg/kg of ABR-217620 (four RCC patients and two PC patients). In the COMBO study, one patient at 22 μg/kg ABR-217620 experienced DLT (sepsis-induced toxic shock syndrome) during cycle 1. DLTs were independent of baseline anti-SAg levels, unlike the earlier study with the predecessor drug ABR-214936.[12] The formal MTD for patients with NSCLC and PC was 26 μg/kg. The observed DLTs in PC patients occurred at doses very close to

Table 3. Pharmacokinetic Parameters of ABR-217620

	MONO				COMBO			
Study	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Treatment cycle/d	1/1		2/1		1/1		2/1	
No. of patients	15		3		13		11	
Dose level, μg/kg	11.6-27.4		8.75-21.0		10.3-22.0		10.3-22.0	
C_{max}/dose, μg/L/μg/kg	13.8	4.65	8.45*	11.1	19.7	27.6	5.18	5.34
AUC/dose, μg $\times$ h/L/μg/kg	13.6	6.63	3.94	3.85	10.8	4.4	4.52	4.51
Terminal half-life, hours	1.38	0.56	0.91	0.25	1.10	0.23	0.90	0.57
CL, L/h/kg	0.11	0.12	6.39	10.7	0.11	0.04	3.74	5.89
V_{ss}, L/kg	0.12	0.09	9.23	15.73	0.11	0.04	1.88	2.64

Abbreviations: MONO ABR-217620 dose escalation monotherapy study; COMBO, ABR-217620 dose escalation combination with docetaxel study; SD, standard deviation; C_{max}, maximum concentration; AUC, area under the time-concentration curve; CL, clearance; V_{ss}, volume of distribution at steady state.
*C_{max} n = 4.

the MTD. Thus, 22 μg/kg was chosen as recommended phase 2 dose for NSCLC and PC. The MTD for RCC was 15 μg/kg.

Dose Reductions and Study Discontinuation

There were no ABR-217620 dose reductions in either study. One dose delay for up to 24 hours was allowed per treatment cycle. In the MONO study, reasons for treatment discontinuation included disease progression (n = 24), DLT (n = 6), consent withdrawal (n = 1), investigator decision (n = 3), adverse event (necrotizing pneumonia, n = 1), death from disease (PC, n = 1), initiated another treatment (n = 1, treatment here included two phase I trials with other biologic agents followed by cytotxic chemotherapy), clinical/laboratory progression (n = 1), and completed protocol (n = 1). In the COMBO study, reasons for treatment discontinuation included disease progression (n = 6), DLT (n = 1), consent withdrawal (n = 1), adverse event (tumor necrosis, n = 1), initiated another treatment (n = 1), and completed protocol (n = 3).

Pharmacokinetics

Pharmacokinetic parameters and plasma concentration time profiles for ABR-217620 are presented in Table 3. Maximum plasma concentrations of ABR-217620 occurred approximately 5 minutes after dosing followed by a rapid decline in plasma concentration with a mean half-life of approximately 1 hour. ABR-217620 showed a small volume of distribution and low plasma clearance (about 0.1 L/kg and 0.1 L/h/kg, respectively) in cycle 1. Pharmacokinetic parameters were unchanged during the treatment cycle and were similar after the first and last dose within a cycle. Pharmacokinetics was linear in the dose range 10 to 27 μg/kg across the three diseases with systemic exposure

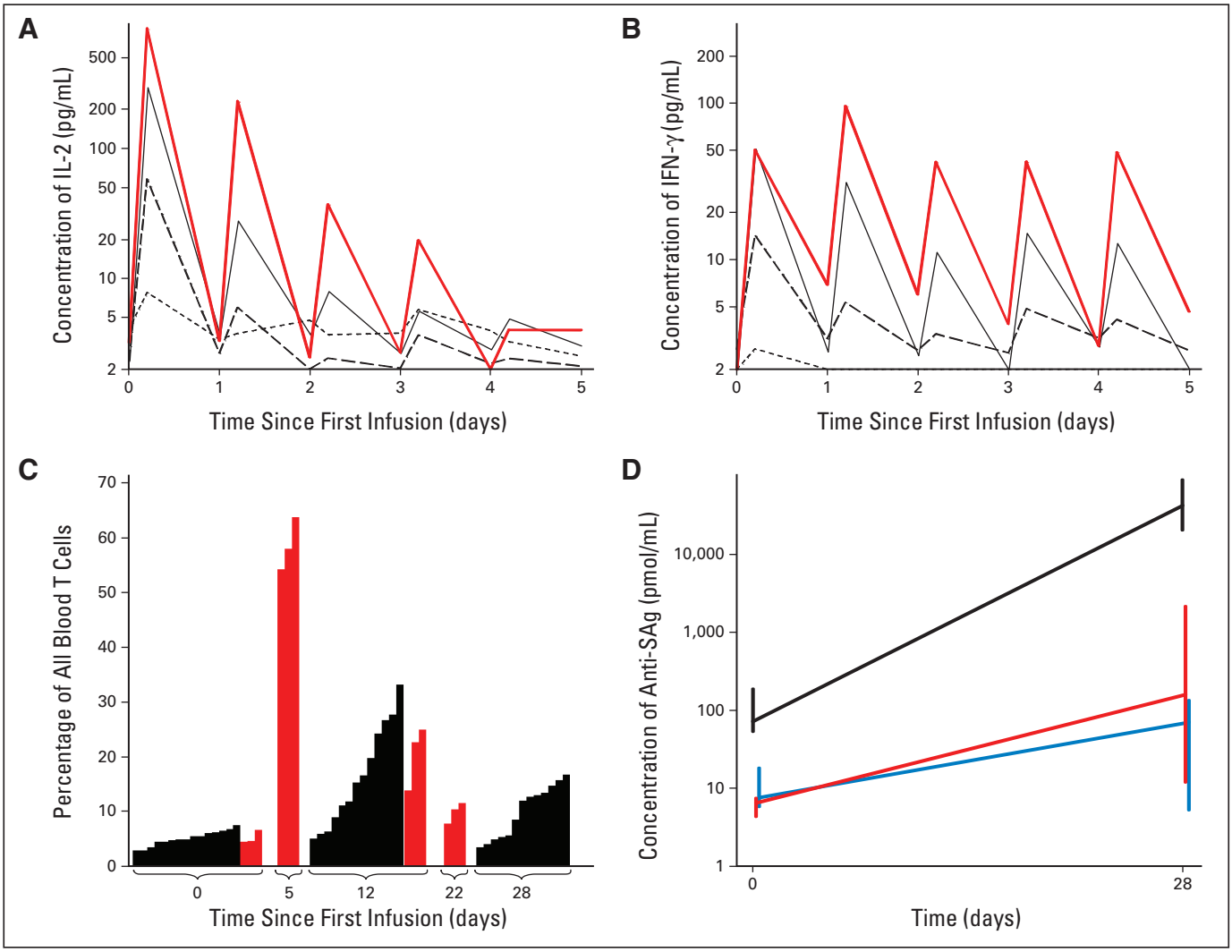

Fig 2. ABR-217620 pharmacology. Geometric mean of (A) interleukin-2 (IL-2) and (B) interferon (IFN)-γ levels in plasma at before and 3 hours after first infusion of ABR-217620 in the MONO (ABR-217620 dose escalation monotherapy) study. Renal cell carcinoma (RCC; n = 9, red lines), non–small-cell lung cancer (NSCLC), and pancreatic cancer (PC; n = 8, black lines) patients receiving more than 15 μg/kg ABR-217620; patients receiving 2.5 to 15 μg/kg (n = 9, dashed lines) and less than 2.5 μg/kg ABR-217620 (n = 12, dotted lines). Plasma samples were taken before and 3 hours after each injection of ABR-217620. (C) Each bar represents an individual subject at the distinct time point. Percentage of T lymphocytes expressing T-cell receptor (TCR)-Vβ6.4 (percentage TCR-Vβ6.4 cDNA of total TCR-Vβ cDNA) in peripheral blood from patients before and after treatment with ABR-217620 in the MONO (black) and COMBO (ABR-217620 dose escalation combination with docetaxel; red) studies. (D) Median with first and third quartiles of anti-SAg antibodies before and 28 days after start of treatment with ABR-214936[13] (anti-staphylococcal enterotoxin E [SEA], black line), ABR-217620 MONO (anti-SEA/E-120, red line), and ABR-217620 COMBO (anti-SEA/E-120, blue line).

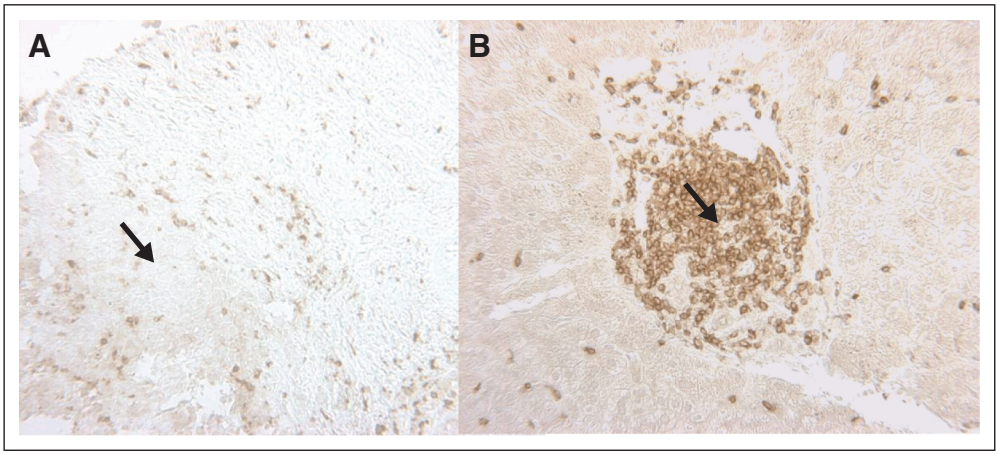

Fig 3. Immunohistochemistry for T-lymphocyte (anti-CD3) infiltration in biopsies taken before treatment (archival tissue) and at the third day of the second cycle treatment with ABR-217620 for patient number 2. The T lymphocytes stain brown and the arrows indicate unstained tumor cells. This patient had a partial response that continues at 30+ months.

to ABR-217620 increasing in a dose proportional manner. In most patients there was a low (0 to 100 pmol/mL) to intermediate (100 to 500 pmol/mL) increase in anti-SAg levels after treatment. In general, systemic exposure to ABR-217620 was lower in treatment cycles 2 and 3, presumably from induced anti-SAg. Systemic exposure to docetaxel was not affected by ABR-217620. The severity of adverse events correlated to dose, concentration at 5 minutes after dosing and area under the time-concentration curve (AUC) of ABR-217620. Changes in laboratory parameters (interleukin-2 [IL-2], interferon-γ [IFN-γ], and lymphocytes) and vital signs (body temperature, pulse, and blood pressure) were found to be significantly correlated to AUC of ABR-217620.

Pharmacodynamics

Cytokines. ABR-217620 infusion caused a dose-dependent increase of cytokines (peaking 2 to 3 hours after bolus) including IL-2 and IFN-γ (Figs 2A and 2B). The induced systemic cytokine levels were greatest after the first treatment. Cytokine production was seen in all treated cancer types and served as a biomarker for T-lymphocyte activation.

Selective T-cell expansion. ABR-217620 caused a pronounced and selective expansion of the superantigen SEA/E-120 reactive T-cell population (TCR-Vβ6.4 expressing T cells) from background levels approximately 5% up to 54% to 64% of T cells with the highest levels observed on day 5 (Fig 2C). This T-cell compartment then returned to baseline level as expected after a phase of stimulation and expansion.

T-cell infiltration of tumor biopsies post–ABR-217620 treatment. Tumor biopsies from two patients (both with NSCLC), in the COMBO study were evaluated by immunohistochemistry. Neither patient had received immunomodulatory drugs (eg, IL-2) between their original biopsies (archival tissue) and treatment with the study drug. Both tumors expressed 5T4. Enhancement of T-cell infiltration of tumor after ABR-217620 treatment was evident in both patients. One patient showed increased numbers of CD3-expressing cells (T lymphocytes) adjacent to tumor cells in a biopsy taken on the third day of treatment in cycle 2. The second patient demonstrated massive tumor-associated infiltration of CD3-positive cells and apparent tumor cell destruction on the cycle 2 day 3 biopsy (Fig 3). Under normal circumstances, there are very few lymphocytes present in most solid tumor unless there is an inflammatory reaction at the tumor site. Thus, we believe that the observed tumor infiltration was due to the study drug.

Immunogenicity. Baseline human antimouse antibodies levels were negligible with mild increases during and after cycle one. Baseline levels of anti-SAg antibodies were low (Fig 2D) with only a low (0 to 100 pmol/mL) to moderate (100 to 500 pmol/mL) increase in concentration in approximately 60% of the patients after treatment in the MONO study. This contrasts with the ABR-214936 experience in which higher concentration of anti-SAg antibodies were induced.[13] Patients in the COMBO study showed lower increases of anti-SAg antibodies. Exposure to ABR-217620 in both studies was lower in subsequent cycles when antibody levels were $\geq$ 100 pmol/mL after cycle 1.

Antitumor activity. In the MONO study, 14 patients (36%) had SD on day 56 (seven NSCLC [25%] and seven RCC [64%]). In the COMBO study, the best overall response was confirmed partial remission for two patients (15%) and SD for five patients (38%). One patient with partial remission received 4 cycles of therapy at 10.3 μg/kg ABR-217620 and the second received 6 cycles of therapy at 22 μg/kg. The latter patient had progressed while receiving prior docetaxel; response continues at 30+ months.

DISCUSSION

We report the results of the first-in-human trials of ABR-217620, a novel immunoconjugate based on the concept of tumor-targeted superantigens (TTS). SAgs bind to major histocompatibility complex class II molecules[17,18] and activate cytotoxic and helper T lymphocytes by interacting with the variable part of the T-cell receptor β chain.[19,20] ABR-217620 has a much reduced binding to preformed antibodies, lower toxicity, higher affinity for the target antigen, and improved tumor cell–killing properties[7] as compared with its predecessor immunotoxin.[12] We hypothesized that a less immunogenic modified SEA could be given for multiple cycles of therapy, and allow dosing of individual patients without regard to pre-existing baseline anti-SEA concentrations.

We used an adaptive (Bayesian) dose escalation scheme.[12,21] Studies of the predecessor immunoconjugate showed a strong

influence of neutralizing pre-existing anti-SAg antibodies on toxicity, yielding a higher MTD in the presence of increased pre-existing antibody concentration. In this study, we showed that the MTD of ABR-217620 was independent of anti-SAg antibody levels across a broad range of concentrations. For reasons that are not clear, the tumor type (RCC *v* NSCLC/PC) influenced the MTD, with no influence on pharmacokinetics. For further development of ABR-217620, 15 μg/kg and 22 μg/kg are the recommended phase 2 doses for RCC and NSCLC/PC, respectively. Pharmacokinetics of ABR-217620 was approximately linear without variation within treatment cycles.

Observed toxicities were mechanism based, mostly grade 1 and 2 and manageable. DLTs—hypotension, fever, vascular leak syndrome, and elevated liver enzymes—were reversible without significant long-term consequences. There were no hypersensitivity reactions or clinically significant hematologic toxicity from ABR-217620. Neutropenia from docetaxel occurred at the expected levels. ABR-217620 was mildly to moderately immunogenic, although not in all patients, and it was possible to administer multiple cycles safely. Deaths in the COMBO trial were due to tumor necrosis and neutropenic sepsis (docetaxel).

Immunohistochemistry evaluation of archival tissue and post-treatment tumor biopsies from two patients showed clear enhancement of tumor-associated T-cell infiltration after ABR-217620 treatment. One patient had an apparent immunological cellular response with T cells in close proximity to tumor cells. The other patient demonstrated massive tumor-associated T-cell infiltration and apparent destruction of tumor cells. Together with dose-dependent T-lymphocyte cytokine induction and substantial expansion of TCR-Vβ6.4–expressing T cells, these observations provide evidence for biologic proof of mechanism.

On day 56, 14 patients had evidence of stable disease by RECIST in the MONO study, all with RCC or NSCLC. In the COMBO study, there were two confirmed partial responses, one with a durable and ongoing PR in hepatic metastases in a patient with documented docetaxel resistance. This latter result could reflect the effects of TTS alone or could indicate the ability of TTS to sensitize the tumor to docetaxel.

In conclusion, ABR-217620 can be safely administered to patients with advanced malignancies at doses of 22 μg/kg in patients with NSCLC and PC and 15 μg/kg in patients with RCC. To evaluate fully the utility of ABR-217620 either as a single agent, or in combination with currently available therapies to enhance immunologic antitumor activity, disease-directed studies are underway and planned.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: Gunnar Hedlund, Active Biotech (C); Göran Forsberg, Active Biotech (C) **Consultant or Advisory Role:** Andre Rogatko, Active Biotech (C) **Stock Ownership:** Göran Forsberg, Active Biotech **Honoraria:** Robert Hawkins, Active Biotech **Research Funding:** Corey Langer, Active Biotech; Robert Hawkins, Active Biotech; Roger B. Cohen, Active Biotech **Expert Testimony:** None **Other Remuneration:** None

AUTHOR CONTRIBUTIONS

Conception and design: Gunnar Hedlund, Göran Forsberg, Andre Rogatko, Roger B. Cohen
Administrative support: Gunnar Hedlund
Provision of study materials or patients: Katherine Alpaugh, Gunnar Hedlund, Corey Langer, Robert Hawkins, Svein Dueland, Ulrik Lassen, Roger B. Cohen
Collection and assembly of data: Hossein Borghaei, Katherine Alpaugh, Gunnar Hedlund, Robert Hawkins, Svein Dueland, Ulrik Lassen, Roger B. Cohen
Data analysis and interpretation: Hossein Borghaei, Katherine Alpaugh, Gunnar Hedlund, Göran Forsberg, Andre Rogatko, Robert Hawkins, Svein Dueland, Ulrik Lassen, Roger B. Cohen
Manuscript writing: Hossein Borghaei, Gunnar Hedlund, Göran Forsberg, Roger B. Cohen
Final approval of manuscript: Hossein Borghaei, Katherine Alpaugh, Gunnar Hedlund, Göran Forsberg, Corey Langer, Andre Rogatko, Robert Hawkins, Svein Dueland, Ulrik Lassen, Roger B. Cohen

REFERENCES

1. Allen TM: Ligand-targeted therapeutics in anti-cancer therapy. Nature Reviews/Cancer 2:750-763, 2002

2. Presta LG: Engineering antibodies for therapy. Curr Pharm Biotechnol 3:237-256, 2002

3. Kreitman RJ, Wang QC, FitzGerald DJ, et al: Complete regression of human B-cell lymphoma xenografts in mice treated with recombinant anti-CD22 immunotoxin RFB4(dsFv)-PE38 at doses tolerated by cynomolgus monkeys. Int J Cancer 81: 148-155, 1999

4. Kreitman RJ, Squires DR, Stetler-Stevenson M, et al: Phase I trial of recombinant immunotoxin RFB4(dsFv)-PE38 (BL22) in patients with B-cell malignancies. J Clin Oncol 23:6719-6729, 2005

5. Sievers EL, Appelbaum FR, Spielberger RT, et al: Selective ablation of acute myeloid leukemia using antibody-targeted chemotherapy: A phase I study of an anti-CD33 calicheamicin immunoconjugate. Blood 93:3678-3684, 1999

6. Forsberg G, Ohlsson L, Brodin T, et al: Therapy of human non-small-cell lung carcinoma using antibody targeting of a modified superantigen. Br J Cancer 85:129-136, 2001

7. Erlandsson E, Andersson K, Cavallin A, et al: Identification of the antigenic epitopes in staphylococcal enterotoxins A and E and design of a superantigen for human cancer therapy. J Mol Biol 333: 893-905, 2003

8. Hole N, Stern PL: A 72 kD trophoblast glycoprotein defined by a monoclonal antibody. Br J Cancer 57:239-246, 1988

9. Starzynska T, Marsh PJ, Schofield PF, et al: Prognostic significance of 5T4 oncofetal antigen expression in colorectal carcinoma. Br J Cancer 69:899-902, 1994

10. Southall PJ, Boxer GM, Bagshawe KD, et al: Immunohistological distribution of 5T4 antigen in normal and malignant tissues. Br J Cancer 61:89-95, 1990

11. Dohlsten M, Abrahmsen L, Bjork P, et al: Monoclonal antibody-superantigen fusion proteins: Tumor-specific agents for T-cell-based tumor therapy. Proc Natl Acad Sci U S A 91:8945-8949, 1994

12. Cheng JD, Babb JS, Langer C, et al: Individualized patient dosing in phase I clinical trials: The role of escalation with overdose control in PNU-214936. J Clin Oncol 22:602-609, 2004

13. Shaw DM, Connolly NB, Patel PM, et al: A phase II study of a 5T4 oncofoetal antigen tumour-targeted superantigen (ABR-214936) therapy in patients with advanced renal cell carcinoma. Br J Cancer 96:567-574, 2007

14. Sundstedt A, Celander M, Wallen Ohman M, et al: Immunotherapy with tumor-targeted superantigens (TTS) in combination with docetaxel results in synergistic anti-tumor effects. Int Immunopharm doi:10.1016/j.intimp.2009.04.013

15. Rosendahl A, Hansson J, Sundstedt A, et al: Immune response during tumor therapy with antibody-superantigen fusion proteins. Int J Cancer 68:109-113, 1996

16. Rosendahl A, Kristensson K, Hansson J, et al: Repeated treatment with antibody-targeted superantigens strongly inhibits tumor growth. Int J Cancer 76:274-283, 1998

17. Fraser JD: High-affinity binding of staphylococcal enterotoxins A and B to HLA-DR. Nature 339:221-223, 1989

18. Fischer H, Dohlsten M, Lindvall M, et al: Binding of staphylococcal enterotoxin A to HLA-DR on B cell lines. J Immunol 142:3151-3157, 1989

19. Dellabona P, Peccoud J, Kappler J, et al: Superantigens interact with MHC class II molecules outside of the antigen groove. Cell 62:1115-1121, 1990

20. Irwin MJ, Hudson KR, Fraser JD, et al: Enterotoxin residues determining T-cell receptor V beta binding specificity. Nature 359:841-843, 1992

21. Giantonio BJ, Alpaugh RK, Schultz J, et al: Superantigen-based immunotherapy: A phase I trial of PNU-214565, a monoclonal antibody-staphylococcal enterotoxin A recombinant fusion protein, in advanced pancreatic and colorectal cancer. J Clin Oncol 15:1994-2007, 1997