Cancer Immunol Immunother (2008) 57:155–163
DOI 10.1007/s00262-007-0357-5

ORIGINAL ARTICLE

# A phase-I trial of the epidermal growth factor receptor directed bispecific antibody MDX-447 without and with recombinant human granulocyte-colony stimulating factor in patients with advanced solid tumors

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Received: 28 March 2007 / Accepted: 13 June 2007 / Published online: 30 June 2007


**Abstract**
*Introduction* MDX-447 is a bispecific antibody directed against the epidermal growth factor receptor (EGFR) and the high affinity Fc receptor (Fc$\gamma$RI). Preclinical data suggest that co-administration of granulocyte-colony stimulating factor (G-CSF) may enhance the tumor cytotoxicity of bispecific antibodies.
*Methods* In group 1, patients received MDX-447 intravenously (IV) weekly. Dose levels of MDX-447 evaluated in group 1 were 1, 3.5, 7, 10, 15, 20, 30, and 40 mg/m$^2$. In group 2, patients received MDX-447 IV weekly with G-CSF (3 mcg/kg/day) subcutaneously (days −3 to +2, 5–9, 12–16, etc.). Dose levels of MDX-447 evaluated in group 2 were 1, 3.5, 7, 10, and 15 mg/m$^2$.
*Results* Sixty-four patients with advanced solid tumors were treated. Forty-one patients received MDX-447 alone (group 1); 23 patients received MDX-447 + G-CSF (group 2). Hypotension was the predominant dose-limiting toxicity (DLT) in both treatment groups, with seven patients experiencing ≥grade 3 events. MDX-447 half-life ($T_{1/2}$) ranged from 1.9 to 8.4 h, with no obvious differences between the two treatment groups. MDX-447 binding to neutrophils and peak levels of circulating tumor necrosis factor α (TNFα) and interleukin-6 (IL-6) were higher in group 2. The MTD for MDX-447 alone was 30 mg/m$^2$. When G-CSF was given with MDX-447, treatment was not well tolerated and group 2 was closed early because of safety concerns, with the last patient being treated at the 7 mg/m$^2$ dose level. There were no objective complete or partial responses in either group.
*Conclusion* MDX-447 alone was generally well tolerated, but did not achieve objective tumor responses. The MTD for MDX-447 alone was 30 mg/m$^2$ weekly. Co-administration of G-CSF with MDX-447 precluded meaningful dose escalation.

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## Introduction

Bispecific antibodies are derived by linking two parental monoclonal antibodies or antibody fragments. The clinical therapeutic strategy with bispecific antibodies is to link immune effector cells to tumor cells directly. One antibody is directed against a tumor antigen and the other antibody is directed against a cytotoxic trigger molecule, such as an Fc receptor, on immune effector cells [1]. In comparison with conventional monoclonal antibodies, bispecific antibodies may have enhanced ability to activate immune-mediated destruction of cancer cells.

MDX-447 is a bispecific antibody directed against the epidermal growth factor receptor (EGFR) and the high affinity Fc receptor (Fc$\gamma$RI) [2]. EGFR is the prototypic member of the ErbB family of transmembrane receptor tyrosine kinases. EGFR is abnormally activated in many epithelial tumors including colorectal, head and neck, bladder, renal, non-small cell lung carcinoma, and others [3]. EGFR directed therapies have included antibodies directed against the cell surface receptor domain and small molecule inhibitors of the intracellular tyrosine kinase domain [3, 4]. Fc$\gamma$RI, a high affinity receptor for monomeric IgG, is expressed on monocytes, macrophages, immature dendritic cells, activated neutrophils, and other hematopoietic cells [5]. Fc$\gamma$R1 helps mediate immune cytotoxic destruction of antibody-bound cells [6].

MDX-447 was created by cross-linking the humanized anti-EGFR monoclonal antibody (H425) F(ab′) fragment and the humanized anti-Fc$\gamma$RI (CD64) monoclonal antibody (H22) F(ab′) fragment [2]. The parent anti-EGFR monoclonal antibody H425 has been administered systemically [7] and by intratumoral infusion [8] in two phase-I studies for patients with glioblastoma multiforme. Systemic treatment was well tolerated, but intratumoral infusion was associated with significant toxicity attributed to local inflammatory reactions. No objective responses were seen in either study [7, 8]. In an effort to improve the clinical efficacy of H425, MDX-447 was developed. In vitro studies have demonstrated that MDX-447 binds to the EGFR and to Fc$\gamma$RI both separately and simultaneously, and that the bispecific antibody mediates antibody-dependent cellular cytotoxicity (ADCC) with lysis of EGFR overexpressing cell lines (R. Curnow, Medarex Inc., unpublished data).

Maneuvers that up-regulate the activity of immune effector cells, such as simultaneous treatment with G-CSF, might enhance the cytotoxicity of bispecific antibodies. A bispecific antibody directed against Fc$\gamma$RI and EGFR killed renal carcinoma cells in vitro more potently in the presence of peripheral blood from G-CSF treated donors, compared with peripheral blood from control donors [9]. In similar experiments using a bispecific antibody against HER-2/neu and Fc$\gamma$RI, destruction of breast carcinoma cells in vitro was enhanced in the presence of peripheral blood from G-CSF treated subjects, versus peripheral blood from control subjects [10]. The enhanced cytotoxicity observed in assays using G-CSF treated peripheral blood was thought to be due to, at least in part, the ability of G-CSF to up-regulate expression of Fc$\gamma$RI on neutrophils [10–12].

We now report the final results of a phase-I study using MDX-447 in adult patients with advanced solid malignancies. This trial contained two groups. Patients in the first group received MDX-447 as a single agent given weekly. Patients in the second group received weekly MDX-447 in addition to 5 days of granulocyte-colony stimulating factor (G-CSF), from 3 days prior to MDX-447 through 1 day after MDX-447. Research bloods were collected to describe pharmacokinetics and to explore flow cytometry as well as peripheral blood cytokine profiles.

## Materials and methods

This phase-I clinical study was approved by the Institutional Review Boards of each participating center. All patients signed an informed consent indicating that they were aware of the investigational nature of the treatment. MDX-447 was supplied by Medarex Inc., Annandale, NJ, USA.

### Patient selection

Patients were adults (age 18 or older) with Karnofsky Performance Status $\geq$60% and histologically documented, advanced stage, primary or recurrent solid malignancy. Patients with breast, ovarian, and bladder cancer must have received at least one prior chemotherapy regimen for metastatic disease. Patients with prostate cancer must have failed primary hormonal intervention. Other eligibility criteria: white blood cell count >3,000 per $mm^3$, hemoglobin >9.0 mg/dl, platelet count >100,000 per $mm^3$, hepatic aminotransferases and gamma-glutamyltranspeptidase <3$\times$ the upper limit of normal for each institution, serum bilirubin <1.5$\times$ the upper limit of normal for each institution, creatinine <1.5$\times$ the upper limit of normal for each institution, and serum calcium <11 mg/dl. Alkaline phosphatase was <3$\times$ the upper limit of normal for each institution, except in patients with documented bony metastases in which case a value <5$\times$ the upper limit of normal for each institution was allowed. All patients of procreative potential agreed to use appropriate contraception for the duration of the trial.

Patients were excluded who had received prior monoclonal antibody therapy; had clinically significant medical comorbidities, psychiatric disease, or history of non-compliance which would interfere with appropriate protocol treatment and monitoring; had known hemorrhagic diathesis or other clinically significant bleeding; or were pregnant or lactating. Prior to enrollment, no chemotherapy, biologic therapy, investigational agent, steroid medication, and/or radiotherapy for disease within 4 weeks (6 weeks for nitrosoureas or mitomycin C) were permitted. Patients must not have received a bone marrow or stem cell transplant within 1 year. Patients must not have discontinued hormonal therapy (with the exception of LHRH antagonists) within 4 weeks prior to enrollment. Patients were also excluded if they were taking medications, such as colchicine, which could impair assessment of immunologic activity or toxicity.

Prior knowledge of the EGFR status of a patient's tumor was not required for patient eligibility.

## Treatment plan

Patients received intravenous MDX-447 infusions on days 1, 8, 15, and 22 of a 28-day cycle according to the dose escalation scheme outlined in Table 1. Patients in group 1 received MDX-447 alone. Patients in group 2 received G-CSF at 3.0 μg/kg for 5 days each week; from 3 days before each dose of MDX-447 through 1 day after each dose (days −3 to +2, 5–9, 12–16, and 19–23).

Patients were premedicated with 650 mg of acetaminophen orally, 25 mg of indomethacin orally, and 50 mg of diphenhydramine orally or intravenously 30 min prior to receiving MDX-447. An alternative antihistamine and/or non-steroidal anti-inflammatory agent could be used at the discretion of the investigator if clinically indicated. Patients received approximately 500 ml of 0.9% normal saline, or an alternative solution if clinically indicated, intravenously prior to receiving MDX-447. Intravenous fluid infusion continued at approximately 150 $cm^3$/h during the subsequent treatment and observation periods.

Prior to the first infusion of MDX-447, patients received an initial test dose of either 10% of the total dose of MDX-447 or 0.2 mg (whichever was smaller) intravenously over 10 min. If the test dose was tolerated without any significant toxicity after 30 min, the remainder of the MDX-447 dose was administered intravenously at a rate of 6 mg/h. The infusion rate could be increased to 9 mg/h if no adverse reactions were noted after the first 30 min and increased to 15 mg/h if there were no adverse reactions for 30 min at 9 mg/h. At the discretion of the investigator, the infusion rate could be increased to 20 mg/h if there were no adverse reactions noted after 2 h at 15 mg/h. Patients were kept under observation for 1 h following treatment.

**Table 1** Exposure to MDX-447

| Dose level | MDX-447 dose (mg/$m^2$) | Group 1, MDX-447 alone (number of patients) | Group 2, MDX-447 + G-CSF (number of patients) |
|---|---|---|---|
| 1 | 1 | 6 | 3 |
| 2 | 3.5 | 6 | 7 |
| 3 | 7 | 3 | 4 |
| 4 | 10 | 3 | 7 |
| 5 | 15 | 6 | 2 |
| 6 | 20 | 6 | – |
| 7 | 30 | 6 | – |
| 8 | 40 | 5 | – |
| Total | | 41 | 23 |

Physical exams and toxicity assessments were performed weekly on each day of scheduled treatment with MDX-447.

Imaging studies were obtained pretreatment, after approximately 4 weeks of treatment, and approximately every 8 weeks thereafter. Criteria for response assessment followed WHO guidelines [13]. Patients demonstrating clinical benefit were allowed to continue receiving weekly infusions of MDX-447 until the time of disease progression or unacceptable toxicity.

## Dose escalation and definition of maximum tolerated dose (MTD)

For each group, three patients were enrolled at a given dose level. Group 1 (MDX-447 alone) was filled first, then group 2 (MDX-447 plus G-CSF). Dose-limiting toxicity (DLT) was defined as any ≥grade 3 non-hematologic adverse event or any ≥grade 4 hematologic adverse event attributed to study treatment during the first 4 weeks (National Cancer Institute Common Toxicity Criteria, NCI CTC Version 2.0). If no patient at a dose level experienced DLT, then three new patients were entered at the next higher dose level. If one of three patients experienced DLT at any dose level, then additional patients up to a total of six were entered at that dose level. If a second patient entered at a given dose level experienced DLT, then the MTD was considered to have been exceeded and a total of six patients were entered at the next lower dose level. The maximum tolerated dose (MTD) was defined as the highest dose at which no more than one of six subjects experienced DLT.

## Pharmacokinetics (PK)

Research blood samples (2 ml venous blood each, EDTA anticoagulant) for pharmacokinetics (PK) were collected on treatment days 1, 8, and 22 of Cycle 1. PK research blood samples were collected at baseline (prior to the initiation of the test dose) and at the following time points after the initiation of drug infusion: 30, 60, and 120 min, and 4, 6, 8, and 24 h. Blood samples were stored at 2–8°C, centrifuged within 15 min of phlebotomy, and then frozen at −80°C until analysis by enzyme linked immunosorbent assay (ELISA) at Medarex Inc. The method used an anti-idiotype antibody (anti-Id 425, lot EMD64616/C11003). All of the research blood samples from an individual subject were analyzed on one microtiter plate. The wells were incubated with a soluble Fc$\gamma$R1–IgM fusion protein that was detected with an anti-human IgM conjugated probe. The lower limit of detection using this method was 0.005 μg/ml. PK parameters derived included observed maximum concentration ($C_{max}$), half-life ($T_{1/2}$), and area under the curve ($AUC_{0-24\,h}$), as determined by non-compartmental analysis using

WinNonLin Version 2.0 software (Pharsight Corporation, Mountain View, CA, USA).

### Flow cytometry

Research blood samples (10 ml venous blood, heparin anticoagulant) were collected during Cycle 1 on treatment days 1 and 22 at the following planned time points: 0, 2, 4, 6, and 24 h. Immunophenotyping was done with directly labeled antibodies for CD3, CD4, CD8, CD13, CD14, CD16, CD64, and HLA-DR. Directly labeled IgG antibodies were used as controls. Washed cells were resuspended in 2% formaldehyde and stored at 4°C until analyzed using a flow cytometer for forward scatter, side scatter, fluorescein isothiocyanate analysis, and phycoerythrin analysis. Neutrophil (CD64/CD13) bound MDX-447 was detected by labeling with a goat anti-mouse immunoglobulin G antibody.

### Cytokine analysis

Research blood samples (2 ml venous blood each, EDTA anticoagulant) for cytokine assays were collected during Cycle 1 on treatment days 1 and 22 at the following time points: pretreatment, and 2, 4, 6, 8, and 24 h after the beginning of the MDX-447 infusion. Blood samples were stored at 2–8°C, centrifuged within 15 min of phlebotomy, and then frozen at −80°C until analysis at Medarex for levels of tumor necrosis factor α (TNF-α), interleukin-6 (IL-6), and interferon-γ (IFN-γ). Cytokine determinations were made on each sample using a commercially available Quantikine ELISA test kit (R&D Systems Incorporated, Minneapolis, MN, USA).

## Results

### Patient characteristics and drug exposure

Patient characteristics are listed in Table 2. A total of 67 patients were enrolled in the study, of which 64 received at least one dose of MDX-447. One patient was withdrawn from the study due to pretreatment laboratory abnormalities. Another patient was withdrawn due to toxicities from G-CSF prior to receiving the study drug, and one had lesions which were shown to decrease in size on the pretreatment scans. Forty-one patients were treated in the group 1 (MDX-447 alone). Because DLTs were seen at lower dose levels in group 2 (MDX-447 plus G-CSF), only 23 patients were treated in that group. The numbers of patients treated at each dose level are shown in Table 1.

### Dose escalation for MDX-447 (group 1)

One of the first three patients treated at the 1 mg/m$^2$ dose level of MDX-447 experienced grade 3 hypotension with the first infusion. The group was expanded to six subjects with no further DLTs observed. At the 3.5 mg/m$^2$ dose level of MDX-447, the third patient experienced grade 3 fever. This group was also expanded to six subjects with no further DLT observed. Three patients were successfully treated on each of the 7, 10, and 15 mg/m$^2$ dose levels without DLT.

The first patient on the 20 mg/m$^2$ dose level experienced grade 3 hypotension, grade 3 tachycardia, and grade 3 hypoxia with the first infusion. Due to the severity of this event, the subsequent three patients were treated at the next

**Table 2** Patient characteristics

| | Group 1 ($N$ = 41 patients) MDX-447 alone | Group 2 ($N$ = 23 patients) MDX-447 + G-CSF |
|---|---|---|
| Gender (M/F) | 30/11 | 11/12 |
| Median age, years (range) | 57 (37–75) | 62 (46–78) |
| Median KPS (range) | 90 (70–100) | 80 (60–90) |
| Prior chemotherapy (%) | 27 (66%) | 15 (65%) |
| Prior radiotherapy (%) | 19 (46%) | 15 (65%) |
| Primary tumor site | | |
| Kidney | 17 (41%) | 10 (43%) |
| Head and neck | 10 (24%) | 5 (22%) |
| Esophagus | 2 | 2 |
| Thyroid | 1 | 1 |
| Lung | 5 | 0 |
| Breast | 1 | 1 |
| Bladder | 1 | 3 |
| Other[a] | 6 | 1 |

Group 1 includes two patients with two primaries (renal/endometrial and larynx/lung)

[a] Other primary tumor sites in one patient each were colon, ovary, uterus, prostate, soft tissue sarcoma, and adenocarcinoma of unknown primary (all in group 1) and skin (group 2)

lower dose level (15 mg/m$^2$). No DLT was seen among these three patients, and the dose was again increased to 20 mg/m$^2$. None of the five additional patients treated at the 20 mg/m$^2$ dose level experienced DLT. At the 30 mg/m$^2$ dose level, the second patient experienced grade 3 hypotension and dysphagia. This dose level was expanded to six patients, without further DLTs.

Three patients were enrolled at the 40 mg/m$^2$ dose level, and the second patient experienced grade 3 hypotension and back pain. The cohort was expanded, with two additional patients beginning treatment within the same week. One patient experienced grade 3 dyspnea, grade 3 hypotension, and grade 3 headache. The next patient at this dose level experienced grade 3 dyspnea. As such, the MTD was exceeded. Because only one of six patients treated at 30 mg/m$^2$ had experienced DLT, the MTD was defined as 30 mg/m$^2$.

## Dose escalation for MDX-447 plus G-CSF (group 2)

The first three patients treated at the 1 mg/m$^2$ dose level of MDX-447 plus G-CSF tolerated the medication without DLT. The first patient treated at the next dose level of MDX-447 (3.5 mg/m$^2$) plus G-CSF experienced grade 3 myalgias thought to be possibly related to study drug, and this dose level was expanded. One subject at this dose level experienced grade 4 constipation and one subject experienced grade 3 elevation of gamma-glutamyl transpeptidase (GGPT), both thought to be unrelated to the study medication. A seventh subject was treated at this dose level for additional safety information; the subject did not experience DLT and escalation to the next dose level was allowed. Three patients were treated at both the 7 and 10 mg/m$^2$ dose levels without DLT.

The first two subjects treated at the 15 mg/m$^2$ dose level both experienced DLT (dyspnea in one subject, hypotension in the other subject). This prompted a return to the 10 mg/m$^2$ dose level, and four additional patients were enrolled. The third patient experienced labile blood pressure after the first infusion, with hypertension followed by grade 3 hypotension. The fourth subject experienced grade 3 dyspnea and grade 3 allergic reaction.

The dose of MDX-447 was reduced to 7 mg/m$^2$ plus G-CSF for the next subject. This patient experienced grade 3 hypotension with the first infusion. Due to concerns about patient safety, no further patients were treated with the regimen of MDX-447 plus G-CSF.

## Adverse events

The most frequently occurring adverse events in the study were chills, fevers, hypotension, headaches, and nausea. Although most patients did experience some adverse side events, these were usually grades 1 to 2 in severity. Table 3 presents cumulative toxicity data for all patients in groups 1 and 2. Adverse events are shown in the table if they were common (occurring in ≥30% of subjects) or if at least one subject experienced ≥grade 3 toxicity, regardless of attribution.

**Table 3** Cumulative adverse events for groups 1 and 2 (all cycles)

| Adverse event | Group 1 ($N$ = 41) MDX-447 alone | | | Group 2 ($N$ = 23) MDX-447 + G-CSF | | |
|---|---|---|---|---|---|---|
| | Gr 1 | Gr 2 | Gr 3/4 | Gr 1 | Gr 2 | Gr 3/4 |
| Chills | 35 | 10 | 0 | 19 | 8 | 0 |
| Fever | 34 | 17 | 2 | 23 | 15 | 0 |
| Nausea | 16 | 4 | 0 | 13 | 3 | 0 |
| Vomiting | 11 | 2 | 0 | 9 | 6 | 0 |
| Hypotension | 14 | 11 | 5 | 5 | 3 | 2 |
| Tachycardia | 10 | 3 | 1 | 10 | 1 | 0 |
| Dyspnea | 9 | 4 | 2 | 9 | 5 | 3 |
| Pain, general | 15 | 5 | 2 | 13 | 6 | 1 |
| Headache | 20 | 12 | 2 | 6 | 1 | 0 |
| Sepsis | 0 | 0 | 1 | 0 | 0 | 0 |
| Hypertension | 9 | 4 | 2 | 6 | 1 | 1 |
| Pulmonary embolism | 0 | 0 | 1 | 0 | 0 | 0 |
| Dysphagia | 2 | 1 | 1 | 2 | 2 | 0 |
| Constipation | 3 | 2 | 0 | 3 | 1 | 1 |
| Diarrhea | 2 | 0 | 0 | 3 | 2 | 2 |
| Hemorrhage, gastrointestinal | 0 | 0 | 0 | 0 | 0 | 1 |
| Dehydration | 2 | 1 | 1 | 0 | 0 | 0 |
| Allergic reaction | 0 | 0 | 0 | 0 | 0 | 1 |
| Dysuria | 1 | 1 | 1 | 0 | 0 | 0 |
| Hematuria | 2 | 1 | 1 | 0 | 0 | 0 |
| Leukopenia | 3 | 2 | 1 | 0 | 0 | 0 |
| Thrombocytopenia | 3 | 2 | 2 | 0 | 0 | 0 |
| Elevated alkaline phosphatase | 3 | 2 | 1 | 0 | 0 | 0 |
| Elevated GGTP | 2 | 0 | 0 | 2 | 2 | 1 |
| Hyperglycemia | 1 | 1 | 1 | 1 | 1 | 1 |

*GGTP* gamma-glutamyl transpeptidase, *Gr* grade, *N* number of subjects

A total of 633 doses of MDX-447 were administered (444 in group 1 and 189 in group 2) which resulted in 41 grade 3 or 4 adverse events (27 in group 1 and 14 in group 2). Grade 3 or 4 events occurring in more than one subject were: hypotension (7), dyspnea (5), pain (3), hypertension (3), headache (2), fever (2), diarrhea (2), thrombocytopenia (2), and hyperglycemia (2). No deaths occurred while a patient was receiving the study medication or within 30 days after a patient's last dose of MDX-447.

## Pharmacokinetics (PK)

Table 4 displays the pharmacokinetic (PK) parameter estimates for groups 1 and 2. The half-life ($T_{1/2}$) ranged between 1.9 and 8.4 h, and a wash out period of seven half-lives is sufficient to clear more than 99.9% of drug. PK parameters were estimated without regard to study day. Table 4 represents pooled data from days 1, 8, and 22. Based upon the half-life of MDX-447, it is reasonable to pool PK data on days 1, 8, and 22 because there should be no carryover effects.

In both groups, the maximum concentration ($C_{max}$) and area under the curve ($AUC_{0–24h}$) increased directly with MDX-447 dose. There was a modest numeric increase in $T_{1/2}$ as well, but $T_{1/2}$ calculation was confounded by longer infusion times at the higher dose levels (up to 7 h). There was marked variability in PK results among patients at a given dose level.

## MDX-447 bound to neutrophils

The percentage of circulating neutrophils bound to MDX-447 was measured at for all patients on day 1 (Table 5). In group 1, binding of MDX-447 was detectable at all time points during the first 24 h after initiation of MDX-447 infusion. In group 2, expression of Fc$\gamma$RI on neutrophils was up-regulated due to G-CSF administration (data not shown). Additionally, the percentage of neutrophils positive

**Table 4** MDX-447 pharmacokinetic parameters

| Dose (mg/m$^2$) | Group 1 | | | Group 2 | | |
|---|---|---|---|---|---|---|
| | $C_{max}$ (μg/ml) | AUC (μg ml/h) | $T_{1/2}$ (h) | $C_{max}$ (μg/ml) | AUC (μg ml/h) | $T_{1/2}$ (h) |
| 1.0 | | | | | | |
| Mean (*n*) | 0.28 | 0.67 | 3.15 | 0.24 | 0.60 | 1.92 |
| SD | 0.21 | 0.45 | 2.90 | 0.10 | 0.20 | 1.21 |
| *N* | 14 | 14 | 12 | 4 | 4 | 3 |
| 3.5 | | | | | | |
| Mean (*n*) | 1.02 | 4.70 | 2.40 | 0.83 | 4.33 | 1.86 |
| SD | 0.83 | 4.30 | 1.16 | 0.34 | 1.60 | 0.54 |
| *N* | 12 | 12 | 10 | 13 | 13 | 11 |
| 7.0 | | | | | | |
| Mean (*n*) | 1.48 | 11.60 | 4.55 | 2.02 | 9.07 | 3.12 |
| SD | 0.37 | 3.35 | 1.69 | 2.15 | 3.86 | 1.03 |
| *N* | 8 | 8 | 8 | 10 | 10 | 8 |
| 10.0 | | | | | | |
| Mean (*n*) | 3.01 | 34.92 | 5.91 | 1.50 | 14.78 | 4.95 |
| SD | 2.38 | 37.79 | 3.74 | 0.90 | 14.50 | 6.74 |
| *N* | 7 | 7 | 6 | 12 | 12 | 8 |
| 15.0 | | | | | | |
| Mean (*n*) | 2.87 | 38.00 | 8.22 | 1.46 | 13.46 | 5.63 |
| SD | 1,22 | 20.94 | 6.61 | 0.54 | 8.69 | 4.39 |
| *N* | 15 | 15 | 10 | 4 | 4 | 4 |
| 20.0 | | | | | | |
| Mean (*n*) | 3.71 | 42.05 | 7.06 | | | |
| SD | 1.09 | 20.58 | 3.36 | | | |
| *N* | 13 | 14 | 10 | | | |
| 30.0 | | | | | | |
| Mean (*n*) | 5.57 | 70.15 | – | | | |
| SD | 2.98 | 40.60 | – | | | |
| *N* | 14 | 14 | – | | | |
| 40.0 | | | | | | |
| Mean (*n*) | 13.96 | 185.54 | 8.37 | | | |
| SD | 4.51 | 90.54 | 1.05 | | | |
| *N* | 7 | 7 | 5 | | | |

*N* number of drug administrations studied, *SD* standard deviation

for MDX-447 was markedly higher for patients in group 2 compared to patients in group 1 (Table 5). Binding of MDX-447 was maximal between the 2 and 6-h time points, and decreased by approximately 20% between 6 and 24 h. Similar results were seen in assays done on day 22 research bloods (data not shown).

### Cytokine levels in peripheral blood

TNFα levels transiently increased after MDX-447 administration in both groups 1 and 2. The time to peak TNFα concentration was generally 2–4 h after drug administration. Table 6 presents the mean plasma concentrations for TNFα for all patients at the 2-h time point on day 1. TNF-α concentrations were markedly higher at all dose levels in group 2. Higher doses of MDX-447 did not appear to be associated with higher peak levels of TNF-α. Similarly, peak IL-6 elevations were higher in group 2 by approximately 3-fold (data not shown). IFN$\gamma$ levels did not appear to be altered by treatment in group 1 or in group 2 (data not shown).

### Clinical efficacy

In group 1, 39 patients were evaluable for response. For best initial response, 24 (61.5%) patients had stable disease and 15 (38.5%) had progression. In group 2, 19 patients were evaluable for response. For best initial response, 11 (57.9%) patients had stable disease and 8 (42.1%) had progression. No objective responses were seen in either group. At time of removal from study, 30 (76.9%) patients in group 1 and 15 (78.9%) patients in group 2 had experienced progression of disease.

**Table 5** MDX-447 bound to neutrophils (mean % positive), day 1

| Dose of MDX-447 (mg/m$^2$) | Hours post-initiation of MDX-447 infusion | | | | |
|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 24 |
| *Group 1* | | | | | |
| 1.0 | 2 | 1 | 2 | 1 | 2 |
| 3.5 | 6 | 20 | 12 | 18 | 18 |
| 7.0 | 2 | 11 | 16 | 38 | 38 |
| 10.0 | 1 | 17 | 18 | 41 | 19 |
| 15.0 | 13 | 13 | 22 | 15 | 30 |
| 20.0 | 2 | 7 | 8 | 6 | 15 |
| 30.0 | 3 | 14 | 23 | 25 | 32 |
| 40.0 | 2 | 5 | 11 | 22 | 59 |
| *Group 2* | | | | | |
| 1.0 + G-CSF | 1 | 40 | 18 | 10 | 1 |
| 3.5 + G-CSF | 12 | 95 | 92 | 86 | 47 |
| 7.0 + G-CSF | 3 | 70 | 92 | 99 | 65 |
| 10.0 + G-CSF | 8 | 95 | 94 | 88 | 66 |
| 15.0 + G-CSF | 1 | 97 | 98 | 88 | 64 |

**Table 6** TNFα levels in peripheral blood at 2 h after initiation of MDX-447 infusion (day 1)

| Dose (mg/m$^2$) | TNFα (pg/ml) | |
|---|---|---|
| | Group 1 | Group 2 |
| 1.0 | | |
| Mean (*n*) | 45.1 (6) | 577.6 (3) |
| SEM | 12.3 | 241.5 |
| 3.5 | | |
| Mean (*n*) | 196.5 (6) | 992.3 (6) |
| SEM | 63.1 | 349.2 |
| 7.0 | | |
| Mean (*n*) | 9.9 (3) | 628.2 (4) |
| SEM | 4.4 | 415.3 |
| 10.0 | | |
| Mean (*n*) | 16.4 (3) | 1,713.5 (7) |
| SEM | 9.2 | 566.3 |
| 15.0 | | |
| Mean (*n*) | 20.1 (6) | 58.4 (2) |
| SEM | 4.7 | 56.8 |
| 20.0 | | |
| Mean (*n*) | 40.6 (6) | |
| SEM | 12.0 | |
| 30.0 | | |
| Mean (*n*) | 24.2 (6) | |
| SEM | 6.7 | |
| 40.0 | | |
| Mean (*n*) | 40.3 (4) | |
| SEM | 21.3 | |

*n* number of subjects, *pg* picogram, *SEM* standard error of the mean

## Discussion

MDX-447 is a bispecific antibody that binds to both human cytotoxic effector cells that express the Fc$\gamma$RI receptor and EGFR on tumor cells. In group 1 of this phase-I study, the MTD of weekly MDX-447 was 30 mg/m$^2$. In group 2, the combination of MDX-447 plus G-CSF was not well tolerated, and this treatment arm was closed before the MTD was established. Also, flow cytometry did demonstrate increased expression of Fc$\gamma$RI, with increased MDX-447 binding, on neutrophils in the peripheral blood of group 2 patients compared with group 1 patients. No objective responses were seen in either group.

The most common DLT was hypotension. The most frequent adverse experiences associated with MDX-447 were fever and chills, followed by headache, hypotension, nausea, and vomiting. No significant hematologic, hepatic, or renal toxicities were encountered. No deaths occurred during the study or within 30 days after treatment.

The basis of the increased toxicity among patients in group 2 is not well understood. There were differences in the patient characteristics between the two groups (lower median KPS, higher median age, and lower male:female ratio in group 2). Among patients receiving MDX-447 plus G-CSF (group 2), peak levels of TNFα and IL-6 were generally higher than at the same dose levels of MDX-447 alone (group 1), which may have contributed to the DLTs seen in group 2. Of note, peak levels of TNFα and IL-6 in the peripheral blood were not related to MDX-447 dose in this study. As such, the data do not suggest that DLTs among patients receiving MDX-447 alone (group 1) were related to circulating levels of these cytokines.

The strategy of combining a bispecific antibody with G-CSF to augment the anti-tumor immune response has been explored in studies of another bispecific antibody, MDX-H210 (anti-HER2 × anti-FcRI). In phase-I studies of MDX-H210 alone, adverse events generally were transient and ≤ grade 2 [2, 14, 15], similar to the experiences of most patients who received MDX-447 alone in the current study. Co-administration of G-CSF plus MDX-H210 generally was well-tolerated in phase-I trials for advanced breast cancer patients, although a simultaneous head-to-head comparison of MDX-H210 with and without G-CSF was not done in those trials [16, 17]. The FcγRI binding component of MDX-H210 is the humanized antibody H22, identical to that for MDX-447 [2]. Therefore, there is no reason to suspect that the divergent clinical toxicity profiles observed with MDX-447 plus G-CSF in the current study versus MDX-H210 plus G-CSF in other studies [16, 17] were driven by differences in the immune effector cell populations targeted by the Fc-binding components of the bispecific antibodies.

The pharmacokinetic data demonstrated dose-dependent increases in $C_{max}$ and AUC, but $T_{1/2}$ did not appear to change appreciably with dose when the longer infusion times at higher doses (up to 7 h) were considered. There was no evidence that co-administration of G-CSF affected the bioavailability of MDX-447. Pharmacokinetic studies of bispecific antibodies commonly demonstrate shorter half-lives than are seen with conventional monoclonal antibodies. In this study, the half-life of MDX-447 was approximately 2–8 h. The half-life of MDX-H210 has been estimated to be approximately 2–4 h [18, 19]. Cetuximab, a conventional monoclonal antibody directed against EGFR, has an estimated half-life of approximately 7 days [20], suggesting different clearance mechanisms.

Although efficacy was not a primary endpoint in this phase-I study, the lack of objective responses with MDX-447 contrasts with the clinical activity of other anti-EGFR monoclonal antibodies. Cetuximab, a bivalent monoclonal antibody, has established roles in the treatment colorectal cancer [21] and head and neck squamous cell carcinoma [22]. There are notable pharmacokinetic and structural differences between MDX-447 and cetuximab that may be relevant in this regard. The relatively short half-life of MDX-447 may limit its anti-tumor activity in patients. It is not known if binding of MDX-447, a monovalent anti-EGFR antibody, has different effects on EGFR activation compared with binding of bivalent antibodies such as cetuximab. MDX-447 and cetuximab may differ in their ability to mediate ADCC. One proposed tumoricidal mechanism for cetuximab is ADCC mediated by FcγRIIa and FcγRIIIa [23], whereas MDX-447 targets FcγRI. With the increasing recognition of interplay between activating Fcγ receptors [24], a trifunctional antibody which binds to FcγRI and FcγRIII receptors as well as HER2 and CD3 is in clinical development [25].

In summary, this study demonstrates that weekly administration of MDX-447 alone is generally well tolerated, and the recommended phase-II dose is 30 mg/m$^2$. Combination of MDX-447 plus G-CSF in group 2 was not well tolerated. Preliminary efficacy data did not indicate that the added toxicity associated with the administration of G-CSF improved efficacy, as no objective responses were seen. Further clinical study of bispecific antibodies is appropriately considered in the current climate of increasing recognition of the therapeutic potential of antibody-based therapy for solid tumors.

**Acknowledgments** We wish to thank Randall Curnow, MD, and Robert Belt, MD, for assistance during the development and conduct of this study.

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