Phase I clinical trial of the bispecific antibody MDX-H210 (anti-FcγRI \times \text{anti-HER-2/neu}) in combination with Filgrastim (G-CSF) for treatment of advanced breast cancer

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A phase I study of the bispecific antibody MDX-H210 in combination with granulocyte colony-stimulating factor (G-CSF) was performed in stage IV breast carcinoma patients, overexpressing HER-2/neu. MDX-H210, constructed by crosslinking antigen binding fragments (Fab\textsuperscript{\textprime}) of monoclonal antibody (mAb) H22 to Fc gamma receptor I (Fc\gammaRI), and mAb 520C9 to HER-2/neu, respectively, mediates the lysis of tumour cells in vitro, and in human Fc\gammaRI transgenic mouse models. The proto-oncogene HER-2/neu is overexpressed in approximately 30% of breast cancer patients, and represents a promising target for antibody-based immunotherapy. Fc gamma receptor I (CD64) is an effective trigger molecule, which is expressed on monocytes/macrophages, immature dendritic cells, and G-CSF-primed polymorphonuclear cells (PMN). Patients received G-CSF (Filgrastim) for 8 consecutive days, and cohorts of three patients were treated on day 4 with escalating, single doses of MDX-H210. A total of 30 patients were included, and treatment was generally well tolerated, without reaching dose-limiting toxicity. Side effects consisted mainly of fever and short periods of chills, which were timely related to elevated plasma levels of interleukin 6 and tumour necrosis factor alpha. In the last two cohorts, MDX-H210 plasma levels exceeded 1 \mu g ml\textsuperscript{−1}, and on circulating myeloid cells >50% saturation of Fc\gammaRI was found until day 4. These effector cells were highly effective in antibody-dependent cell-mediated cytotoxicity. Immunohistochemical analyses of tumour biopsies in individual patients documented infiltration of monocytes and PMN after MDX-H210 infusion. Although the clinical course of the disease was not altered by the single dose of MDX-H210, a favourable toxicity profile – even at high doses – and remarkable biological effects were seen when combined with G-CSF. Therefore, the combination of G-CSF and MDX-H210 should be evaluated in further immunotherapeutical strategies.

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Breast cancer represents a major public health problem in the Western world. In case of localised disease, cure rates have improved, but in women with metastatic disease results are still disappointing (Hortobagyi, 1998). Therefore, novel therapeutical approaches are under investigation. In the last decade, along with new chemothepathetical drugs, developments in the field of immunology and genetic engineering have raised new perspectives. For example, Trastuzumab (Herceptin\textsuperscript{8}), a humanised IgG1 monoclonal antibody (mAb) with specificity for HER-2/neu, has been approved for patients with metastatic breast cancer. As a single agent, the overall response rate was 15% in heavily pretreated patients with HER-2/neu overexpressing metastatic breast cancer (Cobleigh et al, 1999; Baselga, 2001).

The HER-2/neu gene encodes a 185–190 000 molecular weight transmembrane protein with tyrosine kinase activity. Overexpressed HER-2/neu has transforming activity, and in a variety of human carcinomas, gene amplification and protein overexpression have been demonstrated. This includes approximately 30% of breast cancer, in which it has been correlated with the development of metastases and is associated with poor prognosis (Slamon et al, 1987). In addition, HER-2/neu has limited expression on normal tissue, suggesting that it may constitute a valuable target for antibody therapy (Pegram and Slamon, 2000). Although many mechanisms have been proposed to account for the antitumour activities of therapeutic antibodies – including blockade of signalling pathways, activation of apoptosis, and antibody-dependent cell-mediated cytotoxicity (ADCC) – the relevance of...
these mechanisms in humans is still unclear (Houghton and Scheinberg, 2000). In mice, the significance of Fc receptor-bearing effector cells is clearly demonstrated by the reduced efficacy of Trastuzumab to arrest tumour growth in animals deficient in activating Fc receptors (Clynes et al, 2000).

Three different classes of leucocyte receptors for IgG (FcγR) have been distinguished in humans (van de Winkel and Capel, 1993; Ravetch and Bolland, 2001). Fc gamma receptor I (FcγRI) (CD64) is constitutively expressed on myeloid precursors in the bone marrow, monocytes/macrophages, immature dendritic cells, and can be induced on polymorphonuclear cell (PMN) by interferon gamma (IFN-γ) or granulocyte colony-stimulating factor (G-CSF) (Perussia et al, 1983; Repp et al, 1991). FcγRI is unique among FcγRs because of its high affinity for IgG, the limited cell expression solely on cytotoxic effector cells, and because of its inherent capacity to trigger cytotoxic effector cells. Furthermore, FcγRI contains a distinct cytoplasmatic domain, which targets antigens to MHC class II-containing vesicles, and thereby, enhances antigen presentation (van Vught et al, 1999).

Bispecific antibodies (BsAbs) can effectively link target cells to cytotoxic trigger molecules on immune effector cells, and may overcome some of the limitations of conventional mAb (Segal et al, 1999). For example, high concentrations of serum immunoglobulins compete in vivo for binding to FcγRI on immune effector cells. Hereby, the ability of therapeutical antibodies to trigger FcγRI could be impaired. To overcome this problem, the BsAb MDX-H210 was constructed by chemical crosslinking of F(ab′) fragments from humanised mAb 22 (H22), directed to FcγRI, and mAb 520C9, to the proto-oncogene product HER-2/neu, respectively. Monoclonal antibody H22 binds to a site distinct from the ligand-binding site, yet effectively triggers FcγRI-mediated cellular responses (such as phagocytosis, respiratory burst, and ADCC) in the presence of high concentrations of human serum IgG (Guyre et al, 1989; Valerius et al, 1993). To influence the effector cell system specifically, G-CSF was added to the treatment schedule to increase the absolute numbers of PMN, to induce the expression of FcγRI (Repp et al, 1991), and to enhance their functional capacity (Valerius et al, 1993). Preclinical studies demonstrated the binding of MDX-H210 to FcγRI on G-CSF-primed PMN and to HER-2/neu on SK-BR-3 breast carcinoma cells to be comparable to the respective parental mAb. More importantly, in vitro MDX-H210 was highly cytotoxic to HER-2/neu overexpressing cell lines, when FcγRI-positive PMN from G-CSF-treated patients served as effector cells (Stockmeyer et al, 1997; Stockmeyer et al, 2001). In addition, studies in an FcγRI-transgenic animal model reported synergism of FcγRI-directed BsAb and G-CSF, resulting in antitumour activity and in ‘memory’ induction (Honeychurch et al, 2000).

To analyse whether these preclinical observations may translate into the clinical situation, MDX-H210 in combination with G-CSF was tested in a phase I study. The primary objectives of this trial were to define the maximum tolerated dose (MTD), and to identify the optimal biologically active dose of MDX-H210. The secondary objective was to assess the clinical responses.

**PATIENTS AND METHODS**

**Patients**

Patients, aged 18–70 years, with stage IV breast cancer were eligible if their primary tumour or metastases were overexpressing HER-2/neu. The expression of HER-2/neu was determined on paraffin-embedded tissues using standard immunohistochemical methods (Venter et al, 1987). Inclusion in the trial demanded a 2+ or 3+ membrane staining (van de Vijver, 2001). Patients needed to be progressive after at least two standard chemotherapeutic or hormonal regimens, or one hormonal and one chemotherapy regimen. Sufficient blood cell counts, normal hepatic, and renal function, as well as an Eastern Cooperative Oncology Group performance status of <3, and a life expectancy of more than 3 months were required. The protocol was approved by the local Ethics Committee, and written informed consent before treatment was obligatory.

**Study design**

Bispecific antibody MDX-H210 was given as a single, 2-h intravenous (i.v.) infusion, in combination with a fixed subcutaneous (s.c.) dose of 5 μg kg⁻¹ Filgrastim for 8 days in the afternoon, with a minimum of 4 h after the end of MDX-H210 on day 0 (Table 1). Filgrastim was started 3 days before the administration of MDX-H210 to ensure maximal expression of FcγRI on neutrophils (Repp et al, 1991). The dose of MDX-H210 was escalated in cohorts of three patients, and not in an individual patient. For the first cohort of patients, Filgrastim was skipped if the absolute neutrophil count (ANC) was higher than 20 000 μl⁻¹ to prevent toxicity. As no toxicity was observed, the upper limit for ANC was raised to 50 000 μl⁻¹ from the second cohort onwards. In the first two cohorts, the same dose of MDX-H210 (0.35 mg m⁻²) was given. Patients treated in the third to tenth cohort received escalating doses of MDX-H210 from 1 up to 200 mg m⁻² (1, 3, 5, 7, 10, 15, 30, 100, and 200 mg m⁻²). Dose-limiting toxicity (DLT) was defined as the dose where three or more patients in a cohort of six patients experienced grade III or IV toxicity, and the MTD as the dose level below which DLT occurred. Reassessment for tumour response was performed after 30 days. If patients had an objective response or stable disease, a second treatment on compassionate use basis was possible for patients enrolled during the last period according to an amendment. The overall goals of this phase I trial were to determine toxicity, MTD, pharmacokinetics, and pharmacodynamics of MDX-H210, to describe the biological effects of MDX-210 in combination with Filgrastim, and to elucidate the effects of Filgrastim on immune effector cells.

**Production of MDX-H210**

Bispecific antibody MDX-H210 (FcγRI × HER-2/neu) was produced by chemically crosslinking F(ab′) fragments of mAb 22 against FcγRI (CD64) with mAb 520C9 against HER-2/neu, as described by Glennie et al (1987). Briefly, F(ab′)2 fragments were produced by limited proteolysis with pepsin, and were then reduced to provide F(ab′) with free hinge region SH groups. The SH groups on one of the F(ab′)-SH partners were then fully alkylated with excess o-phenylenediamine (o-PDM) to provide free maleimide groups. Finally, the two o-PDM and F(ab′)-SH preparations F(ab′) were combined at a ratio of 1:1 to generate heterochimeric constructs. After purification by size exclusion chromatography, the binding activity of the individual components was verified by flow cytometry, using HER-2/neu-expressing SK-BR-3 cells, FcγRI-expressing human monocytes, and U937 cells as targets. The bispecific nature of the...
construct was confirmed by a solid-phase immunoassay. MDX-H210 was produced according to GMP guidelines by Medarex (Medarex Inc., Annandale, NJ, USA), and shipped as a clear colourless liquid in sterile phosphate-buffered saline for i.v. infusion.

Assessment of adverse reactions
Patients were hospitalised on day –1 and MDX-H210 was administered as a 2-h i.v. infusion on day 0 (Table 1). The vital signs were frequently monitored during and for 24 h after the administration of the BsAb. Afterwards, patients were checked on an outpatient basis. Laboratory tests were performed daily starting on day –3 with additional time points 2, 4, and 8 h after the start of MDX-H210 infusion. Toxicity was graded according to the NCI Common Toxicity Criteria. For dose escalation, transient leucopenia, neutropenia without signs of infection, or fever with mild hypotension were not graded as toxicity, because these symptoms were known to be correlated to MDX-H210 infusion, and to recover spontaneously (Valone et al, 1995).

Pharmacokinetics
Blood samples were obtained before and 2, 4, 8, 24, 48, 72, and 96 h after BsAb infusion. Plasma was immediately separated and stored at –70 °C until analysis. Microritine plates, coated with goat antiserum IgG, were incubated with serial dilutions of the patient’s plasma, or with MDX-H210 diluted in normal human plasma (Nabi Inc., Boca Raton, FL, USA). The captured BsAb was detected by an alkaline phosphatase-conjugated antiserum IgG. Pharmacokinetic analysis was performed using PKPD Tools for Excel Version 1.02 (Charles Minto, Stanford, CA, USA) with a constant i.v. infusion noncompartmental model.

Cytokines, tumour markers, and plasma HER-2/neu levels
Plasma – separated and stored as described above – was analysed for levels of interleukin (IL)-1α, IL-1β, IL-2, IL-6, IL-10, IFN-γ, tumour necrosis factor alpha (TNF-α), G-CSF, soluble IL-2 receptor (sIL-2R), CEA, CA125, and CA15.3 by enzyme-linked immunosorbent assay using kits according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN, USA). Plasma HER-2/neu was measured by enzyme immunosorbent assay using a commercial kit (Chiron Diagnostics, Alameda, CA, USA).

Radioimaging with Technetium-99m (99mTc) MDX-H210
Antibody labelling was performed according to a published kit- formulated NHS-BAT (NHS = N-hydroxysuccinimide and BAT = bis(aminoethanethiol)type) ester conjugation (Eisenhut et al, 1996). Briefly, NHS ester of the BAT ligand, 6-(4’-carboxyphenoxy)butyl)-2,10-dimercapto-2.10-dimethyl-4,8-di- zaun-decane was conjugated with MDX-H210 at ambient temperature, and pH ranging from pH 7.0 to pH 8.5. Unreacted or reducing agent. Within 5 min, labelling yield > 95% was achieved using 1 mg BAT-MDX-H210 comprising about 3 BAT ligands per antibody, and up to 1.5 GBq 99mTc-pertechnetate. Localisation of MDX-H210 in normal and tumour tissues was investigated in a subset of patients who voluntarily participated, using planar whole-body scans, and single photon emission tomography (SPECT) images of the regions of interest.

Granulocyte scintigraphy with Indium-111-oxine (111In)-labelled PMN
Autologous granulocytes were isolated using a Percoll/plasma gradient, and labelled with 111In-oxine as described (Becker et al, 1986). Labelled granulocytes were reinfused either 30 min before, or 72 h after application of MDX-H210. Dynamic imaging was performed with a gamma camera linked to a computer system during 2 h after injection of labelled cells to trace the route of PMN.

Human antibispecific antibody (HABA) response
Microritine plates coated with MDX-H210 were incubated with dilutions of plasma samples. Anti-MDX-H210 antibodies were detected with an alkaline-phosphatase-conjugated goat anti-human IgG Fc-specific probe. The dilution of plasma required to reach the background level (A405 = 0.1) was determined for samples before infusion, and on days 7, 14, and 30 postinfusion. Human antibispecific antibody levels were expressed as x-fold increase over the baseline preinfusion value.

Assessment of biological effects
Isolation of effector cells A measure of 10–20 ml of peripheral blood was drawn from patients or healthy donors (HDs) after informed consent. The total leucocyte counts in whole blood were determined on an EPICS™ Profile flow cytometer (Coulter, Hialeah, FL, USA), and PMN counts in whole blood were calculated from the total leucocyte counts and differentials of Wright-stained blood smears. Isolated PMN were obtained by a method slightly modified from that described in Repp et al (1991). Briefly, citrate anticoagulated blood was layered over a discontinuous Percoll (Seromed, Berlin, Germany) gradient consisting of 70 and 62% for HD, and of 68 and 59% of Percoll for Filgrastim recipients, respectively. After centrifugation, PMN were collected at the interphase between the two Percoll layers, and mononuclear cells from the Percoll/plasma interphase. The remaining erythrocytes were removed by hypotonic lysis. The purity of PMN was determined by cytoospin preparations and exceeded 95%, with few contaminating eosinophils and < 1% mononuclear cells. The viability of cells tested by Trypan blue exclusion was always exceeding 95%.

Immunofluorescence Antibodies 32.2 (FcγRI, CD64; mlgG1) (Guyre et al, 1989), IV.3 (FcγRIII, CD32; mlgG2b) (Looney et al, 1986), 3G8 (FcγRIII, CD16; mlgG1) (Fleit et al, 1982), and 520C9 (HER-2/neu; mlgG1) (Ring et al, 1989) were obtained from Medarex Inc. (Annandale, NJ, USA). Whole blood (100 μl) was incubated with different lineage-specific FITC- or RPE-conjugated antibodies (CD3, CD4, CD8, CD20, CD11b, CD56, CD67) for 30 min at 4°C. Afterwards, samples were subjected to FACS® Lysing solution to lyse red blood cells and fix white cells. Cells were then washed three times in phosphate-buffered saline supplemented with 1% bovine serum albumin, and analysed on an EPICS™ Profile flow cytometer (Coulter). For each cell population, the relative fluorescence intensity (RFI) was calculated as the ratio of mean linear fluorescence intensity of relevant to irrelevant, isotype-controlled antibodies.

ADCC assay Antibody-dependent cell-mediated cytotoxicity was performed as described (Valeriou et al, 1993). Briefly, human SK-BR-3 breast carcinoma cells were used as the target. This cell line was obtained from the American Type Culture Collection (Manassas, VA, USA), and kept in an R10+ medium consisting of RPMI 1640 medium (Gibco BRL, Paisley, Scotland) supplemented with 10% heat-inactivated foetal calf serum, 100 U ml⁻¹ penicillin, 100 U ml⁻¹ streptomycin, and 4 mm l-glutamine (all Gibco BRL). The target cells were labelled with 200 μCi 51Cr for 2 h.
After extensive washing with RF10⁺, cells were adjusted to 10⁶ ml⁻¹. Isolated PMN, effector-to-target ratio 80:1 (E:T, 80:1), or 50 µl of whole blood as effector source, sensitising antibodies and RF10⁻ were added into round-bottom microtitre plates. By adding the target cell suspension, giving a final volume of 200 µl, the assays were started. After 3 h at 37°C, assays were stopped by centrifugation, and ⁵¹Cr release from triplicates was measured in counts per minute (c.p.m.). The percentage of cellular cytotoxity was calculated using the formula:

\[
\% \text{ specific lysis} = \frac{\text{experimental c.p.m.} - \text{basal c.p.m.}}{\text{maximal c.p.m.} - \text{basal c.p.m.}} \times 100
\]

with maximal ⁵¹Cr release determined by adding perchloric acid (3% final concentration) to target cells, and basal release measured in the absence of sensitising antibodies and effector cells. Only very low levels of antibody-mediated noncellular cytotoxicity (without effector cells) were observed under these assay conditions (<5% specific lysis). Low levels of antibody-independent killing were seen in whole-blood assays (Figure 6).

Oxidative burst and phagocytosis The activity of neutrophil respiratory burst was measured by a method modified from Smith and Weidemann (1993). In short, 100 µl of whole blood was incubated for 15 min with 1 µl of 2',7'-dichlorofluorescein diacetate (200 nM solution in ethanol; Molecular Probes, Eugene, OR, USA). After stimulation for 15 min with 1 µg ml⁻¹ PMA (Sigma, Deisenhofen, Germany), red cell lysis and fixation of white cells was obtained by Q-prep (Coulter). Fluorescence of single cells was measured on an EPICS™ Profile flow cytometer (Coulter), and the results were analysed comparing mean fluorescence intensity.

For measurement of phagocytosis, fluorescent polystyrene beads (1.0 µm in diameter; Fluoresbrite, Polysciences Inc., Warrington, PA, USA) were opsonised with human albumin or polyclonal human IgG, respectively, according to the manufacturer’s instructions for coupling proteins to carboxylated polystyrene microparticles (Carbodiimide method, Polysciences Inc., Warrington, PA, USA). A measure of 2 µl of 2.5% carboxylated microparticles were added to 100 µl whole blood, and incubated for 30 min at 37°C. Red cell lysis and fixation of white cells was obtained by Q-prep, and the fluorescence of single cells was measured on an EPICS™ Profile flow cytometer (Coulter). Polymorphonuclear cell populations were gated according to forward light scatter and perpendicular light scatter. Data were expressed as the percentage of PMN-containing FITC-labelled beads and the mean number of beads per PMN (calculated from the fluorescence intensity of PMN with a single particle ingested).

Statistical analysis Group data are reported as mean± standard deviation (s.d.). The differences between groups were analysed by unpaired (or, when appropriate, paired) Student’s t-tests. Levels of significance are indicated. Data presented correspond to the whole patient population, or is indicated when different.

RESULTS

Patient characteristics
In all, 30 women with metastatic breast cancer were treated in this trial. Three patients were treated in each of 10 cohorts received escalating doses of MDX-H210 from 0.35 up to 200 mg m⁻². The first and second cohorts were treated with 0.35 mg m⁻² with an upper limit of the ANC of 20 000 µl⁻¹ in the first and 50 000 µl⁻¹ from the second cohort onwards. Their ages ranged from 40 to 69 years, with a median age of 50 years. The median time from diagnosis was 70.2 months. Patients were heavily pretreated, but had not received previous antibody therapy (Table 2).

Pharmacokinetics
Plasma levels of MDX-H210 were measured up to 4 days after BsAb infusion (Figure 1). The mean t½ values ranged from 4.76 h in the 3.5 mg m⁻² cohort to 17.41 h in the 200 mg m⁻². Cmax values were correlated with the applied dose, and varied from 0.24 mg l⁻¹ at the 1 mg m⁻² level to 155.7 mg l⁻¹ at the 100 mg m⁻² dose level (Table 3). At the 200 mg m⁻² dose level, infusion of MDX-H210 was temporarily stopped in two patients because of chills and vomiting during the infusion period and restarted after about 1 h. Owing to the prolonged infusion time, Cmax was lower compared to the 100 mg m⁻² dose level and was reached after 4 h (Table 3). Both granulocytes and monocytes were opsonised with MDX-H210 at the end of the infusion, and continued to be ‘armed’ with MDX-H210 up to 4 days after the infusion of 200 mg m⁻² (Figure 2), with a comparable expression of FcγRI (CD64) on days 0 and 3 (Figure 4).

Toxicity
In the current study, the combination of MDX-H210 with Filgrastim was well tolerated with minimal to moderate toxicity.

Table 2 Patient characteristics

| Patient demographics | Total number | 30
| Age (years) | 50 (40–69)
| Median time since diagnosis (months) | 70.2 (7.1–181.6)
| Metastatic sites | 2 (1–5)
| Prior therapy | Chemotherapy 2 (0–6) Radiotherapy 1 (0–7) Hormonal treatment 2 (0–4)
| ECOG | 0 4
| 1 20
| 2 6

Figure 1 Plasma levels of MDX-H210. Plasma levels of MDX-H210 in the different patient cohorts were determined 2, 4, 8, 24, 48, 72, and 96 h after BsAb infusion. The related pharmacokinetic parameters are described in Table 3. Data presented relate to all participating patients treated with ≥10 mg m⁻².
up to doses of 200 mg m$^{-2}$ (Table 4). No grade IV toxicity occurred. Nonhaematological toxicities consisted mainly of short periods of chills, low-grade fever, nausea, and vomiting all occurring towards the end of the MDX-H210 infusion. Most side effects resolved spontaneously within 8 h, or could easily be treated with either acetaminophen or antiemetics. Several patients complained about pain at metastatic sites, possibly related to local inflammatory reactions in tumour tissue induced by the BsAb, or to therapy-related cytokine release. A one- to two-fold increase of alkaline phosphatase and γGT was noted, which was maximal after 3–4 days, and resolved spontaneously after 1 week. Haematological changes were mainly related to the treatment medication. After the start of Filgrastim an asymptomatic granulocytosis emerged. At 2 h after infusion of MDX-H210, granulocytes and monocytes decreased dramatically. However, these values returned to preinfusion levels within approximately 12 h. Lymphocytes, which do not express FcγRII, also demonstrated a significant drop after infusion of MDX-H210. Red blood cell counts and platelet numbers did not significantly change (Figure 3).

### Receptor expression

At 72 h after the start of Filgrastim, all blood PMN expressed FcγRII (CD64), declining slowly after G-CSF cessation (Figure 4). A small increase of FcγRII expression was seen on monocytes. Within 2 h after the start of MDX-H210, a transient decrease of FcγRII expression was seen on monocytes and PMN, suggesting a more rapid decline of FcγRII-positive cells. Interestingly, FcγRII (CD32) expression on monocytes and PMN was also increased during Filgrastim application, whereas FcγRIII (CD16) expression of PMN significantly decreased. The expression of CD67 on PMN, and of CD11b on PMN and monocytes increased after the administration of MDX-H210, probably due to concomitant cytokine release.

### Functional assays

**ADCC, phagocytosis, and oxidative burst** In vitro cytotoxicity assays against SK-BR-3 breast cancer cells with isolated PMN demonstrated significantly enhanced cytotoxicity in the presence of MDX-H210 during, but not before or 1 week after the start of Filgrastim application (Figure 5). A small decrease in ADCC activity of PMN on day 1 probably reflected the reduced FcγRII expression on the remaining circulating PMN.

Spontaneous cytotoxicity of whole blood, without the addition of MDX-H210 in vivo could be demonstrated on day 1 in cohorts treated with doses above 10 mg m$^{-2}$, lasting up to day 4 with doses above 100 mg m$^{-2}$ (Figure 6). This spontaneous cytotoxicity documented adequate circulating MDX-H210 levels to induce ADCC, and was in agreement with the measurement of cell-bound MDX-H210 (Figure 2).

Phagocytosis of IgG-coated beads by PMN was increased during Filgrastim application, with a further increase 24 h after MDX-H210 infusion. In contrast, phagocytosis of albumin-coated beads did not change (Figure 7).

Baseline oxidative burst, unstimulated and stimulated with PMA, was comparable to HDs (60.5 ± 22.1 vs 59.8 ± 29.0 and 151.5 ± 25.8 vs 149.6 ± 25.7, respectively). A slight increase of PMA-stimulated oxidative burst was seen during Filgrastim (158.3 ± 23.2; $P = 0.07$), which increased to significant values 24 h after the application of MDX-H210 (163.7 ± 21.6; $P = 0.01$), returning to baseline on day 4.

**Cytokines, HABA** Cytokine plasma concentrations were measured at baseline, and at several time points during the study. Elevated levels of IL-6 and TNF-α were consistently found during the first hour after MDX-H210 infusion (Figure 8), and related to flu-like symptoms. Peak levels of TNF-α and IL-6 did not correlate to the dose of BsAb applied. Whereas peak levels of TNF-α were reached after 2 h, IL-6 levels were maximal after 4 h. The anti-inflammatory cytokine IL-10 also increased, with a maximum after 2 h. Granulocyte colony-stimulating factor plasma levels increased during the application of Filgrastim, with a small, additional increase after MDX-H210 infusion. Soluble IL-2 receptor increased after the start of Filgrastim, and reached its maximum after MDX-H210. Serum levels of IFN-γ, IL-1α, IL-1β, IL-2, and IL-12 were not altered during the study period. Human antibispecific antibody
was detected in 19 patients on day 30, in seven of them ≥16 times above baseline.

**Scintigraphy** The number of circulating PMN rapidly decreased after the start of MDX-H210 infusion. In order to evaluate whether G-CSF-activated PMN could cause additional toxicity, for example, by trapping of PMN in the lung, dynamic granulocyte imaging was performed with $^{111}$In-labelled autologous granulocytes infused 30 min prior to MDX-210 infusion. A normal distribution of granulocytes with no abnormal accumulation at any particular site was found (patient #3). In two patients, either $^{111}$In-labelled (patient #12) or $^{99m}$Tc-HMPAO-labelled autologous granulocytes (patient #23) were infused 72 h after the administration of MDX-H210. Imaging of the liver, spleen, and bone marrow was normal. However, sites of bone metastasis were spared (patient #12), and soft-tissue metastases did not image well. In three patients (patient #6, #11, and #28), 200 mg of $^{99m}$Tc-labelled MDX-H210 was injected simultaneously with unlabelled antibody, resulting in imaging of the liver, spleen, and bone marrow, but without imaging tumour

### Table 4 Maximum nonhaematological toxicity

| Dose level (number of patients) | 0.35 ($n = 6$) | 1 ($n = 3$) | 3.5 ($n = 3$) | 7 ($n = 3$) | 10 ($n = 3$) | 15 ($n = 3$) | 30 ($n = 3$) | 100 ($n = 3$) | 200 ($n = 3$) |
|-------------------------------|----------------|------------|--------------|-------------|-------------|-------------|-------------|---------------|-------------|
| **Toxicity grade**            | 1/2 | 1/2 | 1/2 | 3 | 3 | 3 | 3 | 3 | 3 |
| Nausea                        | 3   | 2   | 2   | 3   | 3   | 3   | 3   | 3   | 1   |
| Vomiting                      | 1   | 2   | 2   | 3   | 3   | 3   | 3   | 3   | 3   |
| Fever                         | 3   | 3   | 3   | 3   | 3   | 3   | 3   | 3   | 2   |
| Chills                        | 3   | 3   | 2   | 3   | 3   | 3   | 3   | 3   | 3   |
| Hepatic                       | 5   | 3   | 3   | 3   | 3   | 3   | 2   | 3   | 1   |
| Respiratory                   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   |
| Tachycardia                   | 1   | 2   | 2   | 1   | 1   | 1   | 1   | 1   | 1   |
| Hypotension                   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   |
| Tumour pain                   | 2   | 2   | 2   | 2   | 2   | 2   | 2   | 2   | 2   |
| Redness of skin               | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   |
| Diarrhoea                     | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   |
| Emotional                     | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   | 1   |

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**Figure 3** Effects of MDX-H210 on circulating white blood cells numbers. In all patients, ANC increased during Filgrastim application. A significant drop of ANC, monocytes, and lymphocytes was observed after infusion of MDX-H210 with a minimum 2–4 h after start of BsAb infusion. Data presented relate to all participating patients.

**Figure 4** Receptor expression of PMN and monocytes during treatment with MDX-H210/Filgrastim. Receptor expression was determined by flow cytometry and expressed as RFI. Changes from days −3 to 0 are attributed to the effect of Filgrastim; further changes were noted after start of the 2 h during infusion of MDX-H210 on day 0. Data presented relate to all participating patients. **Significant changes ($P < 0.05$) were seen for all tested antigens between days −3 and 0 and for all, except CD11b on monocytes between day 0 and the end of MDX-H210 infusion. No significant differences were seen between days −3 and 30.

**Figure 5** ADCC of isolated PMN via MDX-H210. Isolated PMN from HD were compared with PMN from patients in their capacity to mediate cytotoxicity against SK-BR-3 breast cancer cells in the presence of 0.4 $\mu$g $m^{-1}$ MDX-H210 at an E:T ratio of 80:1. Lysis of SK-BR-3 by PMN from patients on days 0, 1, and 4 is significantly higher compared to day −3 (before start of Filgrastim) as well as compared to healthy controls ($P < 0.005$, indicated by *). Data presented (mean ± s.d.) relate to a subset of 26 patients and 26 HDs.
sites. In patient #28, however, asymptomatic brain metastases were detected by SPECT. Since these lesions were also seen with 99mTc-DTPA SPECT without antibody, a nonspecific imaging due to an altered blood–brain barrier has to be considered. In all three groups according to treatment with low (0.35–7 mg m$^{-2}$), medium (10–30 mg m$^{-2}$), and high (100–200 mg m$^{-2}$) doses of MDX-H210. Significant enhanced lysis was seen in patients of the high-dose group (day 1 vs day 0, $P<0.05$, indicated by *). The medium- and high-dose group showed enhanced lysis compared to the low-dose group on day 1 ($\#$: $P<0.05$; ##: $P<0.01$). Patients treated with high doses of MDX-H210 (≥ 100 mg m$^{-2}$) showed a trend toward enhanced lysis of SK-BR-3 even 4 days after MDX-H210 infusion, although not statistically significant.

Figure 6 Spontaneous cytotoxicity. For whole-blood ADCC, citrate anticoagulated, freshly drawn blood was added to $^{51}$Cr-labelled SK-BR-3 cells without the addition of antibody. Patients were divided into three groups according to treatment with low (0.35–7 mg m$^{-2}$), medium (10–30 mg m$^{-2}$), and high (100–200 mg m$^{-2}$) doses of MDX-H210. Significant enhanced lysis was seen in patients of the high-dose group (day 1 vs day 0, $P<0.05$, indicated by *). The medium- and high-dose group showed enhanced lysis compared to the low-dose group on day 1 ($\#$: $P<0.05$; ##: $P<0.01$). Patients treated with high doses of MDX-H210 (≥ 100 mg m$^{-2}$) showed a trend toward enhanced lysis of SK-BR-3 even 4 days after MDX-H210 infusion, although not statistically significant.

Figure 7 Phagocytosis of latex beads by PMN during treatment with MDX-H210. Phagocytosis of 1.0 μl fluorescent polystyrene beads, coated with human albumin and polyvalent human IgG, respectively, was measured by flow cytometry. Mean beads per neutrophil from a patient and an HD measured simultaneously were calculated as described in Material and Methods. The relation of beads per PMN of patient to HD is expressed. Phagocytosis of IgG-coated beads is significantly higher on days 0 and 1 ($P<0.05$, indicated by *) compared to baseline (day −3).

Response

No objective responses were seen in this single dose study in advanced patients. A total of 11 patients had stable, and 19 patients showed no relevant infiltration with macrophages and PMN.

Progressive disease at the end of the study period. Three patients with metastatic skin lesions developed an erythema between days 1 and 4 after the administration of MDX-H210. In one of them, a skin biopsy taken 3 days after the administration of MDX-H210 (patient #21) was stained for macrophages and PMN. Macrophages (arrow) were stained with a CD68 antibody, and PMN (arrow) with chloroacetate esterase. A skin biopsy from the same patient taken before the start of treatment showed no effector cell infiltration.

Figure 8 Cytokine plasma levels. Mean plasma levels of all patients are shown. Plasma levels of IL-6 and TNF-α increase after the infusion of MDX-H210 with a maximum 2 h after the start of infusion. Anti-inflammatory cytokine IL-10 is released with a similar time kinetic. G-CSF levels begin to rise after the start of Filgrastim therapy, with a further significant increase after MDX-H210. Plasma levels of sIL-2R and G-CSF were significantly different between days −3 and 0 ($P<0.001$). Significant differences ($P<0.05$) of plasma levels before (day 0) and after infusion of MDX-H2120 were seen for TNF-α (8 h and 1 day after start of MDX-H210), IL-6 (2 h and 1 day after start of MDX-H210), and sIL-2R (from 2 h up to 3 days after start of MDX-H210). No significant release of IL-1α, IL-1β, IL-2, IFN-γ, and IL-12 was seen.

Figure 9 Immunohistochemical staining of a skin biopsy. A skin biopsy taken 3 days after the administration of MDX-H210 (patient #21) was stained for macrophages and PMN. Macrophages (arrow) were stained with a CD68 antibody, and PMN (arrow) with chloroacetate esterase. A skin biopsy from the same patient taken before the start of treatment showed no relevant infiltration with macrophages and PMN.
As MDX-H210 is designed to trigger phagocytic cells, the transient drop in ANC and monocytes was not surprising. Additional haematological changes were a more than 10-fold increase of circulating PMN, due to Filgrastim, and all PMN expressed FcγRI. Pulmonary toxicity, resulting from entrapment of activated FcγRI-positive PMN in the lungs, was considered to be a potential risk for G-CSF administration. Therefore, in some patients imaging with 111In-labelled granulocytes was performed. However, we did not observe increased clinical pulmonary toxicity nor did we observe abnormal accumulation of PMN in the lung. Three patients were treated because of stable disease after 30 days. Strikingly less side effects were noted in these patients. This desensitisation phenomenon was also reported in other clinical trials with BsAb or mAb (Maloney et al, 1997; Pullarkat et al, 1999; Posey et al, 1999).

In patients with advanced cancer, the cytotoxic capabilities of effector cells are often impaired (Brittenend et al, 1996). In contrast, granulocyte function was not diminished in our patients with regard to phagocytosis and oxidative burst, and was even further enhanced during G-CSF treatment. Isolated PMN were highly cytotoxic in vitro in the presence of MDX-H210, concomitant with the induction of FcγRI expression during G-CSF application. Maximum lysis was achieved at a concentration of 0.4 μg ml⁻¹, with reduced efficacy at higher doses, probably resulting from inhibition by monomeric binding of MDX-H210 to effector and tumour cells (Stockmeyer et al, 1997). Plasma concentrations of MDX-H210 exceeding 1 μg kg⁻¹ were already found in the 3.5 mg m⁻² cohort; with peak levels and AUC up to 200 mg m⁻² with a serum half-life of ~14 h, increasing to 17 h at doses of 200 mg m⁻². Granulocytes and monocytes of patients treated at the 200 mg m⁻² cohort documented complete saturation of FcγRI by BsAb for up to 4 days. These ‘armed’ effector cells are functionally active with high cytolytic activity in an ADCC assay without additional MDX-H210.

**DISCUSSION**

Based on our preclinical data (Stockmeyer et al, 1997), the combination of MDX-H210 and G-CSF was analysed in a phase I study, performed in 30 heavily pretreated breast carcinoma patients overexpressing HER-2/neu. This phase I trial was designed to determine the tolerability, safety, and MTD of BsAb MDX-H210 (anti-FcγRI × anti-HER-2/neu) in combination with Filgrastim. Aside from clinical toxicity, we focused on the biological activity, and pharmacokinetics of the combination. Treatment was generally well tolerated, with a successful dose escalation of MDX-H210 up to 200 mg m⁻² without reaching MTD. Nonhaematologic toxicity appeared within the first 2–4 h, was mainly infusion related, and consisted of transient fever, chills, nausea and vomiting, and hypotension. Tumour necrosis factor alpha and IL-6 levels increased timely related to the side effects with a maximum after 2 and 4 h, respectively. Interestingly, toxicity appeared not to be dose related, in line with comparable peak levels of IL-6 and TNF-α in all cohorts above 0.35 mg m⁻². Despite high peak levels of IL-6 and TNF-α, exceeding 300 pg ml⁻¹, side effects were only moderate, which might be due to the absence of IL-1 and IFN-γ (Waage et al, 1989), and to antagonistic effects of an early release of anti-inflammatory cytokines such as IL-10. Furthermore, G-CSF might contribute to an enhanced anti-inflammatory cytokine response (Hartung et al, 1995). Concomitant administration of MDX-H210 and G-CSF seems to be safe in our study as well as in a multidose trial with MDX-H210 (Pullarkat et al, 1999). Interestingly, the side effects in our study were similar to other MDX-H210 studies, with other cytokines such as IFN-γ or GM-CSF (Posey et al, 1999; Lewis et al, 2001). On the other hand, the addition of G-CSF led to unacceptable toxicity in combination with an anti-FcγRI × anti-EGFR BsAb (MDX-447) (Pfister et al, 1999).

**Figure 10** Plasma levels of soluble HER-2/neu. Soluble HER-2/neu levels were measured during treatment with MDX-H210 and Filgrastim. A significant decrease of s-Her-2/neu was seen between 4 h after the start of infusion and 7 days (P < 0.002). Data are presented as mean ± s.e.m. of all patients.

decrease of 34.8 ± 79.0 U ml⁻¹ (P = 0.03), while on day 30 this was less impressive (a mean decrease of 26.2 ± 76.8 U ml⁻¹; P = 0.083) (Figure 10).

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Immunotherapy. This large cell population can be expanded and activated by G-CSF, which also induced FcεRI expression. Fortunately, concomitant treatment with MDX-H210 and Filgrastim did not lead to limiting toxicity. Although no objective response could be documented in these heavily pretreated patients with progressive breast cancer, biological effects were noted. Thus, MDX-H210 can be safely administered in combination with Filgrastim, and leads to highly efficient, expanded effector cell populations that may well have a significant therapeutic impact when employed in an optimised extended treatment schedule.

REFERENCES

Adams GP, Schier R, McCaul AM, Simmons HH, Horak EM, Alpaugh RK, Marks JD, Weiner LM (2001) High affinity restricts the localization and tumor penetration of single-chain Fv antibody molecules. Cancer Res 61: 4750–4753

Bartsch J (2001) Clinical trials of Herceptin (trastuzumab). Eur J Cancer 37(Suppl. 1): S18 – S24

Becker W, Fischbach W, Reiners C, Borner W (1986) Three-phase white blood cell scan: diagnostic validity in abdominal inflammatory diseases. J Nucl Med 27: 1109 – 1115

Britenden J, Heys SD, Ross J, Erenin O (1996) Natural killer cells and cancer. Cancer 77: 1226 – 1243

Clynes RA, Towers TL, Presta LG, Ravetch JV (2000) Inhibitory Fc receptors modulate in vivo cytokotoxicity against tumor targets. Nat Med 6: 443 – 446

Dillman RO (1999) Infusion reactions associated with the therapeutic use of monoclonal antibodies in the treatment of malignancy. Cancer Metastasis Rev 18: 465 – 471

Eisenhut M, Lehmann WD, Becker W, Behr T, Elser H, Strittmatter W, Ghetie V, Ward ES (2000) Multiple roles for the major histocompatibility complex class 1 receptor CD64. Annu Rev Immunol 18: 739 – 766

Fanger MW, Curnow RT, Kaufman PA, Ernstoff MS (2001) Phase II study of interferon gamma: a multiple-dose phase-I study in patients with metastatic disease. J Clin Oncol 19: 2639

Dillman RO (1999) Infusion reactions associated with the therapeutic use of monoclonal antibodies in the treatment of malignancy. Cancer Metastasis Rev 18: 465 – 471

Eisenhut M, Lehmann WD, Becker W, Behr T, Elser H, Strittmatter W, Steinstraesser A, Baum RP, Valerius T, Repp R, Deo YM (1996) Bispecific NHS-BAT ester for antibody conjugation and stable technetium-99m labeling: conjugation chemistry, immunoreactivity and kit formulation. J Nucl Med 37: 362 – 370

Fleet HB, Wright SD, Unkeless JC (1982) Human neutrophil Fc gamma receptor distribution and structure. Proc Natl Acad Sci USA 79: 3275 – 3279

Ghetie V, Ward ES (2000) Multiple roles for the major histocompatibility complex class 1-related receptor FcRn. Annu Rev Immunol 18: 739 – 766

Glennie MJ, McBride HM, Worth AT, Stevenson GT (1987) Preparation and performance of bispecific F(ab'/2) antibody containing thiourea-linked Fab'; fragments. J Immunol 139: 2367 – 2375

Guyre PM, Graziano RF, Vance BA, Morgenalli PM, Fanger MW (1989) Monoclonal antibodies that bind to distinct epitopes on FcεRI are able to trigger receptor function. J Immunol 143: 1650 – 1655

Hartung T, Docke WD, Gantner F, Krieger G, Sauer A, Stevens P, Volk HD, Wendel A (1995) Effect of granulocyte colony-stimulating factor treatment on ex vivo blood cytokine response in human volunteers. Blood 85: 2482 – 2489

Honechjurc J, Tutt AL, Valerius T, Heinjens IAMF, van de Winkel JG, Glennie MJ (2000) Therapeutic efficacy of FcεRII/CD64-directed bispecific antibodies in B-cell lymphoma. Blood 96: 3544 – 3552

Hortobagyi GN (1998) Treatment of breast cancer. N Engl J Med 339: 974 – 984

Houghton AN, Scheinberg DA (2000) Monoclonal antibody therapies – a ‘constant’ threat to cancer. Nat Med 6: 373 – 374

Lewis LD, Cole BF, Wallace PK, Fisher JL, Waugh M, Guyre PM, Fanger MW, Curnow RT, Kaufman PA, Ernstoff MS (2001) Pharmacokinetic-pharmacodynamic relationships of the bispecific antibody, MDX-H210 when administered in combination with interferon gamma: a multiple-dose phase-1 study in patients with advanced cancer which overexpresses HER-2/neu. J Immunol Methods 248: 149 – 165

Looney RJ, Abraham GN, Anderson CL (1986) Human monocyties and U937 cells bear two distinct Fc receptors for IgG. J Immunol 136: 1641 – 1647

Maloney DG, Grillo-Lopez AJ, White CA, Bodkin D, Schilder RJ, Neidhart JA, Janakiraman N, Toos KA, Liles TM, Dallaire BK, Wey K, Royston I, Davis V, Levy R (1997) IDEC-C2B8 (Rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin’s lymphoma. Blood 90: 2188 – 2195

Pegram M, Slamov D (2000) Biological rationale for HER2/neu (c-erbB2) as a target for monoclonal antibody therapy. Semin Oncol 27: 13 – 19

Perussia B, Dayton ET, Lazarus R, Fanning V, Trinchieri G (1983) Immune interferon induces the receptor for monomeric IgG1 on mononcotic and myeloid cells. J Exp Med 158: 1092 – 1113

Pfister DG, Lipton A, Belt R, Motzer RJ, Winston C, Metz EM, Sherman EJ, Curnow RT (1999) A phase I trial of the epidermal growth factor receptor (EGFR)-directed bispecific antibody (BsAb) MDX-447 in patients with solid tumors. Proc Am Soc Clin Oncol 18: 433a

Posey JA, Rasper R, Verma U, Deo YM, Keller T, Marshall JL, Hodgson J, Mazumder A, Hawkins MJ (1999) A pilot trial of GM-CSF and MDX-H210 in patients with erbB-2-positive advanced malignancies. J Immunother 22: 371 – 379

Pullarkat V, Deo YM, Link J, Spears L, Marty S, Curnow R, Groshen S, Gee C, Weber JS (1999) A phase I study of a HER2/neu bispecific antibody with granulocyte-colony-stimulating factor in patients with metastatic breast cancer that overexpresses HER2/neu. Cancer Immunol Immunother 48: 9 – 21

Ravetch JV, Bolland S (2001) IgG Fc receptors. Annu Rev Immunol 19: 275 – 290

Repp R, Valerius T, Senderl A, Gramatzki M, Iro H, Kalden JR, Platzer E (1999) Neutrophils express the high affinity receptor for IgG (FcεRI, CD64) after in vivo application of recombinant human granulocyte colony-stimulating factor. Blood 78: 885 – 889

Ring DB, Kassel JA, Hsieh-Ma ST, Bjorn MJ, Tringale F, Eaton AM, Reid SA, Frankel AE, Nadji M (1989) Distribution and physical properties of BCA200, a Mr 200,000 glycoprotein selectively associated with human breast cancer. Cancer Res 49: 3070 – 3080

Segal DM, Weiner GJ, Weiner LM (1999) Bispecific antibodies in cancer therapy. Curr Opin Immunol 11: 558 – 562

Slamov DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235: 177 – 182

Smith JA, Weidemann MJ (1993) Further characterization of the neutrophil receptor for IgG (FcγRIIa). J Immunol 150: 4192 – 4198

Stockmeyer B, Gramatzki M, Glennie MJ, van de Winkel JG, Valerius T (2001) Mechanisms of G-CSF- or GM-CSF-primed neutrophils as effector cells against HER-2/neu over-expressing breast cancer. Eur J Cancer 37: 261 – 268

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Valone FH, Kaufman PA, Guyre PM, Lewis LD, Memoli V, Deo YM, Graziano R, Fisher JL, Meyer L, Mrozek-Orlowski M, Wardwell K, Guyre V, Marley TL, Arzivu C, Fanger MW (1995) Phase Ia/Ib trial of bispecific antibody MDX-210 in patients with advanced breast or ovarian cancer that overexpresses the proto-oncogene HER-2/neu. *J Clin Oncol* **13**: 2281 – 2292

van de Vijver MJ (2001) Assessment of the need and appropriate method for testing for the human epidermal growth factor receptor-2 (HER2). *Eur J Cancer* **37** (Suppl. 1): 11 – 17

van de Winkel JGJ, Capel PJA (1993) Human IgG Fc receptor heterogeneity: molecular aspects and clinical implications. *Immunol Today* **14**: 215 – 221

van Vugt MJ, Kleijmeer MJ, Keler T, Zeelenberg I, van Dijk MA, Leusen JHW, Geuze HJ, van de Winkel JGJ (1999) The FcRγIa (CD64) ligand binding chain triggers major histocompatibility complex class II antigen presentation independently of its associated FcR γ-chain. *Blood* **94**: 808 – 817

Venter DJ, Tuzi NL, Kumar S, Gullick WJ (1987) Overexpression of the c-erbB-2 oncoprotein in human breast carcinomas: immunohistological assessment correlates with gene amplification. *Lancet* **2**: 69 – 72

Waage A, Brandtzæg P, Halstensen Å, Kierulf P, Espevik T (1989) The complex pattern of cytokines in serum from patients with meningococcal septic shock. Association between interleukin 6, interleukin 1, and fatal outcome. *J Exp Med* **169**: 333 – 338

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