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ORIGINAL ARTICLE

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The pharmacokinetics of the bispecific antibody MDX-H210 when combined with interferon gamma-1b in a multiple-dose phase I study in patients with advanced cancer

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Abstract *Introduction:* MDX-H210 is a Fab′×Fab′ bispecific antibody (BsAb) constructed chemically by crosslinking Fab′ mAb 520C9 (anti-HER-2/neu) and humanized Fab′ mAbH22 (anti-CD64). *Study design and objectives:* This was a phase I dose-escalation study of intravenous MDX-H210 (1–70 mg/m^2) combined with subcutaneous IFN-γ, 50 μg/m^2 given 24 h before the BsAb, both drugs being given three times a week for 3 weeks. The major objectives of the study were to define the safety, tolerability and pharmacokinetics of MDX-H210 when given with IFN-γ on this schedule. *Results:* The study group comprised 23 patients (19 female, 4 male; median age 51.5 years, range 25–72 years) with advanced HER-2/neu-positive cancers (19 breast, 3 prostate and 1 lung). Inspection of the log plasma MDX-H210 concentrations-time data for both days 1 and 17 of treatment revealed monoexponential decay in the majority of patients with adequate concentration-time data points. The MDX-H210 $T_{1/2}$ ranged from 2.9 to 21.9 h. The MDX-H210 C_{max} on day 1 (means ± SD) increased from 0.30 ± 0.22 μg/ml at the 1-mg/m^2 dose tier to 86.91 ± 6.46 μg/ml at 70 mg/m^2. Equivalent day-17 values were 0.27 ± 0.30 μg/ml increasing to 147.85 ± 40.23 μg/ml. The MDX-H210 T_{max} occurred at or after the end of the infusion for all treatments. The mean MDX-H210 total body clearance (Cl) was in the range 0.01–0.34 ml/min per kg and the mean MDX-H210 apparent volume of distribution at steady-state (Vd_{ss}) in the range 20–170 ml/kg, compatible with distribution primarily limited to the intravascular space. MDX-H210 $T_{1/2}$ increased with dose (ANOVA $P = 0.001$) and Cl decreased with dose (ANOVA $P = 0.006$). There were no significant changes in MDX-H210 C_{max}, AUC, Cl or Vd_{ss} between day 1 and day 17. *Conclusions:* MDX-H210 pharmacokinetics appeared saturable over the dose range 1–70 mg/m^2, and there was no significant change in MDXH210 pharmacokinetics over the course of the study, or evidence of excessive accumulation of MDX-H210 on this multiple dosing schedule. When MDX-H210 was combined with IFN-γ, the estimated MDX-H210 pharmacokinetic parameters were similar to the published data for single-agent MDX-H210.

Keywords Phase I study · MDX-H210 pharmacokinetics

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Introduction

Bispecific antibodies (BsAbs) are chemically or genetically linked antibodies with two heterologous antigen-binding domains [6, 8, 9, 45]. Potentially antineoplastic BsAbs can be prepared by combining specificities for a tumor antigen and cytotoxic trigger molecules on immunoeffector cells. Such antibodies were developed in order to target cytotoxic immunoeffector cells to cancer cells and subsequently facilitate antibody-dependent cell cytotoxicity (ADCC) [6, 46]. BsAbs have clinical activity in the treatment of refractory Hodgkin's disease, CNS tumors, and advanced breast, ovarian and prostate

cancer [5, 17, 20, 34, 44]. MDX-H210 is a partially humanized Fab′×Fab′ BsAb constructed by chemical conjugation of the F(ab′) fragments of the murine monoclonal antibody (mAb) 520C9 (anti-HER-2/neu) with specificity for the cell surface region of the HER-2/neu gene product and H22, a humanized mAb that binds to the human immunoglobulin receptor FcγRI (CD64) found on monocytes and macrophages [13, 23]. The proto-oncogene HER-2/neu encodes a 185-kDa transmembrane phosphoglycoprotein of the epidermal growth factor receptor (EGFR) family with tyrosine kinase activity. On ligand binding, HER-2/neu dimerizes and then undergoes phosphorylation leading to downstream signaling via Ras and MAP kinase pathways [47].

HER-2/neu is overexpressed on many adenocarcinomas, and on approximately 15–40% of early and up to 70% of metastatic breast and ovarian cancers [21, 37, 38], and is an indicator of poor prognosis in breast cancer patients [1, 14]. FcγRI is a 72-kDa protein with high affinity for the Fc portion of IgG. It is one of three Fc receptors constitutively expressed on the membranes of monocytes, macrophages and activated granulocytes [32, 43]. The mAb H22 binds to a site outside the ligand binding domain for IgG. In vitro studies with BsAbs constructed from mAb H22 have confirmed that cytotoxicity is not blocked by IgG or serum [15, 19, 35, 36]. Neutrophils (PMNs) express FcγRI following activation with interferon gamma (IFN-γ) [29] or granulocyte colony-stimulating factor (G-CSF) [10, 24, 33] in vitro suggesting that FcγRI may be upregulated in vivo following treatment with either of these cytokines. Several studies have indicated that neutrophils play an important role in ADCC [18, 27, 28, 40].

A single-dose phase I study of the nonhumanized version of this BsAb (MDX-210) has been performed in patients with tumors that overexpressed HER-2/neu [41]. This BsAb was found to be well tolerated, and immunologically active as shown by cytokine release after administration as well as localization to cutaneous tumor metastases. The maximum tolerated dose of MDX-210 was reported to be 7–10 mg/m^2, with a dose-limiting toxicity of transient grade III hypotension. In this study, MDX-210 plasma concentration time curves showed monoexponential decay. The MDX-210 pharmacokinetics were linear over the 0.35–10 mg/m^2 dose range with a mean ($n = 10$) terminal elimination half-life of 5.4–7.7 h (range 1.6–12.6 h) and a volume of distribution compatible with the intravascular space. Another phase Ia/Ib clinical trial [21] of three infusions of MDX-210 at doses of 7 and 10 mg/m^2 given over 1 or 3 weeks has revealed MDX-210 pharmacokinetic parameters similar to those in the single-dose study [41] and without evidence of time dependency. The partially humanized MDX-H210 BsAb was shown to have dual specificity and immunological activity in vitro. It causes lysis of HER-2/neu-expressing cell lines mediated by monocytes and IFN-γ- and G-CSF-activated PMNs [23].

We therefore investigated the combination of IFN-γ and MDX-H210 based on the hypothesis that IFN-γ would activate and upregulate the expression of FcγRI on monocytes, macrophages and neutrophils. This combination potentially increases the probability of an interaction between FcγRI-expressing immune-effector cells and tumor cells expressing HER-2/neu, with the likely outcome of enhanced tumor cell kill. The primary objectives of the study were to determine (1) the safety and maximum tolerated dose of MDX-H210 when combined with IFN-γ, (2) the multiple dose pharmacokinetics of MDX-H210, and (3) the immunohematological responses to multiple doses of MDX-H210 when combined with IFN-γ in patients with advanced or refractory solid tumors that overexpressed HER-2/neu. The detailed clinical aspects [22] and pharmacokinetic-pharmacodynamic relationships [25] observed in this study have been previously reported. The detailed pharmacokinetics of MDX-H210 observed in this study are reported here.

Materials and methods

Expression and determination of HER-2/neu on tumor tissue

Expression of HER-2/neu was determined on paraffin-embedded tissues, generally from the primary carcinoma. After peroxidase blocking, optimal staining was achieved at a dilution of 1:50 for CB-11. An avidin-biotin complex technique was utilized, with appropriate controls, along with the chromogenic substrate diaminobenzidine, and counterstaining with hematoxylin (Bio-Tek, Santa Barbara, Calif.). Slides were scored in regard to both the percentage of positive tumor cells ($< 10\%$, 10–50%, and $> 50\%$) and membrane staining intensity (negative, moderate, strong). All patients included in this study were scored positive and showed moderate to strong staining.

Patient population

The study protocol was approved by the Norris Cotton Cancer Center Clinical Cancer Research Committee and the Dartmouth Medical School Committee for the Protection of Human Subjects (local IRB). All patients gave written informed consent prior to being registered and receiving treatment. Patients were recruited from the ambulatory clinics of the Norris Cotton Cancer Center. Patient eligibility criteria for this study included a diagnosis of metastatic carcinoma of any primary site for which no proven curative therapy was known, and overexpression of HER-2/neu as determined by immunohistochemical techniques utilizing mAb CB-11 (see above and reference 4). A maximum of three prior chemotherapy regimens for metastatic disease was permitted. Prior high-dose chemotherapy with autologous bone marrow or stem cell support was allowed provided 1 year had elapsed. A minimum of 4 weeks was to have elapsed since any cancer-specific treatment or radiation therapy, and the patient must have fully recovered from any significant treatment-related toxicities prior to study entry. Adequate hematologic, hepatic, and renal function was required, as was an ECOG performance status of 0-1. Exclusion criteria included prior exposure to murine mAbs with significant human antimurine antibody (HAMA) response, active infection or inflammatory disease, and other serious medical conditions.

Study design and treatment plan

Eligible patients were treated with subcutaneous injections of 50 μg/m^2 IFN-γ on days 0, 2, 4, 7, 9, 11, 14, 16, and 18 (24 h prior

to MDX-H210). MDX-H210 was administered intravenously on days 1, 3, 5, 8, 10, 12, 15, 17, and 19 as a 2-h infusion. Treatment was given for a planned 3-week cycle, as above, with 3 weeks "off treatment" between cycles. Treatment was planned for one cycle of therapy, with allowance for a subsequent cycle based on clinical assessment. All patients were pretreated with acetaminophen (650 mg orally), diphenhydramine (25–50 mg orally), and lorazepam (0.25–1.0 mg orally). When necessary, patients received meperidine HCl 25–50 mg intravenously for rigors. In successive patient cohorts, based on the toxicity observed, the dose of MDX-H210 was escalated as follows: 1.0, 3.5, 7.0, 10.0, 15.0, 30.0, 50.0 and up to 70.0 mg/m^2.

The original protocol design planned for treatment of cohorts of three patients. If two patients in the same dose cohort experienced dose-limiting toxicity, based on the Cancer and Leukemia Group B (CALGB) toxicity criteria, the number of patients treated at that level was expanded to six. If only two of these six patients experienced dose-limiting toxicity, dose escalation continued. If three or more patients had dose-limiting toxicity, dose escalation would stop. As higher doses were studied, however, it became apparent that the toxicity of this regimen was minimal, and that a more rapid dose escalation of MDX-H210 was appropriate. The dose-escalation schema, therefore, was subsequently modified from the originally defined schema to incorporate a rapid dose-escalation schedule from 15 up to 70 mg/m^2 of MDX-H210, at which dose the study was closed.

MDX-H210 and IFN-γ

MDX-H210 was prepared by the method of Glennie et al. [13]. Briefly the F(ab′)$_2$ fragments of humanized mAbs H22 (anti-FcγRI) and 520C9 (anti-HER-2/neu) were reduced to Fab′ by incubation with 10 m*M* mercaptoethanolamine. The Fab′-SH fragments were conjugated using *N*′*N*′-*o*-phenylenedimaleimide (OPDM), acetylated with iodoacetamide, and purified by chromatography on Superdex 200. The binding activities of the individual components of the bispecific antibody were checked by fluorescence activated cell sorter (FACS) analysis [16] using the HER-2/neu-expressing cell line SKBR3 [2] and FcγRI-expressing cell lines [26]. In addition, the bispecific nature of the molecule was verified by a flow cytometric assay that simultaneously detected both ends of the molecule. This assay detected intact bispecific antibodies bound to HER-2/neu expressed on SKBR3 cells and a soluble FcγRI fused to the heavy chain of human IgM.

MDX-H210 was supplied as a clear, colorless solution at a concentration of 1 mg/ml in sterile phosphate-buffered saline for intravenous injection (Medarex, Annandale, N.J.). MDX-H210 was diluted in sterile saline to a concentration of 0.1 μg/ml prior to intravenous administration. Recombinant IFN-γ was purchased from Genetech Corporation (San Francisco, Calif.) and was supplied as a clear, colorless solution for subcutaneous administration containing 100 μg IFN gamma-1b (3 MU) per 0.5-ml vial.

Evaluation of toxicity

Toxicity was assessed by clinical examination and medical laboratory testing. This was performed on an ongoing basis throughout the duration of therapy and for at least 4 weeks afterwards. The Cancer and Leukemia Group B (CALGB) modified National Institute of Health Common Toxicity Criteria were used to grade the observed adverse events.

Blood sampling schedule for MDX-H210 pharmacokinetics

Venous blood (2 ml) was collected into heparinized tubes before and at specified time-points up to 48 h after infusion of MDX-H210 on days 1 and 17. Specifically, blood samples were obtained at preinfusion, and at 2 (end of infusion), 3, 4, 6, 8, 24 and 48 h (and 12 and 16 h if the patient was an inpatient) after initiating the infusion. On day 19 (the last day of MDX-H210 therapy) blood samples were only obtained in the majority of patients preinfusion (48 h after day 17 therapy), at the end of the MDX-H210 infusion (2 h after initiating the infusion) and only up to either 4 or 6 h after initiation of the infusion and assayed for plasma MDX-H210 concentration.

Measurement of plasma MDX-H210 concentrations

Plasma was separated from blood samples by centrifugation at 1500 *g* for 10 min and stored at –70°C until determination of plasma MDX-H210 concentrations. Microtiter plates coated with goat anti-murine IgG probe were incubated with dilutions of patient plasma or MDX-H210 prepared in normal human plasma (Nabi, Boca Raton, Fl.). The captured BsAb was detected by ELISA using an alkaline phosphatase-conjugated goat anti-murine IgG Fab′ probe. The means of duplicate sample measurements were used to determine MDX-H210 plasma concentrations. These were read in the linear part of the standard curve (0.05–1.0 μg/ml) or diluted until they fell within this range. Duplicate samples that varied by >15% were remeasured. The limit of quantitation of the assay was 0.05 μg/ml. The interday assay coefficient of variation over the linear part of the standard curve, concentration range 0.05–1.0 μg/ml, fell within the range 4.6–11.7% and the intraday assay coefficient in the range 5.4–9.7%.

Pharmacokinetic data analysis

The plasma MDX-H210 concentration-time curves were plotted on a semilogarithmic basis and inspected. These data were then analyzed using the WinNonlin pharmacokinetic program (Pharsight Corporation, Mountain View, Calif.). The analysis performed was that of a constant intravenous infusion open noncompartmental model (WinNonlin model 202) without weighting. For any individual patient dataset, the elimination rate constant (β) was estimated by linear regression of a minimum of four and up to eight of the terminal plasma MDX-H210 concentration-time data points. The maximum plasma concentration (C_{max}) and the time of the maximum plasma concentration (T_{max}) are reported as the observed values. The area under the concentration-time curve from time zero to infinity [$AUC_{(0-\infty)}$] was estimated using the log-linear trapezoidal rule and the terminal part of the curve extrapolated to infinity using the Wagner-Nelson correction, i.e. C_{last}/β. The total body clearance (Cl) was estimated from dose/$AUC_{(0-\infty)}$. The mean residence time (MRT) was estimated from the equation:

$$\left(\left[AUMC_{infinity}/AUC_{(0-\infty)}\right] - \left[T_{infusion}/2\right]\right)$$

where $AUMC_{infinity}$ is the area under the first moment of the curve (integral of dC_t/dt) extrapolated to infinity and $T_{infusion}$ is the duration of the intravenous infusion. The apparent volume of distribution at steady state (Vd_{ss}) was estimated from MRT×Cl.

The accumulation ratio (R) for MDX-H210 was estimated from the equation:

$$\frac{\text{Day17}AUC_{(0-\tau)}}{\text{Day1}AUC_{(0-\infty)}}$$

where $AUC_{(0-\tau)}$ represents the area under the plasma concentration curve from time zero to the end of the dosing interval (τ).

Statistical methods

Differences between pharmacokinetic parameters with different doses were initially investigated using ANOVA followed by Bonferroni corrected *t*-test for multiple comparisons. Differences over the duration of the study were investigated where appropriate with a one-way repeated measures analysis of variance (MANOVA) or a Student's paired *t*-test if only two datasets were compared. Two-sided *P*-values <0.05 were taken as indicative of statistical significance.

Results

Patient characteristics

A total of 24 patients with advanced solid tumors overexpressing HER-2/neu were registered for the study. Patient no. 8, who had colon cancer, had rapid clinical progression of the cancer and was registered for entry into the study, but did not receive treatment on this study. Thus the 23 patients treated ranged in age from 25 to 72 years, and their additional demographic data are shown in Table 1.

Table 1. Demographics for all patients treated in the study. One patient was registered for the study but never received treatment, thus only 23 patients were treated in this study

Number of patients	23 (19F, 4M)
Age (years)	
Median	51.5
Range	25–72
Tumor types	
Breast	19
Prostate	3
Lung	1
Karnofsky performance status	
Median	80
Range	70–90
Prior treatment	
Surgery	18
Radiotherapy	12
More than two chemotherapy regimens	6

MDX-H210 pharmacokinetic data

The pharmacokinetic data reported here for all patients treated relates only to their first cycle of therapy. The mean log plasma MDX-H210 concentration-time profiles for the different dose cohorts on day 1 or day 17 of therapy are shown in Figs. 1 and 2. The log plasma MDX-H210 concentration over time plots for the majority of patients on both days 1 and 17 showed a monoexponential decay. The detailed MDX-H210 pharmacokinetic data derived from these plots, using an open model noncompartmental analysis are shown in Table 2 for day 1 and Table 3 for day 17. The MDX-H210 plasma concentration-time data on day 19 were insufficient to allow accurate estimation of either the terminal elimination decay of plasma MDX-H210 concentrations or the AUC and thus pharmacokinetic parameters beyond the C_{max} and T_{max} could not be estimated for day 19 of treatment.

The MDX-H210 T_{max} occurred at the end of or shortly after the end of the intravenous infusion on all treatment occasions. The MDX-H210 C_{max} and $AUC_{(0-\infty)}$ increased with dose on days 1 and 17 (all ANOVA $P < 0.00001$). The data plots of MDX-H210 C_{max} and $AUC_{(0-\infty)}$ versus dose on days 1 and 17 best fitted a nonlinear regression model (see Figs. 3 and 4). The relationship between dose and MDX-H210 pharmacokinetics was studied by a regression analysis of MDX-H210 dose tier and the MDX-H210 terminal elimination half-life ($T_{1/2}$), Cl and apparent Vd_{ss}. MDX-H210 $T_{1/2}$ increased with dose (day 1 ANOVA $P < 0.0001$, day 17 ANOVA $P = 0.001$). Cl decreased with dose on day 1 ($P = 0.006$) and showed a trend toward decreased clearance on day 17 ($P = 0.07$). There was also a marginally significant reduction in Vd_{ss} with MDX-H210 dose on day 1 (ANOVA $P = 0.021$) and on day 17 ($P = 0.06$).

A potential effect of time on the pharmacokinetics of MDX-H210 over the duration of the study was investigated by comparing the pharmacokinetic parameters on day 1 and day 17. These analyses revealed a borderline significant difference in MDX-H210 $T_{1/2}$ (Bonferroni corrected *t*-test two-tailed $P = 0.04$), but no significant differences in C_{max} ($P = 0.8$), AUC ($P = 0.75$), Cl ($P > 0.9$) or Vd_{ss} ($P = 0.7$) between day 1 and day 17. These results were complemented by a one-way MANOVA of MDX-H210 C_{max} on days 1, 17 and 19 which revealed no significant change in C_{max} over these days ($P = 0.44$, $n = 18$ datasets). The accumulation index (or accumulation ratio, R), the ratio of MDX-H210 AUC on day 17 and to that on day 1, had a mean value of 1.26 (median 1.23, range 0.37–2.17, $n = 17$ paired datasets).

Clinical toxicity – nonhematological and hematological

This aspect of the study has been reported in greater detail elsewhere [22]. In summary, the treatment regimen was well tolerated with predominantly grade I/II nonhematological toxicity. This consisted most frequently of patients who developed nausea and/or fevers and chills after infusion on day 1, and was relieved with antipyretic agents and/or minimized by premedication. On treatment days subsequent to day 1, these symptoms were considerably less marked. Mild fatigue (grade I) was noted during the last week of therapy. Hematological toxicity was tolerable and transient: eight patients developed grade III neutropenia, one patient developed grade IV neutropenia, and one patient developed grade III thrombocytopenia. Transient monocytopenia occurred in all patients, developed rapidly, was maximal 2–4 h after starting MDX-H210 therapy and had resolved by 8 h. This time-course was also noted for the neutropenia.

Discussion

This report focuses on the MDX-H210 pharmacokinetic parameters estimated in this phase I study, which combined IFN-γ with MDX-H210 in 23 patients with advanced cancer that overexpressed HER-2/neu. Over the dose range studied (1.0–70.0 mg/m^2) there was a dose-dependent nonlinear increase in MDX-H210 C_{max} and AUC (Figs. 3 and 4) with a concordant increase in

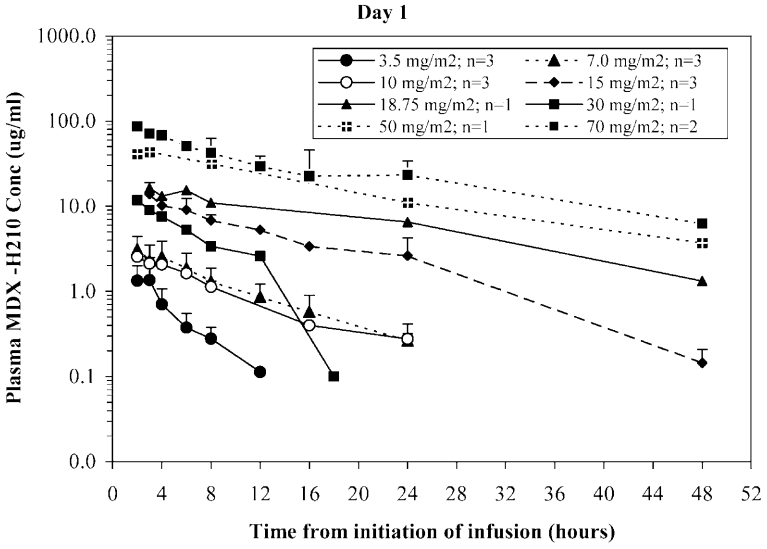

Fig. 1. Log plasma MDX-H210 concentration versus time data (mean + SD) for all dose cohorts studied on day 1 of therapy. The number of patients in each cohort is indicated in the insert. The lowest dose cohort (MDX-H210 1.0 mg/m^2, $n = 6$) is not shown as there were insufficient measurable plasma MDX-H210 concentration data points to estimate standard pharmacokinetic parameters in these patients

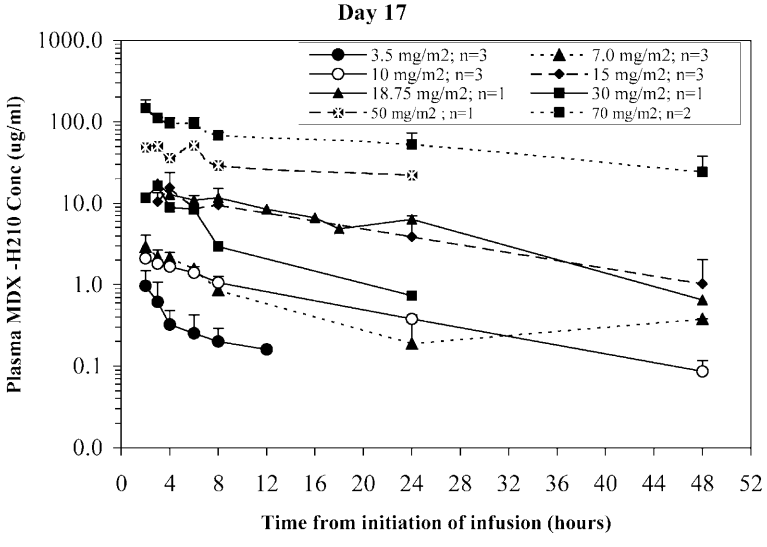

Fig. 2. Log plasma MDX-H210 concentration versus time data (mean + SD) for all dose cohorts studied on day 17 of therapy. The number of patients in each cohort is indicated in the insert. The lowest dose cohort (MDX-H210 1.0 mg/m^2, $n = 6$) is not shown as there were insufficient measurable plasma MDX-H210 concentration data points to estimate standard pharmacokinetic parameters in these patients

elimination $T_{1/2}$ and decrease in Cl with dose (see Tables 2 and 3). These data suggest that the MDX-H210 clearance mechanisms are saturable. The MDX-H210 apparent Vd_{ss} was, in the majority of patients, equal to or less than the intravascular volume and dose dependent. Comparison of day-1 and day-17 MDX-H210 AUCs revealed slight accumulation of MDX-H210, particularly in the two highest dose cohorts in which the accumulation ratio was 1.30 (50 mg/m^2, $n = 1$) and 1.71–1.93 (70 mg/m^2, $n = 2$).

Schwaab et al. [34] have reported a phase I study of single-agent multiple-dose MDX-H210 in seven prostate cancer patients who received doses of MDX-H210 ranging from 1.0 to 8.0 mg/m^2 three times a week for two consecutive weeks. Adequate plasma MDX-H210 concentration versus time data to permit pharmacokinetic parameter estimation was available for only four of these patients. When comparing similar MDX-H210 doses, the reported C_{max} (range 0.3–7.8 μg/ml) and elimination half-life (range 1.2–10.2 h) are similar to the data from this study (C_{max} 0.27–3.11 μg/ml, $T_{1/2}$ 2.9–7.8 h). Further comparison of the pharmacokinetic data between the study presented here and that of Schwaab et al. [34] reveals considerable overlap in total body clearance (range 0.02–0.89 vs 0.13–0.34 ml/min per kg) and the apparent volume of distribution. Due to the small number of patients with pharmacokinetic data, Schwaab et al. [34] did not investigate the relationships between C_{max} or AUC and dose. Therefore, the MDX-H210 pharmacokinetic data defined in our study over the dose range 1–8 mg/m^2 are similar to those reported by Schwaab et al. [34]. This suggests that IFN-γ did not markedly alter the pharmacokinetic profile of MDX-H210 at doses between 1.0 and 8.0 mg/m^2.

In a multidose phase I study in patients with stage IV breast cancer MDX-H210 was combined with G-CSF [30]. In this study patients received weekly MDX-H210 (dose range 1.0–40.0 mg/m^2) for 3 weeks followed by a 2-week dose-free interval and then three more weekly doses of MDX-H210. G-CSF was administered for

Table 2. Day 1 MDX-H210 pharmacokinetic parameters (mean and SD) for MDX-H210 summarized for each dose tier ranging from 1.0 to 70.0 mg/m^2. T_{max} is summarized as the median value where adequate data permitted

Dose (mg/m^2)		$T_{1/2}$ (h)	C_{max} (μg/ml)	T_{max} (h)	$AUC_{(0-\infty)}$ (μg·h/ml)	MRT (h)	Vd_{ss} (ml/kg)	Cl (ml/min/kg)
1.0 ($n=6$)[a]	Mean	–	0.30	2.0[b]	–	–	–	–
	SD	–	0.22		–	–	–	–
3.5 ($n=3$)	Mean	2.9	1.78	3.0[b]	6.4	4.0	55.0	0.23
	SD	1.2	0.31		1.7	0.5	14.4	0.06
7.0 ($n=3$)	Mean	5.9	3.11	2.0[b]	28.2	7.9	64.2	0.13
	SD	0.1	1.33		13.3	1.1	20.7	0.04
10.0 ($n=3$)	Mean	6.3	2.56	2.0[b]	25.6	8.5	81.5	0.16
	SD	0.4	0.06		3.2	0.5	8.1	0.02
15.0 ($n=3$)	Mean	6.9	13.87	3.0[b]	210.7	11.5	26.6	0.11
	SD	0.7	5.14		128.9	3.1	10.0	0.04
18.75 ($n=1$)		10.4	17.38	2.0	266.5	13.9	27.2	0.03
30.0 ($n=1$)		6.5	11.77	2.0	93.4	6.0	47.7	0.11
50.0 ($n=1$)		12.5	43.45	3.0	852.6	16.4	18.2	0.02
70.0 ($n=2$)	Mean	14.7	86.91	2.1[b]	1402.2	18.7	23.9	0.02
	SD	3.2	6.46		483.0	2.5	5.4	0.002

[a]Six patients were treated in the 1.0 mg/m^2 dose tier, only four of whom had measurable plasma MDX-H210 concentrations. The majority of plasma MDX-H210 concentration versus time data points for the patients in this dose cohort were below the level of detection of the MDX-H210 assay and therefore there were insufficient data to estimate the standard pharmacokinetic parameters

[b]Median value

Table 3. Day 17 MDX-H210 pharmacokinetic parameters (mean and SD) for MDX-H210 summarized for each dose tier ranging from 1.0 to 70.0 mg/m^2. T_{max} is summarized as the median value where adequate data permitted

Dose (mg/m^2)		$T_{1/2}$ (h)	C_{max} (μg/ml)	T_{max} (h)	$AUC_{(0-\infty)}$ (μg·h/ml)	MRT (h)	Vd_{ss} (ml/kg)	Cl (ml/min/kg)
1.0 ($n=6$)[a]	Mean	–	0.27	3.0[b]	–	–	–	–
	SD	–	0.30		–	–	–	–
3.5 ($n=3$)	Mean	7.8	0.97	2.0[b]	5.6	8.9	178.9	0.34
	SD	5.6	0.51		3.2	4.5	114.0	0.23
7.0 ($n=3$)	Mean	5.7	2.94	2.0[b]	25.6	8.7	68.4	0.13
	SD	2.7	1.07		7.0	5.7	39.2	0.05
10.0 ($n=3$)	Mean	10.0	2.10	2.0[b]	29.1	13.0	106.6	0.14
	SD	0.9	0.21		3.1	1.4	11.1	0.01
15.0 ($n=3$)	Mean	11.4	15.60	4.0[b]	258.4	15.3	31.6	0.04
	SD	3.5	8.19		173.9	4.5	17.2	0.03
18.75 ($n=1$)		13.0	16.33	3.0	352.3	17.9	26.5	0.02
30.0 ($n=1$)		5.1	16.30	3.0	101.8	6.2	46.1	0.12
50.0 ($n=1$)		19.8	51.42	6.0	1355.0	28.9	20.1	0.01
70.0 ($n=2$)	Mean	21.9	147.85	2.0[b]	3459.7	30.2	16.0	0.01
	SD	7.7	40.23		1198.8	12.1	3.6	0.002

[a]Six patients were treated in the 1.0 mg/m^2 dose tier, only three of whom had measurable plasma MDX-H210 concentrations. The majority of plasma MDX-H210 concentration versus time data points for the patients in this dose cohort were below the level of detection of the MDX-H210 assay and therefore there were insufficient data to estimate the standard pharmacokinetic parameters

[b]Median value

either 3 or 5 days following MDX-H210 administration on days 8, 15, 43 and 50. No G-CSF was administered on day 1 or day 36. The pharmacokinetic data for MDX-H210 showed that the terminal elimination half-life for day 1 of treatment ranged from 4.4 to 9.3 h, compared with 2.9 to 13.0 h observed in our study. Over the dose range 1.0–20.0 mg/m^2, the reported MDX-H210 C_{max} on day 1 was 0.06–13.46 μg/ml, which is similar to the day-1 C_{max} range in our study (0.3–17.38 μg/ml).

The log MDX-H210 AUC versus dose data reported by Pullarkat et al. [31] suggest a dose-dependent non-linear increase in AUC, consistent with the observations in our study. Interestingly, both our data and those of Pullarkat et al. reveal decreasing MDX-H210 total body clearance with increasing dose and a prolongation of $T_{1/2}$ with dose, compatible with saturable clearance mechanisms. Furthermore, the estimated apparent Vd_{ss} in both studies suggests that MDX-H210 was mainly distributed in the intravascular space. In vivo, the MDX-H210 distribution/trafficking mechanisms are hypothesized to result from the binding of the bispecific antibody to FcγRI receptors on monocytes/macrophages and neutrophils with subsequent movement of these BsAb-bound cells out of the central vascular compartment. This mechanism may also represent a

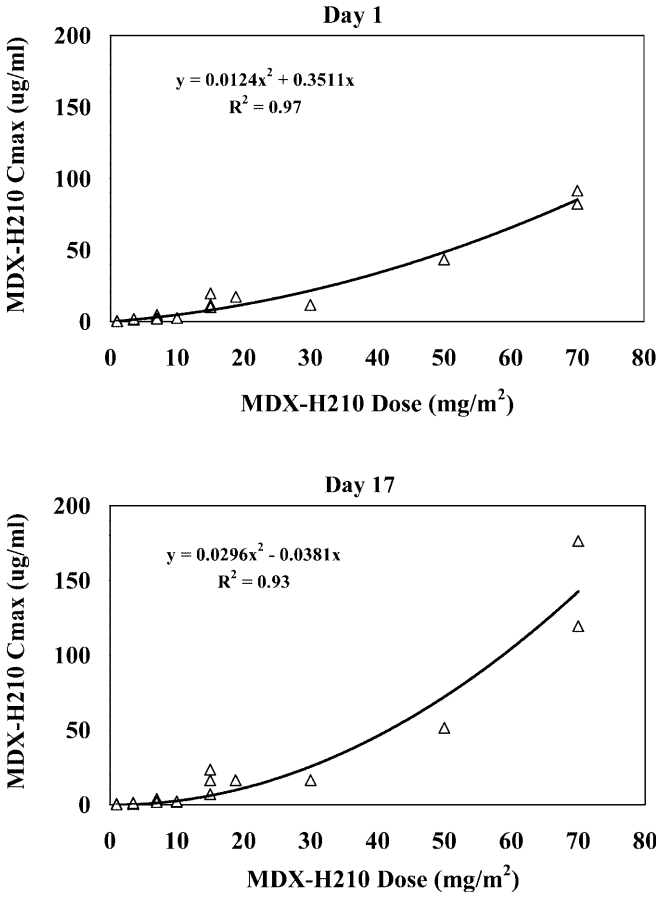

Fig. 3. Relationship between the MDX-H210 C_{max} and dose (mg/m^2) on days 1 and 17. The data best fitted a nonlinear regression model, indicated by the trend lines. The equations for the lines are second-order polynomial

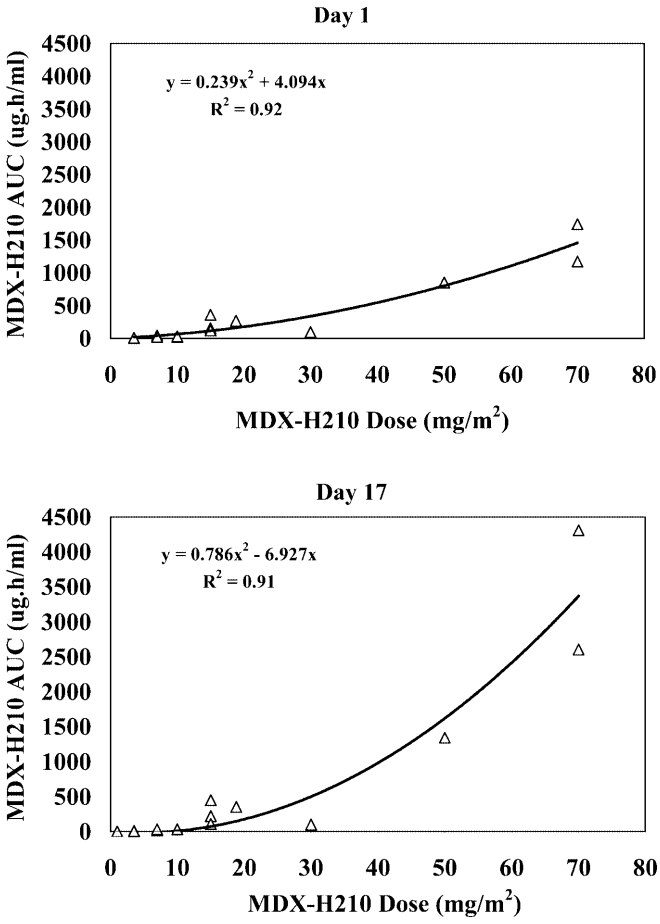

Fig. 4. Relationship between the MDX-H210 AUC and dose (mg/m^2) on days 1 and 17. The data best fitted a nonlinear regression model, indicated by the trend lines. The equations for the lines are second-order polynomial

considerable portion of MDX-H210 pharmacokinetic clearance.

Saturation of monocyte/macrophage/neutrophil and/or tissue binding sites may, at least in part, explain the decrease in Vd_{ss} with increasing dose noted in our study. Pullarkat et al. [31] did not report MDX-H210 Vd_{ss}, nor did they observe any alteration in MDX-H210 AUC with time and stated that the mean MDX-H210 AUC for a given dose cohort did not change with G-CSF administration. The data from these two studies suggest that when MDX-H210 is combined with either G-CSF or IFN-γ, the pharmacokinetics of this bispecific antibody are similar.

Detailed information on the pharmacokinetics of MDX-H210 is sparse. Therefore, it is worthwhile and pertinent to compare the MDX-H210 pharmacokinetics with the pharmacokinetic data of a related mAb, trastuzumab. Trastuzumab (Herceptin) is an intact, humanized IgG_1 mAb directed against HER-2/neu. It does not have specificity for the FcγRI receptor. Baselga et al. [3] have reported pharmacokinetic data on trastuzumab from a phase II clinical trial of weekly intravenous administration of trastuzumab (250 mg loading dose, followed by 100 mg weekly for 10 weeks) to patients with breast cancer overexpressing HER-2/neu. Dose-dependent nonlinear pharmacokinetics were observed (n = 45) with a mean (± SD) terminal elimination half-life of 8.3 ± 5 days. Similar results have also been observed in a phase I dose-escalation study of weekly trastuzumab therapy in patients with metastatic breast cancer overexpressing HER-2/neu [39]. Trastuzumab pharmacokinetic data showed a dose-dependent nonlinear increase in AUC and a decrease in total clearance with dose (range 1–8 mg/kg). The mean trastuzumab terminal elimination half-life increased from 2.7 days in the 1-mg/kg dose cohort to 10.4 days in the 8-mg/kg dose cohort. Dose-dependent nonlinear pharmacokinetics suggest that the clearance mechanisms for trastuzumab are saturable, a finding that is common to trastuzumab and MDX-H210. The mean apparent Vd_{ss} of trastuzumab was 52–70 ml/kg, similar to that noted for MDX-H210 in our study. The in vivo $T_{1/2}$ of MDX-H210, however, is much shorter than that of trastuzumab (hours vs days) suggesting different clearance mechanisms.

One potential explanation for the longer half-life of IgG analogs, including trastuzumab, may be related to

cellular processing of IgG analogs. FcRn, a MHC class I receptor, is expressed by many cells including human endothelial and epithelial cells. FcRn recognizes and binds the Fc region of human IgG_1 at low pH and releases it at neutral pH [11, 12]. When IgG antibodies are engulfed by a cell, they are captured in an acidic endosome by FcRn and transported back to the cell surface where they are released into the plasma. No such recycling pathways exist for bispecific antibodies such as MDX-H210, although their precise clearance mechanisms have yet to be fully elucidated in humans.

Interestingly, from our data, the inflection point in the nonlinear relationship between MDX-H210 dose and AUC occurred at relatively low MDX-H210 doses (i.e. 7.0 mg/m^2) and the nonlinear relationship became more pronounced at doses above this. One potential, though unproven, explanation for the nonlinear pharmacokinetics of MDX-H210 could be saturation of the FcγRI binding sites on the different pools of immunoeffector cells (i.e. monocytes, macrophages, IFN-γ-stimulated neutrophils). Data supporting this hypothesis are that saturation of monocyte binding of MDX-H210 occurs in vitro at concentrations >0.1 μg/ml [23] and data from our study show that saturation of binding to monocytes occurs at a C_{max} of approximately 2 μg/ml and at 10 μg/ml for neutrophils [25].

The data from our study permitted comparison of the MDX-H210 pharmacokinetic parameters over time between day 1 and day 17 of therapy. The one statistically significant difference suggesting a time effect was the observed change in $T_{1/2}$, which was greater on day 17 (Bonferroni corrected *t*-test $P=0.04$). Analysis of MDX-H210 C_{max}, AUC, total body clearance and apparent Vd_{ss} for the patients with adequate data on both days ($n=17$) did not show a significant difference between day 1 and day 17. Furthermore, our data did not show a significant difference between day 1, day 17 and day 19 C_{max} (MANOVA $P=0.44$).

Taken in their entirety these data suggest no change in MDX-H210 pharmacokinetics over time, which is consistent with the data from other phase I studies with this bispecific antibody and its murine (nonhumanized) predecessor MDX-210 [41, 42]. The borderline significant change in $T_{1/2}$ between day 1 and day 17 most likely represents a type 1 statistical error, as importantly there was no significant change in MDX-H210 clearance or Vd_{ss}, one or the other of which must change if there is a real change in $T_{1/2}$ because of their strong interrelationship.

In conclusion, the MDX-H210 pharmacokinetic data reported here show that over the dose range investigated (1.0–70.0 mg/m^2), MDX-H210 showed dose-dependent nonlinear pharmacokinetics that did not show time dependency. The MDX-H210 pharmacokinetic profile supports a dose of 15 mg/m^2 of MDX-H210 given regularly (up to three times per week) with IFN-γ as a dose that did not accumulate excessively and was well tolerated clinically. In addition, this dose maintained MDX-H210 trough plasma concentrations above 0.1 μg/ml, which is likely to produce maximal saturation of FcγRI binding sites on immunoeffector cells. The previously reported pharmacokinetic-pharmacodynamic analysis of the data from this study [25] also supports the further clinical development of the 15-mg/m^2 dose of MDX-H210 in combination with cytokines from a perspective of maximum saturation of binding to FcγRI on immunoeffector cells in the vascular (central) compartment. This recommended phase II dose of MDX-H210 is neither based solely on a maximum tolerated dose, as is classically the case for anticancer agents, nor on its pharmacokinetic profile, but on a combination of the pharmacokinetic and pharmacodynamic data. Encouraging data from a phase II study combining MDX-H210 (15 mg/m^2) with granulocyte macrophage colony-stimulating factor in prostate cancer patients has already been reported [20] and other phase II studies of this combination in colorectal and renal cell cancer are soon to be completed.

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References

1. Allred DC, Clark GM, Tandon AK, Molina R, Tormey DC, Osborne CK, Gilchrist KW, Mansour EG, Abeloff M, Eudey L, McGuire WL (1992) HER-2/neu in node-negative breast cancer: prognostic significance of overexpression influenced by the presence of in situ carcinoma. J Clin Oncol 10:599
2. Backman KA, Guyre PM (1994) Gamma-interferon inhibits Fc receptor II-mediated phagocytosis of tumor cells by human macrophages. Cancer Res 54:2456
3. Baselga J, Tripathy D, Mendelsohn J, Baughman S, Benz CC, Dantis L, SklarinNT, Seidman AD, Hudis CA, Moore J, Rosen PP, Twaddell T, Henderson IC, Norton L (1996) Phase II study of weekly intravenous recombinant humanized anti-p185^{HER2} monoclonal antibody in patients with HER-2/neu-overexpressing metastatic breast cancer. J Clin Oncol 14:737
4. Corbett IP, Henry JA, Angus B, Watchorn CJ, Wilkinson L, Hennessy C, Gullick WJ, Tuzi NL, May FE, Westley BR, Horne CH (1990) NCL-CB11, a new monoclonal antibody recognizing the internal domain of the c-erbB-2 oncogene protein effective for use on formalin-fixed, paraffin-embedded tissue. J Pathol 161:15
5. Curnow RT (1997) Clinical experience with CD64-directed immunotherapy: an overview. Cancer Immunol Immunother 45:210
6. Fanger MW, Guyre PM (1991) Bispecific antibodies for targeted cellular cytotoxicity. Trends Biotechnol 9:375
7. Fanger MW, Graziano RF, Shen L, Guyre PM (1989) Fc gamma R in cytotoxicity exerted by mononuclear cells. Chem Immunol 47:214

8. Fanger MW, Segal DM, Romet-Lemonne JL (1991) Bispecific antibodies and targeted cellular cytotoxicity. Immunol Today 12:51
9. Fanger MW, Graziano RF, Guyre PM (1994) Production and use of anti-FcR bispecific antibodies. Immunomethods 4:72
10. Gericke GH, Ericson SG, Pan L, Mills LE, Guyre PM, Ely P (1995) Mature polymorphonuclear leukocytes express high-affinity receptors for IgG (FcγRI) after stimulation with granulocyte colony stimulating factor (G-CSF). J Leukoc Biol 57:455
11. Ghetie V, Ward ES (1997) FcRn: the MHC class I-related receptor that is more than an IgG transporter. Immunol Today 18:592
12. Ghetie V, Ward ES (2000) Multiple roles for the major histocompatibility complex class I-related receptor FcRn. Annu Rev Immunol 18:739
13. Glennie MJ, McBride HM, Worth AT, Stevenson GT (1987) Preparation and performance of bispecific F(ab′γ)2 antibody containing thioether-linked Fab′γ fragments. J Immunol 139:2367
14. Gusterson BA, Gelber RD, Goldhirsch A, Price KN, Säve-Söderborgh J, Anbazhagan R, Styles J, Rudenstam CM, Golouh R, Reed R, Martinez-Tello F, Tiltman A, Torhorst J, Grigolato P, Bettelheim R, Neville AM, Bürki K, Castiglione M, Collins J, Lindtner J, Senn HJ (1992) Prognostic importance of c-erbB-2 expression in breast cancer. J Clin Oncol 10:1049
15. Guyre PM, Graziano RF, Vance BA, Morganelli PM, Fanger MW (1989) Monoclonal antibodies that bind to distinct epitopes on Fc gamma RI are able to trigger receptor function. J Immunol 143:1650
16. Guyre PM, Campbell AS, Kniffin WD, Fanger MW (1990) Monocytes and polymorphonuclear neutrophils of patients with streptococcal pharyngitis express increased numbers of type I IgG Fc receptors. J Clin Invest 86:1892
17. Hartmann F, Renner JC, Jung W, Deisting G, Juwana M, Eichentopf B, Kloft M, Pfreundschuh M (1997) Treatment of refractory Hodgkin's disease with an anti-CD16/CD30 bispecific antibody. Blood 89:2042
18. Heijnen IA, Rijks LJ, Schiel A, Stockmeyer B, van Ojik, HH, Dechant M, Valerius T, Keler T, Tutt AL, Glennie MJ, van Royen EA, Capel PJ, van de Winkel JG (1997) Generation of HER-2/neu-specific cytotoxic neutrophils in vivo: efficient arming of neutrophils by combined administration of granulocyte colony-stimulating factor and Fcgamma receptor I bispecific antibodies. J Immunol 159:5629
19. Hsieh Ma ST, Eaton AM, Shi T, Ring DB (1992) In vitro cytotoxic targeting by human mononuclear cells and bispecific antibody 2B1, recognizing c-erbB-2 protooncogene product and Fc gamma receptor III. Cancer Res 52:6832
20. James N, Atherton P, Jones J., Howie AJ, Tchekmedyian S, Curnow RT (2001) A phase II study of the bispecific antibody MDX-H210 (anti-HER2 × CD64) with GM-CSF in HER2+ advanced prostate cancer. Br J Cancer 85:152
21. Kaufman PA, Guyre PM, Lewis LD, Valone FH, Memoli V, Wells W, Deo YM, Ernstoff MS, Fisher J, Mrozek-Orlowski M, Phipps K, Fanger MW (1996) HER-2/neu targeted immunotherapy: a pilot study of multi-dose MDX-210 in patients with breast or ovarian cancers that overexpress HER-2/neu and a report of an increased incidence of HER-2/neu overexpression in metastatic breast cancer. Tumor Targeting 2:17
22. Kaufman PA, Guyre PM, Barth RJ, Lewis LD, Memoli V, Curnow RT, Deo Y, Harris R, Valone F, Wallace PK, Wells W, Schned A, Fisher J, Waugh M, Phipps K, Mackay K, Fanger MW, Ernstoff MS (1997) Phase-I trial of interferon gamma (IFNγ) and MDX-210 (anti-HER-2/neu x anti-FcγRI) in patients (pts) with metastatic carcinomas that overexpress HER-2/neu: preliminary findings (abstract 1542). Proc Am Soc Clin Oncol 16:430a
23. Keler T, Graziano RF, Mandal A, Wallace PK, Fisher J, Guyre PM, Fanger MW, Deo YM (1997) Bispecific antibody-dependent cellular cytotoxicity of HER2/neu-overexpressing tumor cells by Fc gamma receptor type I-expressing effector cells. Cancer Res 57:4008
24. Kerst JM, van de Winkel JG, Evans AH, de Haas M, Slaper-Cortenbach IC, de Wit TP, von dem Borne AE, van der Schoot CE, van Oers RH (1993) Granulocyte colony-stimulating factor induces hFc gamma RI (CD64 antigen) positive neutrophils via an effect on myeloid precursor cells. Blood 81:1457
25. Lewis LD, Cole BF, Wallace PK, Fisher JL, Waugh MW, 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-I study in patients with advanced cancer which overexpresses Her-2/neu. J Immunol Methods 248:149
26. Looney RJ, Abraham GN, Anderson CL (1986) Human monocytes and U937 cells bear two distinct Fc receptors for IgG. J Immunol 136:1641
27. Lopez AF, Nicola NA, Burgess AW, Metcalf D, Battye FL, Sewell WE, Vadas M (1983) Activation of granulocyte cytotoxic function by purified mouse colony-stimulating factors. J Immunol 131:2983
28. Matsumoto Y, Saiki I, Murata J, Okuyama H, Tamura M, Azuma I (1991) Recombinant human granulocyte colony-stimulating factor inhibits the metastasis of hematogenous and non-hematogenous tumors in mice. Int J Cancer 49:444
29. Perussia B, Dayton ET, Lazarus R, Fanning V, Trinchieri G (1983) Immune interferon induces the receptor for monomeric IgG1 on human monocytic and myeloid cells. J Exp Med 158:1092
30. Posey JA, Raspet 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
31. Pullarkat V, Deo Y, Link J, Spears L, Marty V, Curnow R, Groshen S, Gee C, Weber JS (1999) A phase I study of a HER-2/neu bispecific antibody with granulocyte-colony-stimulating factor in patients with metastatic breast cancer that overexpresses HER-2/neu. Cancer Immunol Immunother 48:9
32. Ravetch JV, Kinet JP (1991) Fc receptors. Annu Rev Immunol 9:457
33. Repp R, Valerius T, Sendler A, Gramatzki M, Iro H, Kalden JR, Platzer E (1991) Neutrophils express the high affinity receptor for IgG (Fc gamma RI, CD64) after in vivo application of recombinant human granulocyte colony-stimulating factor. Blood 78:885
34. Schwaab T, Lewis LD, Cole BF, Deo Y, Fanger MW, Wallace P, Guyre PM, Kaufman PA, Heaney JA, Schned AR, Harris RD, Ernstoff MS (2001) Phase I pilot trial of the bispecific antibody MDXH210 (anti-FcγRI X anti-HER-2/neu) in patients whose prostate cancer overexpresses HER-2/neu. J Immunother 24:79
35. Shen L, Guyre PM, Anderson CL, Fanger MW (1986) Heteroantibody-mediated cytotoxicity: antibody to the high-affinity Fc receptor for IgG mediates cytotoxicity by human monocytes which is enhanced by interferon-gamma and is not blocked by human IgG. J Immunol 137:3378
36. Shen L, Graziano RF, Fanger MW (1989) The functional properties of Fc gamma RI, II and III on myeloid cells: a comparative study of killing of erythrocytes and tumor cells mediated through the different Fc receptors. Mol Immunol 26:959
37. Slamon 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
38. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A, Press MF (1989) Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 244:707
39. Tokuda Y, Watanabe T, Omuro Y, Ando M, Katsumata N, Okumura A, Ohta M, Fujii H, Sasaki Y, Niwa T, Tajima T (1999) Dose escalation and pharmacokinetic study of a

humanized anti-HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer. Br J Cancer 81:1419
40. Valerius T, Repp R, de Wit TP, Berthold S, Platzer E, Kalden JR, Gramatzki M, van de Winkel JG (1993) Involvement of the high-affinity receptor for IgG (Fc gamma RI; CD64) in enhanced tumor cell cytotoxicity of neutrophils during granulocyte colony-stimulating factor therapy. Blood 82:931
41. Valone FH, Kaufman PA, Guyre PM, Lewis LD, Memoli V, Deo Y, Graziano R, Fisher JL, Meyer L, Mrozek-Orlowski M, Wardwell K, Guyre V, Morley TL, Arvizu 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
42. Valone FH, Kaufman PA, Guyre PM, Lewis LD, Memoli V, Eernstoff MS, Wells W, Barth R, Deo Y, Fisher J, et al (1995) Clinical trials of bispecific antibody MDX-210 in women with advanced breast or ovarian cancer that overexpresses HER-2/neu. J Hematother 4:471
43. Van de Winkel JG, Anderson CL (1991) Biology of human immunoglobulin G Fc receptors. J Leukoc Biol 49:511
44. Van Ojik HH, Repp R, Groenewegen G, Valerius T, van de Winkel JG (1997) Clinical evaluation of the bispecific antibody MDX-H210 (anti-Fc gamma RI × anti-HER-2/neu) in combination with granulocyte-colony-stimulating factor (filgrastim) for treatment of advanced breast cancer. Cancer Immunol Immunother 45:207
45. Van Spriel AB, van Ojik HH, van de Winkel JG (2000) Immunotherapeutic perspective for bispecific antibodies. Immunol Today 21:391
46. Watanabe M, Wallace PK, Keler T, Deo YM, Akewanlop C, Hayes DF (1999) Antibody dependent cellular phagocytosis (ADCP) and antibody dependent cellular cytotoxicity (ADCC) of breast cancer cells mediated by bispecific antibody, MDX-210. Breast Cancer Res Treat 53:199
47. Woodburn J (1999) The epidermal growth factor receptor and its inhibition in cancer therapy. Pharmacol Ther 82:241