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## Clinical experience with CD64-directed immunotherapy. An overview

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**Abstract** The class I IgG receptor (Fc $\gamma$ RI or CD64 receptor), which is present on key cytotoxic effector cells, has been shown to initiate the destruction of tumor cells in vitro and has been hypothesized to play a role in the destruction of antibody-coated cells such as platelets in idiopathic thrombocytopenia purpura (ITP). This overview summarizes the clinical experience with CD64-directed immunotherapy in cancer patients with the bispecific antibodies MDX-447 [humanized Fab anti-CD64  $\times$  humanized Fab anti-(epidermal growth factor receptor, EGFR)] and MDX-H210 (humanized Fab anti-DC64  $\times$  Fab anti-HER2/neu), and with the anti-CD64 monoclonal antibody (mAb) MDX-33 (H22) in the modulation of monocyte CD64 in vivo. In an ongoing phase I/II open-label trial with progressive dose escalation (1–15 mg/m<sup>2</sup>), patients with treatment refractory EGFR-positive cancers (renal cell carcinoma (RCC), head and neck, bladder, ovarian, prostate cancer and skin cancer) are treated weekly with intravenous MDX-447, with and without granulocyte-colony-stimulating factor (G-CSF). MDX-447 has been found to be immunologically active at all doses, binding to circulating monocytes and neutrophils (when given with G-CSF), causing monocytopenia and stimulating increases in circulating plasma cytokines. MDX-447 is well tolerated, the primary toxicities being fever, chills, blood pressure lability, and pain/myalgias. Of 36 patients evaluable for response, 9 have experienced stable disease of 3–6 month's duration. The optimal dose and the maximal tolerated dose (MTD) have yet to be defined; dose escalation continues to define better the dose, toxicity, and the potential therapeutic role of this bispecific antibody. Three MDX-H210 phase II trials are currently in progress, all using the intravenous dose of 15 mg/m<sup>2</sup> given with granulocyte/macrophage (GM-CSF). These consist of one trial each in the treatment of RCC

patients, patients with prostate cancer, and colorectal cancer patients, all of whom have failed standard therapy. At the time of writing, 11 patients have been treated in these phase II trials. Four patients have demonstrated antitumor effects. Patients demonstrating responses include 2 with RCC and 2 with prostate cancer. One RCC patient has had a 54% reduction in size of a hepatic metastatic lesion and the other has had a 49% decrease in the size of a lung metastasis with simultaneous clearing of other non-measurable lung lesions. Regarding the two patients with prostate cancer, one has had a 90% reduction in serum prostate-specific antigen (PSA; 118–11 ng/ml), which has persisted for several months; the other patient with prostate has had a 70% reduction of serum PSA (872 ng/ml to 208 ng/ml) within the first month of treatment. Both patients have also demonstrated symptomatic improvement. In a completed phase I and in ongoing phase I/II clinical trials, patients with treatment-refractory HER2/neu positive cancers (breast, ovarian, colorectal, prostate) have been treated with MDX-H210, which has been given alone and in conjunction with G-CSF, GM-CSF, and interferon  $\gamma$  (IFN $\gamma$ ). These trials have been open-label, progressive dose-escalation (0.35–135 mg/m<sup>2</sup>) studies in which single, and more often, multiple weekly doses have been administered. MDX-H210 has been well tolerated, with untoward effects being primarily mild-to-moderate flu-like symptoms. The MTD has not yet been defined. MDX-H210 is immunologically active, binding to circulating monocytes, causing monocytopenia, as well as stimulating increases in plasma cytokine levels. Furthermore, some patients have evidence of active antitumor immunity following treatment with MDX-210. Antitumor effects have been seen in response to MDX-H210 administration; these include 1 partial, 2 minor, and 1 mixed tumor response; 15 protocol-defined stable disease responses have occurred. In a completed phase I trial, MDX-33 was administered as a single intravenous dose to 17 normal subjects in order to assess its potential as an immunomodulator for the treatment of idiopathic thrombocytopenia purpura and other immune disorders. Doses of 1.5, 3.0, 5.0, and 7.5 mg/m<sup>2</sup> were administered. The variables evaluated in response to

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MDX-33 were circulating monocyte and neutrophil counts, monocyte CD64-mediated phagocytosis, monocyte CD64 modulation, MDX-33 pharmacokinetics, and various safety parameters. MDX-33 is well tolerated at doses of 5.0 mg/m<sup>2</sup> or less, the primary toxicities being chills, low-grade fever, headache, and muscle aches. Persistent binding of MDX-33 to 80–99 % of circulating monocytes is seen for at least 6 days; down-modulation of monocyte CD64 occurs and also lasts more than 6 days. Monocyte CD64-mediated phagocytosis is significantly inhibited at all doses of MDX-33. At the 3.0 mg/m<sup>2</sup> and 5.0-mg/m<sup>2</sup> dose, phagocytosis is fully inhibited for at least 6 days, returning to baseline levels by 20 days after dosing. These results clearly demonstrate that immunomodulation of monocyte CD64 by the mAb MDX-33 can be accomplished with minimal clinical toxicity, and further indicate the potential of MDX-33 in the treatment of ITP and other auto-immune disorders. In conclusion, the results from completed and ongoing clinical trials with the CD64-directed bsAb MDX-447 and MDX-H210 demonstrate excellent tolerability in association with promising antitumor effects in tumors that have become refractory to all available therapies. Also promising are the results from the trial of the CD64-directed mAb, MDX-33, which show the ability to modulate monocyte CD64 in the clinical setting. Studies are currently being conducted to elucidate the full potential of these and other approaches using CD64-directed immunotherapy.

**Key words** CD64 · MDX-H210 · MDX-447 · MDX-33 · ITP · Cancer

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## Introduction

The class I IgG receptor (FcγRI or CD64 receptor), which is present on key cytotoxic effector cells, has been shown to initiate the destruction of tumor cells [6] *in vitro* and has been hypothesized to play a role in the destruction of antibody-coated cells such as platelets in idiopathic thrombocytopenia purpura (ITP) [4]. In addition, bispecific antibodies (bsAb) targeting CD64 on cytotoxic effector cells to tumor antigens on malignant cells effectively promote lysis of tumor cells *in vitro* [5]. This overview summarizes the published and selected unpublished clinical experience with CD64-directed immunotherapy in cancer patients with the bispecific antibodies MDX-447 [humanized Fab anti-CD64 × humanized Fab anti-(epidermal growth factor receptor, EGFR)] and MDX-H210 (humanized Fab anti-CD64 × Fab anti-HER2/neu), and with the anti-CD64 mAb MDX-33 in the modulation of monocyte CD64 *in vivo*.

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## MDX-447 Clinical Experience

MDX-447 is a BsAb constructed by cross-linking F(ab') fragments of mAb H22 to CD64 and mAb H425 to the

epidermal growth factor receptor (EGFR). *In vitro*, MDX-447 effects lysis of EGFR-overexpressing cell lines [3]; CD64-positive neutrophils constitute a major effector cell population during granulocyte-colony-stimulating factor (G-CSF) therapy.

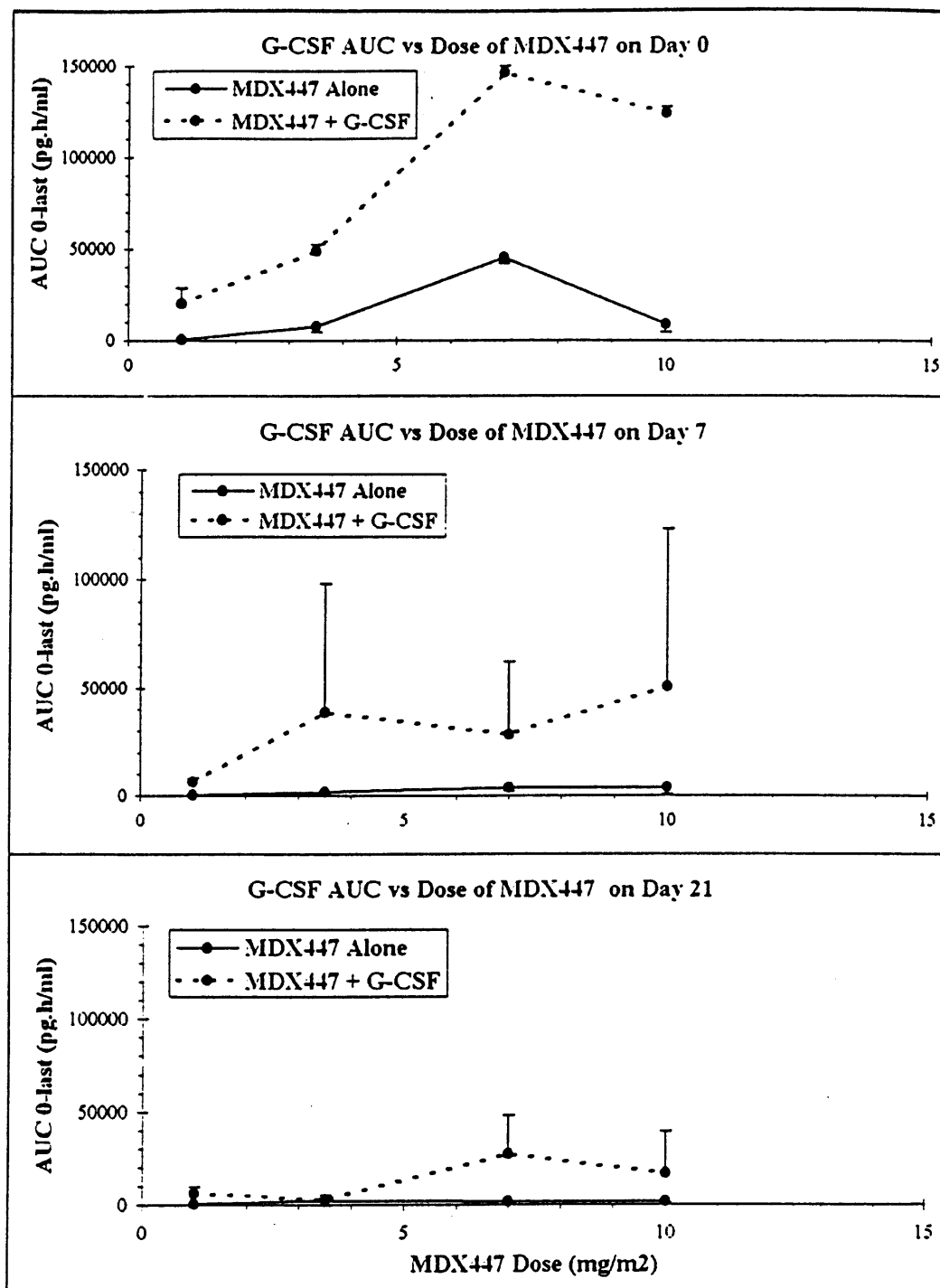
Intravenously administered MDX-447 is currently being evaluated, with and without the concurrent administration of G-CSF, in a phase I/II clinical trial at Memorial Sloan-Kettering Cancer Center. Patients evaluated in the trial have treatment-refractory, EGFR-overexpressing tumors, which include head and neck, kidney, bladder, and prostate cancers. Successive groups of 3–6 patients have received MDX-447 intravenously weekly alone, or with G-CSF (3 μg kg<sup>-1</sup>day<sup>-1</sup>) subcutaneously 3 days before and on the day of MDX-447 administration. Doses of MDX-447 evaluated thus far include 1.0, 3.5, 7.0 and 10 mg/m<sup>2</sup>; evaluation of 15.0 mg/m<sup>2</sup> is in progress [2]. Primary toxicities encountered include fever, chills, blood pressure tolerability, pain/myalgias, and grade 2 increase in 5'-nucleotidase: most toxicities abated within 12 h and have not been more frequent or severe with higher doses or pretreatment with G-CSF. In all patients, toxicities were less severe on the second and subsequent administrations.

*In vivo* binding of MDX-447 to monocytes occurred in all patients, but to a significant degree to neutrophils only in G-CSF-treated patients. Increased plasma levels of tumor necrosis factor α (TNFα), interleukin-6 (IL-6), and G-CSF occurred following MDX-447 administration. Figure 1 shows the area-under-the-curve (AUC) values for plasma G-CSF concentrations on the first (day 0), second (day 7) and fourth (day 21) days following MDX-447 administration at the doses of 1, 3.5, 7.0, and 10 mg/m<sup>2</sup> in those patients who did and did not receive G-CSF. In patients who did not receive G-CSF, AUC values increased in a dose-dependent fashion between the doses of 1.0 mg/m<sup>2</sup> and 7.0 mg/m<sup>2</sup>.

Pre-treatment with G-CSF dramatically increased the MDX-447-stimulated rises in plasma G-CSF values. The rises in plasma G-CSF on day 7 and day 21 were markedly attenuated in those not receiving G-CSF, at all doses of MDX-447, compared to day 0. In those patients receiving G-CSF, this attenuation was much less.

MDX-447 administration also provoked a dose-dependent increase in plasma IL-6 concentrations. As with plasma G-CSF values, these increases were also attenuated at days 7 and 21. G-CSF administration had little effect on IL-6 responses to MDX-447 (data not shown). The effect of MDX-447 on plasma TNFα concentrations was more variable than for plasma G-CSF or IL-6 concentrations; however, as with plasma G-CSF, G-CSF administration markedly increased the maximum rises in plasma TNFα in response to MDX-447. Table 1 shows the mean C<sub>max</sub> values, with and without G-CSF administration, for plasma TNFα following MDX-447 administration on day 0.

Of 36 patients evaluable for response, 9 have experienced stable disease for 3–6 months. The optimal dose and maximum tolerated dose (MTD) have yet to be defined; dose escalation continues to better define the dose, toxicity, and the potential therapeutic role of MDX-447.



**Fig. 1** Area-under-curve concentrations of granulocyte-colony-stimulating factor (G-CSF) plotted against dose of MDX-447 on days 0, 7 and 21 of MDX-447 treatment. Values are shown in patients treated with MDX-447 alone and patients treated with MDX-447 plus G-CSF

### MDX-H210 CLINICAL EXPERIENCE

MDX-H210 is a F(ab') × F(ab') bispecific molecule comprised of the F(ab') of anti-HER2/neu murine antibody 520C9 and F(ab') of anti-CD64 humanized antibody H22. The safety and efficacy of MDX-H210 in patients with various treatment-refractory HER2/neu-overexpressing malignancies have been evaluated in one completed phase I clinical trial [9], five ongoing phase I/II trials, and three ongoing phase II trials (Table 2).

The phase I and I/II trials have been open-label, progressive dose escalation (0.35–135 mg/m<sup>2</sup>) studies in which single and, more often, multiple weekly doses have been given; MDX-H210 has been evaluated when given alone and in conjunction with the subcutaneously administered cytokines, G-CSF, granulocyte/macrophage-CSF, and INF $\gamma$ .

**Table 1** Plasma tumor necrosis factor  $\alpha$  (TNF $\alpha$ )  $c_{max}$  values (mean  $\pm$  SD) on day 0. G-CSF granulocyte-colony-stimulating factor

G-CSF addition	TNF $\alpha$ (pg/ml) after an MDX-447 dose of:			
	1.0 mg/m <sup>2</sup>	3.5 mg/m <sup>2</sup>	7.0 mg/m <sup>2</sup>	10.0 mg/m <sup>2</sup>
Without G-CSF	129 $\pm$ 68 (n = 4)	458 $\pm$ 404 (n = 6)	22 $\pm$ 3 (n = 3)	34 $\pm$ 32 (n = 3)
With G-CSF	829 $\pm$ 875 (n = 3)	1360 $\pm$ 1425 (n = 7)	1019 $\pm$ 1657 (n = 3)	2363 $\pm$ 1102 (n = 3)

**Table 2** Institutions conducting MDX-H210 clinical trials. INF  $\gamma$  interferon  $\gamma$ 

Phase I/II	
National Cancer Institute (NIH; USA) (MDX-H210)	
Dartmouth University (USA) (MDX-H210 plus IFN $\gamma$ )	
Georgetown University(USA) (MDX-210 plus GM-CSF)	
University of Southern California (USA) (MDX-H210 plus G-CSF)	
University of Erlangen (Germany) (MDX-H210 plus G-CSF)	
Phase II	
Birmingham University (UK) (MDX-H210) plus GM-CSF	Prostate carcinoma
	Renal cell carcinoma
Karolinska Institute (Sweden) (MDX-H210 plus GM-CSF)	
	Colorectal carcinoma

Results from the phase I and phase I/II trials have demonstrated that MDX-H210 treatment is well tolerated, with patients experiencing transient (1–12 h duration) low-grade fevers, malaise, blood pressure lability, pain at tumor sites (especially bone metastases), dyspnea, nausea, vomiting and headache [7–9].

Doses of MDX-H210 of 3.5 mg/m<sup>2</sup> or more saturate at least 80% of monocyte CD64 and produce peak plasma concentrations of at least 0.9  $\mu$ g/ml, which is greater than the concentration of optimal monocyte/macrophage activation in vitro [9]. Elevated plasma levels of TNF $\alpha$ , IL-6, and G-CSF have consistently been observed, with maximum levels at doses of 7.0 mg/m<sup>2</sup> or above [7, 9]. Transient monocytopenia and lymphopenia occur 1–2 h after infusion [9]. Treatment with successive doses of MDX-H210 results in desensitization with regard to the development of monocytopenia, as well as the increase in plasma TNF $\alpha$ , IL-6, and G-CSF. Treatment with successive doses is also associated with marked decreases in the number and severity of toxicities experienced by the patients. MDX-H210 has also been shown to reduce elevated plasma HER2/neu [8].

It has been hypothesized that MDX-210, by directing macrophage phagocytosis of tumor cells, might result in presentation of tumor antigens and the induction of a humoral immune response; consistent with this hypothesis, some patients have shown evidence of active antitumor immunity following therapy with MDX-210 [7]. Biopsies of metastatic lesions before and after treatment suggest tumor localization of MDX-H210 and potential local immunological activity [7–9].

In these dose-ranging clinical studies, evidence of anti-tumor activity has been seen. This includes 1 partial (metastatic ovarian cancer) and 1 mixed (metastatic breast cancer) tumor response, which were observed among 10 assessible patients given MDX-210 alone, [9] 2 minor responses (metastatic colorectal carcinoma; J. Possey per-

sonal communication), in patients given GM-CSF, and 15 protocol-defined responses of stable disease.

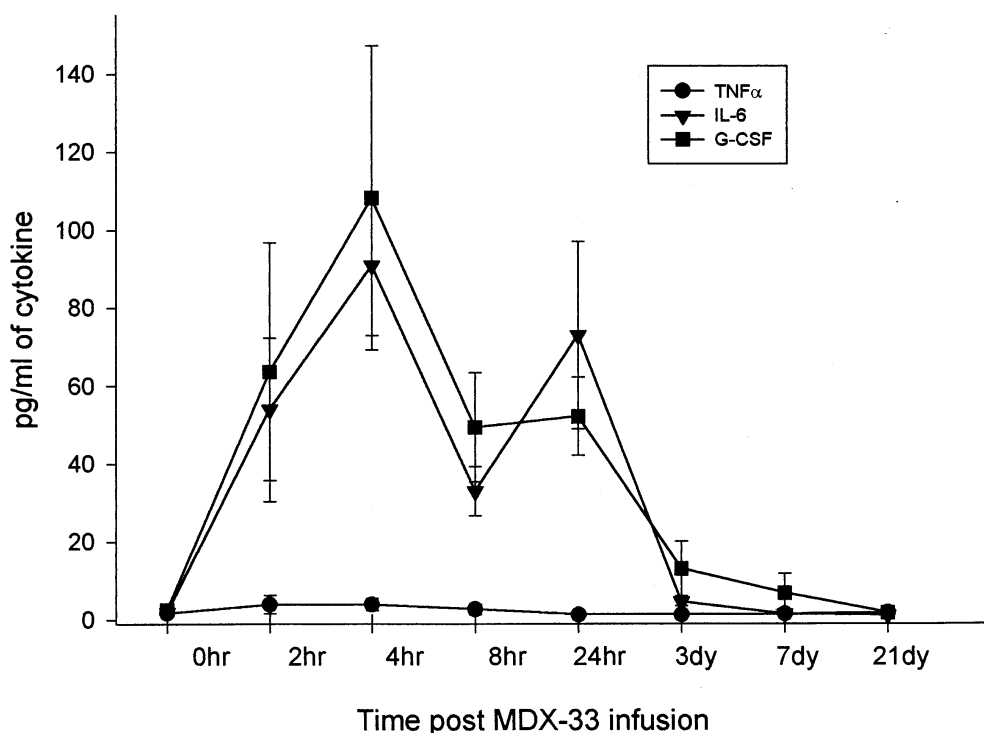
Three MDX-H210 phase II trials are currently in progress, all at the intravenous dose of 15 mg/m<sup>2</sup>, given with GM-CSF. These consist of one trial each in the treatment of RCC patients, prostate cancer patients, and colorectal cancer patients, all of whom have failed standard therapy. At the time of writing, 11 patients have been treated in these phase II trials. Four patients have demonstrated antitumor effects. Patients demonstrating responses include 2 with RCC and 2 with prostate cancer. One patient with renal cell carcinoma has had a 54% reduction in size of a hepatic metastatic lesion and the other has had a 49% decrease in the size of a lung metastasis with simultaneous clearing of other non-measurable lung lesions. Regarding the two patients with prostate cancer, one has had a 90% reduction in serum prostate-specific antigen (PSA; 118–11 ng/ml), which has persisted for several months [1]; the other patient with prostate cancer has had a 70% reduction of serum PSA (872–208 ng/ml) within the first month of treatment. Both patients also have demonstrated symptomatic improvement. Accordingly, these early-stage findings clearly indicate that MDX-H210 has exciting potential in the therapy of at least two otherwise refractory cancers.

### MDX-33 clinical experience

MDX-33 is a humanized CD64 specific MoAb that is being evaluated in the treatment of ITP and other autoimmune disorders in man. Several lines of evidence suggest that cytotoxic immune effector cells (e.g. monocytes and macrophages) that express Fc receptors for IgG (Fc $\gamma$ R) play an important role in platelet destruction [4]. Thus the platelets coated with IgG are probably either phagocytosed, lysed or cleared by CD64-bearing effector cells. Cross-linking an CD64 by CD64-specific antibodies has been shown to down-modulate CD64 significantly. Therefore, such CD64-specific mAb may have therapeutic utility in the treatment of ITP.

In a phase I clinical trial, MDX-33 was administered as a single intravenous dose to 17 normal subjects in order to assess its potential as an immunomodulator for the treatment of ITP and other immune disorders. Doses of 1.5, 3.0, 5.0, and 7.5 mg/m<sup>2</sup> were administered. The variables evaluated in response to MDX-33 were circulating monocyte and neutrophil counts, plasma cytokines (TNF $\alpha$ , IL-6, and G-CSF), monocyte CD64-mediated phagocytosis, monocyte CD64 modulation, MDX-33 pharmacokinetics, and various safety parameters. MDX-33 is well tolerated

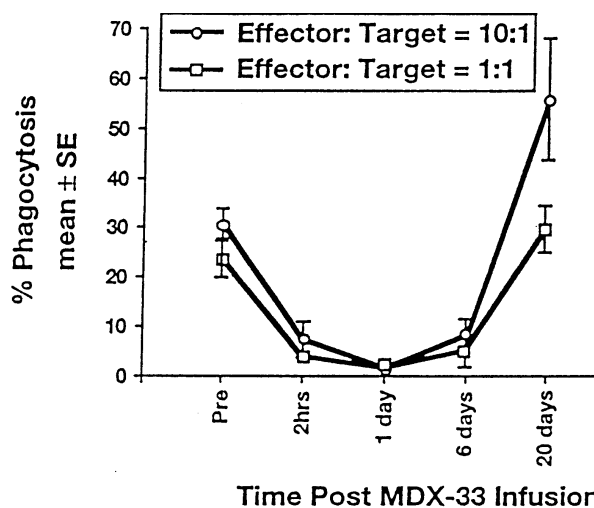
**Fig. 2** Plasma cytokine levels in subjects infused with 5mg/m<sup>2</sup> MDX-33 (*n* = 8)



at doses of 5.0 mg/m<sup>2</sup> or less, the primary toxicities being chills, low-grade fever, headache, and muscle aches.

Figure 2 shows the effect of the 5-mg/m<sup>2</sup> dose of MDX-33 on the plasma cytokines. Plasma IL-6 and G-CSF increase rapidly, peak levels being reached after 4 h at both doses; both plasma cytokines remained elevated for 24 h, returning to pre-treatment levels by 48 h. Plasma TNF $\alpha$  levels were not changed at either dose in response to MDX-33.

Persistent binding of MDX-33 to 80%–99 % of circulating monocytes is seen for at least 6 days; down-modulation of monocyte CD64 occurs and also lasts more than 6 days. Monocyte CD64-mediated phagocytosis is significantly inhibited at all doses of MDX-33. At the 3.0-mg/m<sup>2</sup> and 5.0-mg/m<sup>2</sup> doses, phagocytosis is fully inhibited for at least 6 days, returning to baseline levels by 20 days after dosing (see Fig. 3 for 3.0 mg/m<sup>2</sup> results). These results clearly demonstrate that immunomodulation of monocyte CD64 by the mAb MDX-33 can be accomplished with minimal clinical toxicity, and further indicate the potential of MDX-33 in the treatment of ITP and other auto-immune disorders.



**Fig. 3** CD64-mediated phagocytosis of monocytes taken at the indicated assay times from subjects infused with 3 mg/m<sup>2</sup> MDX-33

the ability to modulate monocyte CD64 in the clinical setting. Studies are currently being conducted to further elucidate the full potential these and other approaches using CD64-directed immunotherapy.

## Conclusions

The results from completed and ongoing clinical trials with the CD64-directed bsAb MDX-447 and MDX-H210 demonstrate excellent tolerability in association with promising antitumor effects in tumors that have become refractory to all available therapies. Also promising are the results from the trial of the CD64-directed mAb, MDX-33, which show

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