Pergamon

European Journal of Cancer, Vol. 35, Suppl. 3, pp. S25–S28, 1999

Printed in Great Britain
0959-8049/99/$ - see front matter

PII: S0959-8049(99)00086-6

Original Paper

GM-CSF as Adjuvant for Immunotherapy with Bispecific Antibodies

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MONOCLONAL ANTIBODIES IN ONCOLOGY

Approximately 100 years after P. Ehrlich's idea of using antibodies as 'magic bullets' for tumour therapy, and 20 years after Köhler and Milstein's description of the hybdridoma technology, immunotherapy with monoclonal antibodies (MAbs) is now becoming an additional treatment option in oncology. For example, treatment with antibody 17-1A to the EpCAM molecule, which is overexpressed on the majority of colorectal cancers, reduced mortality of patients with resected Dukes' C colon carcinoma by 32% [1]. C2B8—a human/mouse chimeric IgG1 antibody to the B cell differentation antigen CD20—induced clinically relevant responses in approximately 50% of patients with relapsed low grade lymphoma [2]. This response rate is in the range of what can be achieved with conventional chemotherapy in these patients. Furthermore, the combination of chemotherapy and 4D5—a humanised IgG1 antibody against the proto-oncogen product HER-2/neu, which is overexpressed in approximately 30% of patients with breast cancer, but also in other common cancers such as prostate, lung and colorectal—significantly improved survival of patients with metastatic breast cancer compared to chemotherapy alone [3].

MAbs mediate their antitumour effects either by directly acting on tumour cells, e.g. by blocking growth factors, inhibiting cell proliferation, inducing programmed cell death or dormancy—or by recruiting immune effector mechanisms such as cell- or complement-dependent cytotoxicity. The hypothesis that cell-mediated effects are an important mechanism of antibody action *in vivo* is strongly supported by studies with isotype switch variants, which showed a positive correlation between the antibodies' capacity to induce cell-mediated lysis *in vitro*, and their therapeutic efficacy *in vivo*. In addition, the therapeutic efficacy of antibody therapy was abrogated in animals, in which the signalling machinery of immunoglobulin receptors was genetically disrupted [4]. In order to induce cell-mediated effects, MAbs interact via their constant Fc regions with activating immunoglobulin receptors on immune effector cells. Depending on their specificity for the heavy chains of IgA, IgE or IgG, these Fc receptors are grouped as Fcα, Fcε, or Fcγ receptors, respectively. The majority of Fc receptors consists of ligand specific α-chains, which associate with shared molecules for signalling. A total of 12 different leucocyte Fcγ receptor isoforms is grouped into two classes of low affinity receptors—named FcγRII (CD32) and FcγRIII (CD16), respectively—and into FcγRI (CD64), the latter containing one high affinity isoform [5]. For IgA, one class of receptors (FcαRI, CD89) has been identified on myeloid cells, which consists of five different isoforms [6].

In vitro, the capacity for antibody-mediated tumour cell lysis has been demonstrated for NK-cells, monocytes/macrophages as well as neutrophilic and eosinophilic granulocytes—all having characteristic patterns of Fc receptor expression. For example, monocytes/macrophages express cytolytic isoforms of FcγRI, FcγRII, FcγRIII and FcαRI, whereas on NK-cells FcγRIII is the only cytotoxic Fc receptor. Neutrophils—the most abundant Fc receptor expressing effector cells—constitutively express the myeloid receptor for IgA, and the two low affinity IgG receptors FcγII and FcγIII. However, the most strongly expressed FcγRIII on neutrophils is GPI-linked (FcγRIIIb) and not cytolytic, in contrast to the transmembrane FcγRIIIa isoform on monocytes/macrophages and NK-cells. Importantly, neutrophils additionally express the high affinity FcγRI receptor after exposure to G-CSF or IFN-γ, which also acts as cytotoxic trigger molecule on these activated cells [7]. The contribution of each of these effector cell types and their respective Fc receptor classes to antibody efficacy *in vivo* is unclear at the moment and is being addressed in several laboratories. *In vitro*, we found neutrophils to be the major effector cell population for conventional monoclonal and for FcγRI-directed bispecific antibodies against HER-2/neu-expressing breast cancer cells [8]. However, neutrophils could not kill malignant B cells in the presence of conventional antibodies to CD20 or to other 'classical' B cell antigens, although B cells were susceptible to the killing machinery of neutrophils, as indicated by the effective lysis of lymphoma cells in the presence of antibodies to HLA class II [9].

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RATIONALE TO USE BISPECIFIC ANTIBODIES

Human IgG1—the clinically most widely used antibody isotype—effectively activates human complement, interacts well with FcγRIIIa on NK-cells and macrophages, and has an extended half-life *in vivo*, because IgG1 is protected from degradation by binding to the IgG protection receptor FcRn. However, human IgG1 was less effective in recruiting PMN—the most populous Fc-receptor expressing effector cell in the blood. This could be explained by competition of therapeutic antibodies with high concentrations of natural immunoglobulins for binding to Fc receptors. This issue is especially critical for FcγRI, because this high affinity receptor binds monomeric IgG and is, therefore, not available as a cytotoxic trigger molecule in the presence of serum concentrations of human IgG. In addition, therapeutic antibodies may bind to Fc receptors on non-effector cells (e.g. platelets or B cells), or to Fc receptors on effector cells, which do not trigger cytolytic cascades (e.g. FcγRIIb or FcγRIIIb). Fc receptor-directed bispecific antibodies represent an elegant solution to many of these problems [10]. These genetically or chemically constructed molecules combine specificity for a tumour cell epitope with reactivity for a cytotoxic trigger molecule on immune effector cells, thereby allowing specific engagement of activating Fc receptors on cytotoxic cells. By selecting antibodies, which bind with their variable regions to Fc receptor epitopes distinct from the Ig binding site, competition with serum Ig can be avoided, and full activity in the presence of natural antibodies is conserved. *In vitro*, FcγRI-directed bispecific antibodies against several target antigens were found to be effective at 5-fold lower concentrations than their conventional MAbs. Recently, we demonstrated that in addition to the clinically tested IgG receptors FcγRI and FcγRIII also the myeloid IgA receptor FcαRI constitutes an interesting cytotoxic trigger molecule for bispecific antibody therapy [11].

REASONS TO COMBINE BISPECIFIC ANTIBODIES WITH MYELOID GROWTH FACTORS

The cytotoxic activity of FcαRI-directed bispecific antibodies has been found to be dramatically increased when blood from patients during therapy with G-CSF or granulocyte macrophage-colony stimulating factor (GM-CSF) was compared with blood from control donors not receiving these growth factors (Figure 1). G-CSF additionally enhanced killing with FcγRI-directed constructs, whereas the activity of FcγRIII-directed bispecific antibodies was not enhanced by either cytokine. When blood was then fractionated into plasma, mononuclear cells and granulocytes, the major effector cell population for both FcγRI- or FcαRI-directed bispecific antibodies was found to reside in the neutrophil fraction, whereas FcγRIII-directed bispecific antibodies mainly recruited NK-cells from the mononuclear cell fraction. As therapy with G-CSF or GM-CSF significantly enhanced neutrophil numbers, increased effector to target cell ratios were identified as one mechanism for enhanced cytotoxicity during growth factor therapy. This effect was particularly pronounced for the combination of G-CSF and FcγRI-directed bispecific antibodies, because G-CSF therapy induced the expression of this cytotoxic trigger molecule on otherwise FcγRI-negative neutrophils. GM-CSF was found predominately to activate tumour cell killing via FcαRI-directed bispecific antibodies. This enhanced cytotoxicity during GM-CSF involved both increased effector cell numbers and improved functional activity of individual cells. Most importantly, the combination of GM-CSF primed neutrophils and FcαRI-directed bispecific antibodies killed tumour cells, such as the prostate cancer cell line DU145, which were otherwise very resistant to cell-mediated lysis by conventional or Fcγ-receptor directed bispecific antibodies (Figure 1). Furthermore, malignant B cells were effectively killed by GM-CSF primed neutrophils in the presence of FcαRI-directed bispecific antibodies against CD20, demonstrating that neutrophil's antigen restriction in killing of malignant B cells, which was observed with Fcγ receptor-directed antibodies [9], can be overcome by this interesting combination (data not shown).

In addition to mediating phagocytosis and direct killing of tumour cells, Fc receptors are well documented to enhance the capacity of professional antigen presenting cells to activate CD4-positive T helper cells. For Fcγ receptors type II and III, this function required cooperation of the ligand binding α-chain with the shared γ-chain [12]. Interestingly, FcγRI—but not other Fc receptors—contains a distinct intracellular motif, which directs antigen/antibody complexes to HLA class II vesicles and thus effectively enhances presentation of antigens to helper T cells. Thus, the combination of GM-CSF, which is known to enhance antigen presentation in several experimental systems, and FcγRI-directed bispecific antibodies seems particularly promising to boost the patients' immune response against their tumour.

Until recently, Fc receptor-directed immunotherapy could not be tested in syngenic animal models, because no specific antibodies for individual murine Fcγ or Fcα receptors are available. To circumvent these technical limitations, animals transgenic for human FcγRI, FcαRI, or both were generated. These transgenic mice showed myeloid specific expression of

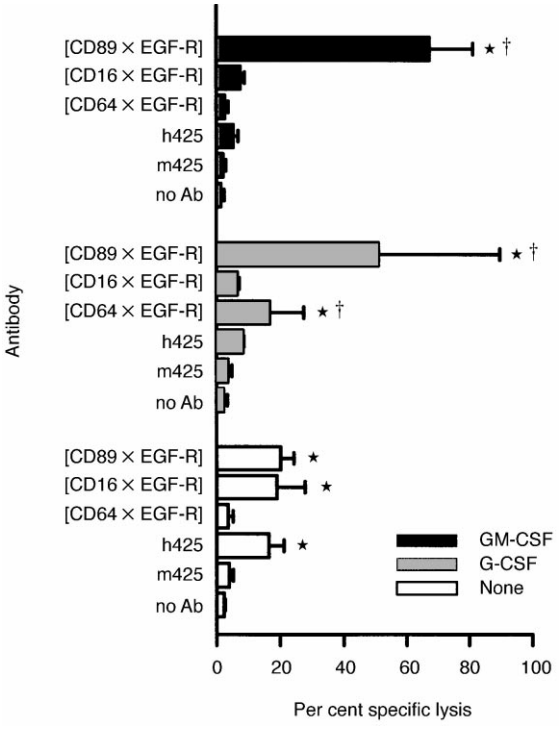

Figure 1. Comparing killing of prostate cancer cell line DU145 by EGF-R directed bispecific antibodies with specificites for FcγRI (CD64), FcγRIII (CD16) or FcαRI (CD89), respectively, using blood from cytokine-treated or control donors.

Table 1. *Clinical trials with bispecific antibodies in combination with GM-CSF or G-CSF*

Phase	Bispecific antibody	Cytokine	Patient population	Doses and schedule	Institution and location
I	MDX-H210	G-CSF	Breast	0.35–200 mg/m^2, single	Universities Erlangen (Germany) and Utrecht (The Netherlands)
I/II	MDX-H210	G-CSF	Breast	1–140 mg/m^2, multiple	University, Southern California (U.S.A.)
II	MDX-H210	GM-CSF	All	20 mg/m^2, multiple	University, Georgetown (U.S.A.)
II	MDX-H210	GM-CSF	Prostate and renal	15 mg/m^2, multiple	Multiple
II	MDX-H210	GM-CSF	Colorectal	15 mg/m^2, multiple	Karolinska, Stockholm (Sweden)
I	MDX-447	±G-CSF	All	1–10 mg/m^2, multiple	Sloan-Kettering, New York (U.S.A.)

the respective transgenes under their endogenous promoters. As in humans, huFcγRI expression in mice was properly regulated by exposure to the cytokines IFN-γ, G-CSF, IL-4 and IL-10, and neutrophil FcγRI was increased during inflammation [13]. Importantly, both human FcγRI and FcαRI were shown to be functional in murine effector cells as evidenced by phagocytosis and ADCC experiments with corresponding bispecific antibodies. Moreover, number and functional activity of transgenic effector cells were increased by treating mice with G-CSF or GM-CSF, respectively. First *in vivo* results from syngenic lymphoma models showed synergistic therapeutic effects of G-CSF and FcγRI-directed bispecific antibodies in huFcγRI-transgenic but not in control animals. Thus, these animals are valuable for further analysing the *in vivo* potential of myeloid growth factors in combination with Fc receptor directed bispecific antibodies.

CLINICAL EXPERIENCE WITH BISPECIFIC ANTIBODIES IN COMBINATION WITH MYELOID GROWTH FACTORS

FcγRI-directed bispecific antibodies with tumour-directed specificities for HER-2/neu, EGF-R or the myeloid CD15 antigen have entered clinical trials. Results from these phase I/II clinical trials showed acceptable toxicity profiles—with fever/chills, nausea/vomiting and pain in tumour areas as most common side-effects, all of which could be managed by symptomatic therapy. As evidence for biological activity of these bispecific antibodies, elevated levels of pro-inflammatory cytokines such as IL-6 or TNF-α were measured shortly after bispecific antibody application [14]. The above summarised evidence suggested to combine FcγRI-directed bispecific antibodies with GM-CSF or G-CSF. Table 1 summarises these trials.

In a phase I trial with bispecific antibody MDX-H210 (FcγRI×HER-2/neu) in combination with G-CSF in metastatic breast cancer patients, the dose of MDX-H210 could be escalated up to 200 mg/m^2 without dose-limiting toxicity. Side-effects were not closely dose-related, occurred mainly with the first antibody infusion, and were similar to those observed with MDX-H210 alone [15]. MDX-H210 is currently being evaluated in combination with GM-CSF in several clinical phase II studies. So far, the most promising results were observed in patients with hormone refractory HER-2/neu-positive prostate cancer, some of which showed objective tumour responses. Side-effects of this combination were generally mild, but in some of the responders grade 3 toxicity was noted [16]. Bispecific antibody MDX-447 (FcγRI×EGF-R) was evaluated with and without G-CSF in patients with EGF-R-expressing tumours. Interestingly, here the combination of G-CSF and bispecific antibody was more toxic than the bispecific antibody alone, which was probably caused by higher levels of endogenous TNF-α release after application of the combination [17]. A phase I trial of an FcαRI-directed bispecific antibody against CD20 is expected to commence soon.

CONCLUSIONS

The combination of Fc receptor-directed bispecific antibodies and myeloid growth factors such as GM-CSF or G-CSF is a promising approach to further improve the efficacy of antibody therapy.

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Acknowledgements—This work was supported by the Deutsche Forschungsgemeinschaft (Va 124/1-3).