In vitro and In vivo Characterization of MDX-1401 for Therapy of Malignant Lymphoma

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Abstract Purpose: This study was undertaken to evaluate the effects of MDX-1401, a nonfucosylated fully human monoclonal antibody that binds to human CD30, and to determine whether it exhibits greater in vitro and in vivo activity than its parental antibody. Experimental Design: Assays measuring antibody binding to CD30-expressing cells and FcγRIIIa (CD16) transfectants as well as antibody-dependent cellular cytotoxicity (ADCC) were conducted. Antitumor activity was determined using a Karpas-299 systemic model. Results: The binding of MDX-1401 to CD30 antigen was identical to fucose-containing parental anti-CD30 antibody (MDX-060). In contrast, MDX-1401 showed increased binding affinity to FcγRIIIa-transfected cells resulting in increased effector function. MDX-1401 greatly improved ADCC activity as evidenced by a decrease in half-maximal effective concentration (EC50) and an increase in maximum cell lysis when compared with MDX-060. Increased ADCC activity was observed among a panel of cell lines, including one with very low CD30 antigen expression in which parental antibody failed to induce any detectable ADCC. MDX-1401 activity with all FcγRIIIa polymorphic variants, including less active Phe/Phe158 and Phe/Val158 effector cells, was shown. Furthermore, MDX-1401 was efficacious in inhibiting tumor growth in CD30+ lymphoma xenografts. Conclusions: The low doses of antibody required for ADCC activity irrespective of donor genotype, the ability to mediate ADCC in target cells expressing low levels of CD30, and increased in vivo efficacy support the development of MDX-1401 for treatment of malignant lymphoma.

Monoclonal antibodies (mAb) have proven to be an effective class of new antitumor agents for several tumor types and are especially useful in hematologic cancers such as leukemias and lymphomas. CD30, a member of the tumor necrosis factor superfamily, is highly expressed on several neoplasms, including Hodgkin’s lymphoma (HL) and anaplastic large cell lymphoma (ALCL), but has low expression on normal tissues where it is restricted to activated lymphocytes (1–5). CD30 has therefore attracted attention as a potential target for immunotherapy of HL and ALCL, and several mAbs specific for CD30 have been developed and tested in the clinic with varying degrees of success (6–10).

Antibody therapeutics have been shown to use several different mechanisms to kill tumor cells, including direct induction of apoptosis, activation of complement-mediated cytotoxicity, and Fc receptor (FcR)–mediated processes such as antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis (11, 12). Multiple lines of evidence indicate that Fcγ-mediated killing is particularly important. For example, an antitumor antibody with potent antitumor activity in a wild-type mouse model showed less efficacy in mice lacking activating FcγRI and FcγRIII (13). Similar findings were made with clinically successful antibodies rituximab and trastuzumab, which had no detectable effect in mice lacking activating FcRs, but showed high levels of antitumor activity in mice deficient in inhibitory receptor FcγRIIB (14).

Four classes of FcRs have been identified, with FcγRIIIa (CD16) designated as the major FcR expressed on natural killer cells and macrophages (15). FcγRIIIa has two polymorphic isoforms at residue 158: Val158 and Phe158 (16, 17). The FcγRIIIa-Val158 allele shows higher binding affinity for human IgG1 than the FcγRIIIa-Phe158 allele, and this increased binding correlates with enhanced activity in ADCC assays using effector cells from FcγRIIIa-Val158 donors (18). Analysis of the distribution of these polymorphic variants in patients...
MDX-1401, a second-generation anti-CD30 antibody with a nonfucosylated Fc region, has been generated and is characterized in this study. Its higher binding affinity to FcγRIIIa as well as improved ADCC activity among a large panel of cell lines with a broad range of CD30 receptor expression, irrespective of FcγRIIIa genotype, are clearly shown. Furthermore, MDX-1401 is shown to have superior antitumor activity in vivo using a systemic lymphoma xenograft model.

Materials and Methods

Antibodies and cell lines. MDX-060 and MDX-1401 are fully human IgG1 (κ) isotype mAbs specific for human CD30 antigen. MDX-060 was obtained by cloning of heavy and light chain cDNA from hybridoma 5F11 and reexpression in Chinese hamster ovary (CHO) cells (28). Nonfucosylated MDX-1401 was obtained by expression of 5F11 heavy and light chain cDNA in a Ms704-PF FUT8 (α-1,6-fucosyl transferase) knockout CHO cell line (29). A variant of MDX-060 was also expressed in which site-directed mutagenesis was used to remove the site for N-linked carbohydrate addition from the Fc region by mutating the asparagine at position 297 to glutamine (N297Q). This antibody was used as a study control. Antibodies were subsequently produced and purified using standard mammalian cell cultivation and chromatographic purification techniques.

The Karpas-299 (ACC 31) human ALCL T-cell lymphoma line as well as L540 (ACC 72), L428 (ACC 197), and L1236 (ACC 530) human HL cell lines were purchased from the German Resource Centre for Biological Material. Ms704-PF CHO cells were obtained from BioWa.

Oligosaccharide characterization of mAbs by capillary electrophoresis with laser-induced fluorescence. N-linked oligosaccharides were released from IgG samples (100 μg) by overnight incubation of the samples with 12.5 milliunits PNGaseF (Prozyme) at 40°C. Following ethanol precipitation to remove protein, the supernatant containing the glycans was dried by vacuum centrifugation and resuspended in 19 mmol/L APTS (Beckman) in 15% acetic acid and 1 mol/L sodium cyanoborohydride in THF (Sigma-Aldrich). The glycan labeling reaction was allowed to continue overnight at 40°C followed by 25-fold dilution of sample in water. APTS-labeled glycans were applied to a capillary electrophoresis system (P/ACE MDQ CE; Beckman) with laser-induced fluorescence and reverse polarity using a 50-μm internal diameter N-CHO–coated capillary (Beckman) with 50 cm effective length. Separation was carried out at 20°C using Carbohydrate Separation Gel Buffer (Beckman) at 25 kV for 20 min and monitored using a 3 mW argon laser with excitation and emission wavelengths of 488 nm and 520 nm, respectively.

Monosaccharide analysis by high-performance liquid chromatography with high-performance anion-exchange chromatography with pulsed amperometric detection. IgG samples (200 μg) were subjected to acid hydrolysis using either 2 mol/L trifluoroacetic acid (for estimating neutral sugars) or 6 mol/L HCl for estimating amino sugars at 100°C for 4 h. Samples were dried by vacuum centrifugation at ambient temperature and reconstituted in 200 μL water before analysis by high-performance anion-exchange chromatography with pulsed amperometric detection ( Dionex). Monosaccharides were separated using a CarboPac PA10 4 × 250 mm column with precolumn Amino Trap and Borate Trap ( Dionex). Procedures were followed according to Dionex Technical Note 53. Monosaccharide peak identity and relative abundance were determined using monosaccharide standards ( Dionex).

Binding of MDX-060 and MDX-1401 to tumor cell surface CD30. Aliquots of 5 × 10⁴ cells per well were incubated for 30 min at 4°C with either MDX-060 or MDX-1401 primary antibody at increasing concentrations ranging from 0.012 to 50 μg/mL. Cells were washed twice in PBS with 2% fetal bovine serum (FBS; Life Technologies-Invitrogen) before addition of goat anti-human IgG phycoerythrin-labeled secondary antibody (Jackson ImmunoResearch) for 30 min at 4°C. Cells were again washed twice in PBS with 2% FBS and assayed by fluorescence-activated cell
sorting (FACS) with a FACSCalibur or FACSArray system (BD Biosciences). Binding curves were plotted for geometric mean fluorescent intensity (GMFI) versus antibody concentration using nonlinear regression analysis, sigmoidal dose response (GraphPad Prism software).

**Binding of MDX-060 and MDX-1401 to human FcγRIIIa-Phel158-expressing and FcγRIIIa-Val158-expressing cells.** CHO cells transfected with either human FcγRIIIa-Phel158 or human FcγRIIIa-Val158 cDNA (1 × 10^5 per well) were incubated for 30 min at 4°C with increasing concentrations of biotinylated MDX-060 or biotinylated MDX-1401. Cells were washed twice with PBS containing 2% FBS before addition of streptavidin-phycoerythrin conjugate (BD Biosciences) for 30 min at 4°C. Cells were then washed as above and resuspended in propidium iodide solution. Data acquisition on a FACSArray system was done by FACS gating on propidium iodide-negative cells. GMFI was plotted against the various concentrations of antibodies used. Data were analyzed using nonlinear regression analysis, sigmoidal dose response (GraphPad Prism software).

**ADCC assay.** Human peripheral blood mononuclear effector cells were purified from either heparinized whole blood (AllCells) or Buffy coat (Stanford Blood Center) by standard Ficol-Paque separation. Cells (2 × 10^9) were washed in PBS and sent for genotyping. The remaining cells were then resuspended at 1 × 10^9/mL in RPMI 1640 containing 10% FBS and 50 units/mL human interleukin-2 (Research Diagnostics) and incubated overnight at 37°C. After ~18 h, the resulting effector cells were washed once and resuspended in culture medium. Tumor target cells were labeled at 37°C with either BATDA [bis(acetoxy-methyl)2,2′,6,2′-terpyridine-6,6′-dicarboxylate] reagent (Perkin-Elmer) for 20 min or 100 μg/ml Na_2^{15}O_4 (Perkin-Elmer) for 1 h. Target cells were washed thrice before addition to effector cells in 96-well plates (1 × 10^4 target cells and 5 × 10^5 effector cells per well) at a final E:T ratio of 1:50. Serial dilutions of MDX-060, MDX-1401, or isotype control antibody were added to the effector/target cell mixture. Maximal lysis was determined by incubation of target cells in 3% detergent (Lysol) and spontaneous release assessed by incubation of target and effector cells in the absence of antibody. Supernatant from BATDA-labeled target cell wells was harvested after 1-h incubation at 37°C and mixed with Europium solution (Perkin-Elmer) before time-resolved fluorescence analysis using a Perkin-Elmer Fusion Alpha reader with a 400-μs delay and 330/80 and 620/10 excitation and emission filters, respectively. Supernatant from 15^Cr-labeled target cell wells was harvested after 4-h incubation at 37°C and read on a Cobra II gamma counter (Perkin-Elmer). Specific lysis was calculated from raw emission event counts with the following equation: (experimental release – spontaneous release)/(maximal release – spontaneous release) × 100. Data were analyzed by nonlinear regression, sigmoidal dose response (variable slope) using GraphPad Prism software. CD16 blockade of ADCC activity was achieved with anti-human CD16 antibody (clone 3G8; BD Biosciences) at a concentration of 80 μg/mL for 15 min at room temperature.

**In vivo antitumor activity.** Male severe combined immunodeficient (SCID) mice (Taconic Farms) were maintained under specific pathogen-free conditions at Medarex Animal Facility, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. Karpas-299 cells were prepared from cultures in log growth phase. Cells were harvested, washed, and resuspended in serum-free medium to provide ~10^7 cells per 200 μL DMEM. On day 0, 10^7 Karpas-299 cells were i.v. introduced in the tail vein of each study animal. On day 1, mice were randomized into four groups (n = 8) and each animal was injected i.p. with ~200 μl MDX-060, MDX-1401, anti-CD30 mutant N297Q, or human IgG1 isotype control antibody to achieve a dose of 1 mg/kg. Response was measured as a function of body weight loss and/or death. Body weight measurements were obtained weekly and mice were monitored daily for clinical symptoms until day 64. Mice were euthanized when they showed >20% weight loss, in accordance with institutional guidelines. Statistical analysis of in vivo survival data was conducted using the Mantel-Haenszel log-rank test (GraphPad Prism software).

**Results**

**Generation and carbohydrate analysis of nonfucosylated MDX-1401.** The human antibody to CD30, MDX-060, was selected based on high binding affinity and specificity for CD30, potent ADCC activity in vitro, and antitumor activity in in vivo models (28). MDX-1401 was produced by expression of cDNA encoding the MDX-060 heavy and light chains in Ms-704PF cells, which were knocked out for FUT8 (ref. 29). Purified MDX-1401 was analyzed biochemically to determine the nature of its carbohydrate content compared with that of parental MDX-060, which was obtained from expression of the same cDNA in a standard CHO cell line with active FUT8. Both monosaccharide and oligosaccharide analyses were carried out. As expected, MDX-060 contained a mixture of oligosaccharide structures. Genotype analysis of a CHO cell line with active FUT8 showed that MDX-1401 had similar similar structures with variable levels of galactose but no detectable fucosylated oligosaccharides. Analysis of monosaccharides released by acid hydrolysis confirmed the absence of fucose on MDX-1401 (data not shown).

**Identical antigen-binding properties of fucosylated and nonfucosylated anti-CD30 antibodies.** To determine whether nonfucosylated MDX-1401 retained the binding characteristics of MDX-060, binding affinity to soluble recombinant antigen as well as cell surface CD30 was measured. Binding affinity to recombinant CD30 by surface plasmon resonance showed that MDX-060 and MDX-1401 had similar affinities with K_D values of 0.80 and 0.83 nmol/L, respectively (data not shown). The affinity of MDX-060 or MDX-1401 for cell surface CD30 was determined in saturation binding studies using L540 cells. Data analyses of binding affinities between the two antibodies (data not shown).

**Flow cytometric analysis of several CD30^+ cell line lines (L540, L428, Karpas-299, and L1236) was also done.** Dose-dependent binding was seen for all cell lines tested, showing that both MDX-1401 and MDX-060 recognized CD30^+ cells (Fig. 2A). The differences in maximum GMFI between the cell lines suggested that the number of receptors per cell varied widely. The rank order of CD30 expression was highest on L540 cells followed by Karpas-299, L428, and L1236 cells, with respective values of approximately 1,200, 800, 500, and 50 GMFI. No remarkable differences were seen in the binding between MDX-1401 and MDX-060 in any of the cell lines tested, indicating that the nonfucosylated antibody retained the antigen binding properties of the parental antibody. The binding of anti-CD30 antibody containing an N297Q mutation was also tested by flow cytometry and was nearly identical to the binding of MDX-1401 and MDX-060 to Karpas-299 cells (Fig. 2B).

**MDX-1401 enhanced affinity for Phel158 and Val158 polymorphic variants of human FcγRIIa.** To determine the binding activity of MDX-1401 and MDX-060 to polymorphic variants of human FcγRIIa, a cell-based binding assay was done using CHO cells transfected with human FcγRIIa expressing either the Val158 or Phel158 allele. Clones having similar FcγRIIa surface expression level, as determined by anti-CD16 3G8 binding.
were selected and dose-dependent binding of both anti-CD30 mAbs was measured by flow cytometry. As expected, the binding of MDX-060 was very weak to low affinity FcγRIIIa-Phe158 and FcγRIIIa-Val158. However, the binding of MDX-1401 to both FcγRIIIa-Val158-transfected and FcγRIIIa-Phe158-transfected cells was notably higher (Fig. 2C and D). The maximal binding values (GMFI) to FcγRIIIa-Val158-transfected cells were 3,150 and 1,750 for MDX-1401 and MDX-060, respectively. Similarly, binding to FcγRIIIa-Phe158-transfected cells was 1,450 for MDX-1401 and 339 for MDX-060, whereas the background of secondary antibody alone GMFI was ∼25. Although the anti-CD30 antibody with an N297Q mutation bound well to CD30+ cells, this antibody failed to bind to FcγRIIIa-Val158 or FcγRIIIa-158Phe. No binding to parental CHO cells was observed (data not shown). These results clearly showed an enhanced binding affinity of nonfucosylated MDX-1401 antibody to both polymorphic variants of human FcγRIIIa.

**Superior MDX-1401-mediated FcγRIIIa-dependent ADCC activity in vitro.** In vitro ADCC activities of MDX-1401 and MDX-060 antibodies were measured in a standard 51Cr release assay. Both MDX-1401 and MDX-060 induced dose-dependent killing of L540 cells (Fig. 3). Blocking antibody to CD16 (3G8) inhibited MDX-060-mediated and MDX-1401-mediated ADCC, whereas the isotype control had no effect, showing that lysis was mediated by FcγRIIIa. MDX-1401 was more potent and efficacious than parental MDX-060. In fact, EC50 values were 0.009 and 0.018 µg/mL and percentage maximum cell lysis values were 87% and 63% for MDX-1401 and MDX-060, respectively. These results agreed with published reports that describe other antibody antigen systems in which removal of fucose improved both the potency and efficacy of antibodies (14).

To determine whether the superior ADCC activity mediated by MDX-1401 is observed in cells with varying CD30 antigen expression, MDX-1401 and MDX-060 antibodies were tested using a panel of CD30+ cells expressing different levels of CD30 (Figs. 2 and 4). In this series of experiments, the maximum percentage lysis induced by MDX-1401 was 68% in L540 cells, 50% in Karpas-299 cells, 43% in L428 cells, and 13% in L1236 cells, suggesting that there is a positive correlation between antigen density and maximal ADCC responses. EC50 values for MDX-1401 and MDX-060 were, respectively, 0.008 and 0.04 µg/mL for L540 cells, 0.03 and 0.25 µg/mL for Karpas-299 cells, and 0.009 and 0.1 µg/mL for L428 cells. For L1236 cells, MDX-1401 exhibited an EC50 value of 0.04 µg/mL, whereas MDX-060 had no measurable ADCC activity. Likewise, maximum lysis was consistently higher for MDX-1401 when compared with MDX-060. The improvements in potency, as measured by EC50, and in efficacy, as measured by maximum percentage lysis, showed that enhanced activity of MDX-1401 over parental MDX-060 is observed regardless of the number of CD30 receptors per cell. Of particular interest, MDX-060 induced only minimal lysis in L1236 cells that express low levels of CD30, whereas MDX-1401 displayed dose-dependent ADCC activity.

To confirm that the enhanced affinity of MDX-1401 for both FcγRIIIa-Phe158 and FcγRIIIa-Val158 correlates with ADCC activity, increasing concentrations of MDX-1401 and MDX-060 antibodies were incubated with either homozygous (FcγRIIIa-Phe/Phe158) or heterozygous (FcγRIIIa-Phe/Val158) human effector cells and B整理ized peptide L540 target cells (Fig. 5). MDX-1401 greatly increased ADCC activity compared with MDX-060 irrespective of donor genotype. For FcγRIIIa-Phe/Phe158 effector cells, the EC50 values were 0.01 and 0.03 µg/mL for MDX-1401 and MDX-060, respectively.
whereas the corresponding maximal percentage lysis values were 42% and 15%. For FcγRIIIa-Phe/Val158 effector cells, the EC50 values were 0.01 and 0.06 μg/mL for MDX-1401 and MDX-060, respectively, whereas the corresponding maximal percentage lysis values were 42% and 31%. The ADCC enhancement mediated by MDX-1401 was more pronounced with FcγRIIIa-Phe/Val158 effector cells.

**MDX-1401 improved antitumor activity in vivo.** In the mouse, the most homologous FcR to human FcγRIIIa is FcγRIV, also known as mCD16-2 (30). To examine whether MDX-060 and MDX-1401 were capable of binding differentially to this receptor, a binding assay was carried out using soluble recombinant protein. In this instance MDX-1401 exhibited slightly greater binding to FcγRIV than MDX-060 (data not shown). Previous studies showed that MDX-060 induces tumor cell killing in vitro through ADCC and direct growth inhibition but not complement-mediated cytotoxicity (28). Likewise, no complement-mediated cytotoxicity activity was shown with MDX-1401 (data not shown). To test the reliance of antibody therapeutics on FcR-directed effector function in the tumor model, another variant of anti-CD30 antibody, N297Q, was produced. In this variant, a mutation was introduced to remove the site for attachment of N-linked carbohydrate. N297Q was shown to be devoid of carbohydrate and to bind equivalently to CD30-expressing cells but not FcRs, and was therefore as expected inactive in ADCC and phagocytosis assays, although activity in the direct induction of apoptosis was retained (data not shown).

The mean survival time was 29 days for the isotype control group, 29 days for the N297Q-treated group, 32.5 days for the MDX-060–treated group, and 57 days for the MDX-1401–treated group (P = 0.0012 for N297Q versus MDX-1401 survival analysis using a log-rank test). Pharmacokinetic analysis has indicated that MDX-1401 and MDX-060 antibodies have a similar half-life in SCID mice (data not shown), suggesting that the superior antitumor activity of MDX-1401 was due to enhanced FcR-mediated effects. In addition, N297Q was completely ineffective in the Karpas-299 tumor model, showing that the efficacy of MDX-060

![Fig. 2. Flow cytometric analysis of MDX-060 and MDX-1401 antibodies binding to human CD30 antigen on tumor cells and FcγRIIIa-transfected CHO cells.](image-url)
and MDX-1401 antibodies relied on FcR-dependent functional activity (Fig. 6). It should be noted that in a s.c. implanted Karpas-299 model, similar efficacy of MDX-060 and MDX-1401 was shown, whereas N297Q was not effective. In addition, at higher antibody doses (3 mg/kg), the efficacy of MDX-1401 and MDX-060 in the Karpas-299 systemic model was identical (data not shown).

**Discussion**

FcR-mediated functional activity, including ADCC, is an important mechanism for the \textit{in vivo} activity of many therapeutic antibodies (11, 12). A promising strategy to further optimize the efficacy of mAbs is to increase FcR-dependent activity. One way to improve binding to FcγRIIIa and consequently increase antibody functional activity is to remove fucose from the Fc domain, enhancing ADCC-mediated antitumor cytotoxicity (18, 25, 26). In this study, a glyco-optimization strategy was successfully used to improve the efficacy of parental anti-CD30 mAb MDX-060, which showed limited activity as a single agent in phase 1/2 trials of patients with relapsed and refractory CD30+ lymphomas (6). The data presented clearly show that the newly generated nonfucosylated MDX-1401 mAb has dramatically improved activity relative to its fucosylated counterpart, MDX-060.

The interaction of anti-CD30 mAbs with human FcγRIIIa was investigated because (a) it is the major FcR present on two types of cytotoxic effector cells, natural killer cells, and macrophages (15); (b) lack of fucose improves antibody binding to human FcγRIIIa while having no effect on binding to other human FcRs (24); and (c) polymorphism in human FcγRIIIa is predictive of clinical response to IgG1 mAbs such as rituximab (19–22). The higher clinical responses of FcγRIIIa-Val158 patients compared with FcγRIIIa-Phe158...
patients are attributed to enhanced affinity of FcγRIIIa-Val158 receptors for IgG1 antibodies and may also result from higher FcγRIIIa-Val158 expression levels (31), although this finding is controversial (32).

As observed in the cell-based binding assay, removal of fucose dramatically improved anti-CD30 mAb affinity for FcγRIIIa irrespective of donor genotype. Similarly, the enhanced ADCC activity of MDX-1401 mAb was observed in both homozygous FcγRIIIa-Phe/Phe158 and heterozygous FcγRIIIa-Phe/Val158 effector cells. Interestingly, the ADCC enhancement mediated by MDX-1401 was more pronounced in FcγRIIIa-Phe/Val158 effector cells than in those of the heterozygote. This observation is particularly relevant from a clinical standpoint because the allelic frequency of FcγRIIIa-Phe158 is much higher than the frequency of FcγRIIIa-Val158 in all ethnic groups (16,33). Thus, MDX-1401 may be especially beneficial to the majority of patients who are poorly responsive to traditional mAb therapy.

In addition to a correlation of ADCC with the number of CD30 antigens per cell, an important observation in this study was MDX-1401 enhanced ADCC activity among a large panel of tumor cell lines with a broad range of CD30 receptor expression, including one cell line with a low number of receptors per cell in which the parental fucosylated mAb failed to induce ADCC. These data are consistent with previous reports describing other antibody/antigen systems (34). Because CD30 expression on human tumors is often low and heterogeneous, MDX-1401, like other nonfucosylated antibodies, may be therapeutically advantageous.

Demonstration of improved in vivo efficacy of nonfucosylated antibodies over their fucosylated counterparts has proven to be challenging because mouse natural killer cells have similar binding affinities to fucosylated and nonfucosylated antibodies. Indeed, human peripheral blood mononuclear cell engraftment into SCID mice has been necessary to show improved potency with nonfucosylated antibodies in vivo (26). However, murine FcγRI has been reported to show improved binding to nonfucosylated antibodies (35). FcγRI is homologous to human FcγRIIIa (30) but is present largely on macrophages, monocytes, and neutrophils (36).

The similar in vivo efficacy of MDX-060 and MDX-1401 in s.c. implanted tumors (data not shown) prompted a study in a systemic model. An ALCI model was established by i.v. injection of human Karpas-299 lymphoma cells in SCID mice. In this model, the efficacy of anti-CD30 mAbs relied on FcR-dependent activity. This was shown by the fact that the N297Q mutant variant of MDX-060 mAb was completely ineffective, although in vitro the N297Q mutant retained the ability to kill tumor cells by direct signaling leading to apoptosis (data not shown). The improved efficacy of MDX-1401 in this systemic ALCI model may be due to FcR-mediated function based on the increased binding of MDX-1401 to murine FcγRI in this system. Zhang and colleagues (37) have shown that the activity of anti-CD30 antibody He-Fi-1 in the Karpas-299 xenograft model does not require ADCC. This discrepancy is not fully understood but can be explained, in part, by differences in the use of nonobese diabetic SCID versus CB17 SCID mice or by differences in the antibodies that were used in the two studies. Here,
it was shown that at a suboptimal prophylactic dose of 1 mg/kg nonfucosylated MDX-1401 significantly prolonged survival, whereas mutant N297Q mAb was ineffective. This result shows improved in vivo antitumor efficacy of MDX-1401 achieved at a low dose and suggests a potential clinical advantage of nonfucosylated mAbs over traditional fucosylated mAbs. However, as stated earlier, demonstration of superior antitumor activity due to enhanced effector function is extremely difficult in mouse models and confirmation must come from testing MDX-1401 in lymphoma patients.

A phase 1/2 clinical study with MDX-060 showed that the antibody is well tolerated up to 15 mg/kg. As a single agent, MDX-060 had limited activity; however, the data were intriguing in that 25 of 72 treated patients had stable disease, implying that either combination therapy or an enhanced antibody approach might be warranted. Likewise, a phase 1 multidose study was done with another anti-CD30 antibody, SGN-30, and modest activity was also noted (7), indicating that trials with an improved antibody might be justified. Additional strategies to enhance the efficacy of therapeutic antibodies are being pursued. An antibody-drug conjugate in which a cytotoxic compound is linked to CD30 antibody is in clinical development (38).

In conclusion, the newly generated nonfucosylated MDX-1401 antibody binds with higher affinity to FcRyIIIA receptors, has greatly improved in vitro ADCC activity, and is more efficacious in an in vivo tumor model relative to its fucosylated counterpart, MDX-060. The low doses of antibody required for in vitro ADCC and in vivo tumor efficacy, together with improved activity among cell lines with a broad range of CD30 expression, irrespective of donor genotype, suggest that clinical studies with MDX-1401 are warranted.

References


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