In vivo effects of targeting CD79b with antibodies and antibody-drug conjugates

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Abstract

Antibodies directed against B cells are in use for the treatment of non-Hodgkin's lymphoma and autoimmune disorders. The B-cell–restricted surface antigen CD79b, a signaling component of the B-cell receptor, has been shown as a promising antibody target in mouse efficacy models of systemic lupus erythematosus. Anti-CD79b antibody-drug conjugates (ADC), cytotoxic drugs linked through specialized chemical linkers to antibodies, are effective in mouse xenograft models of non-Hodgkin's lymphoma. We were interested in evaluating the systemic effects of anti-CD79b antibodies and ADCs in normal animals as a step toward the development of these molecules as therapeutics. As we were unable to identify any cell surface binding anti-human CD79b antibodies that were cross-reactive to other species, we developed an antibody to cynomolgus monkey (Macaca fascicularis) CD79b (anti-cyCD79b). The anti-cynomolgus antibody, anti-cyCD79b (10D10), and the maytansine (tubulin inhibitor)–conjugated ADC, anti-cyCD79b (10D10)-MCC-DM1, were administered to cynomolgus monkeys at ~30 mg/kg for two doses 3 weeks apart. Anti-cyCD79b and anti-cyCD79b-MCC-DM1 resulted in peripheral blood B-cell depletion of ~65% and ~94%, respectively. In addition, anti-cyCD79b-MCC-DM1 resulted in near-complete absence of splenic germinal centers, an observation supporting an effect on dividing B cells. Both molecules were well tolerated, with minimal findings for the antibody and findings for the ADC limited to the lymphoid and hematopoietic systems, liver, and peripheral nerves. These preclinical data suggest that targeting CD79b with antibodies or ADCs may provide safe and effective therapies for B-cell malignancies and autoimmune diseases.

Introduction

Antibody-based therapeutic approaches have revolutionized the treatment of non-Hodgkin’s lymphoma and other B-cell malignancies. However, given the biological and clinical heterogeneity of the diseases, a large variability in clinical response has been observed. Currently, patients with refractory or relapsed lymphomas have limited therapeutic options; therefore, the need to develop effective new treatments remains. A promising approach for the treatment of non-Hodgkin’s lymphoma that has yet to achieve clinical success is the use of antibody-drug conjugates (ADC), cytotoxic drugs covalently linked to antibodies through specialized chemical linkers (1–4). In addition, antibodies directed to B-cell targets such as rituximab and epratuzumab are in clinical trials for the treatment of autoimmune disorders (5, 6). The presumed mechanism of action for these antibodies when used in autoimmune indications is depletion of B cells, thus down-modulating the immune reaction.

CD79, the signaling component of the B-cell receptor (BCR), is a promising target of antibodies and ADCs for the treatment of B-cell malignancies and autoimmune diseases. CD79 is a heterodimer, consisting of CD79a and CD79b, and is an attractive target because it is B cell specific and is expressed in almost all forms of non-Hodgkin’s lymphoma and most forms of chronic lymphocytic leukemia (7–9). CD79 is particularly attractive for use as an ADC because the receptor is trafficked to a lysosome-like compartment as part of antigen presentation (10), thereby providing a mechanism for enhancing the release of drug from the ADC.

We have shown that anti-human CD79b (huCD79b) antibodies conjugated on lysine residues to the maytansinoid N(N′)-(2′)-deacetyl-N(2′)-(3-mercapto-1-oxopropyl)-maytansine (DM1), an antimitotic tubulin inhibitor, through the thioether linker succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (MCC; refs. 11, 12) are effective in xenograft models of non-Hodgkin’s lymphoma (13). Single doses of anti-huCD79b (SN8)-MCC-DM1 resulted in complete regression of Burkitt’s, follicular, and mantle-cell lymphoma xenograft tumors, although several of these cell lines express relatively low levels of surface CD79b. Furthermore, anti-CD79 antibodies have been shown to inhibit autoimmune disease in mouse models of systemic lupus erythematosus (14).

Despite the appeal of this target for the treatment of B-cell disorders, the only data on the in vivo effects of anti-CD79 antibodies and ADCs come from mouse efficacy models (13, 14). B-cell-depleting antibodies such as anti-CD20 (Rituxan)
have been successfully developed for clinical use (15) and the ADC trastuzumab (Herceptin)-MCC-DM1 has shown promising safety and efficacy in early clinical trials (16). However, there are several additional safety considerations for an antibody or ADC developed against CD79b. As the signaling component of the BCR, it is possible that binding of CD79b by an antibody could trigger signaling events that result in the release of cytokines, although the signaling by anti-CD79b antibodies is relatively weak (17, 18). Other potential concerns in ADC-mediated targeting of CD79b include possible exacerbation of hematologic toxicities by increasing the accumulation of the cytotoxic drug in the bone marrow and the possibility of increasing the nonspecific release of cytotoxic drug following clearance of ADC-bound B cells by the mononuclear phagocyte system. We therefore designed studies to evaluate the effects of anti-CD79b antibodies and ADCs in vivo. Our initial aim was to test an antibody directed against the huCD79b that cross-reacted with an animal species routinely used in toxicology studies. However, because we could not identify any cell surface binding anti-huCD79b antibodies that were cross-reactive, we developed antibodies to cynomolgus monkey (Macaca fascicularis) CD79b (cyCD79b). For nonclinical studies, we selected an anti-CD79b antibody with similar properties to anti-huCD79b (SN8), an antibody that has been described previously as a particularly effective ADC in non-Hodgkin’s lymphoma xenograft models (13). The results of studies in cynomolgus monkeys, following the administration of this antibody and an ADC made from this antibody, show the potential of CD79b as target antibody and ADCs for the treatment of B-cell disorders.

Materials and Methods

Antibodies and ADCs

Anti-huCD79b (SN8) was obtained from Ben Seon; anti-huCD79b (2F2), anti-huCD79b (17A7), and anti-cyCD79b (10D10) were generated at Genentech as described previously; and anti-huCD79b (AT-105.1; AbD Serotec) and anti-huCD79b (CB3.1; BD Pharmingen) were purchased commercially. Anti-cyCD79b (10D10), anti-huCD79b (2F2), and anti-huCD79b (SN8) were generated as mouse/human chimeric antibodies as described previously (13). Antibody-MCC-DM1 conjugates were made as described previously (13).

Flow Cytometry and Peptide Competition

To assay the CD79b expression on xenograft tumors, recovered tumors were minced and put through a 30 μm cell strainer (BD Biosciences) to achieve a single-cell suspension. The tumor cells were subsequently prepared by the standard density centrifugation over lymphocyte separation medium (MP Biomedical). The resulting single-cell suspension was stained with either naked antibodies or biotinylated antibodies followed by rat anti-mouse IgG1-PE or streptavidin-APC (BD Bioscience) mixed with anti-CD20-FITC and 7-amino-actinomycin D. Data were analyzed using FlowJo (Tree Star), and graphs were plotted from the CD20+ and 7-amino-actinomycin D-negative population.

For the competition assay, both anti-huCD79b and anti-cyCD79b antibodies were preincubated with an excess amount of synthesized peptide (10×) or purified human or cynomolgus monkey CD79b proteins (3×) for 30 min at 4°C in the dark before they were added to the cells for standard staining. Rat anti-mouse IgG1-PE antibody was used as the secondary detecting reagent.

Cloning of cyCD79b and Generation of a cyCD79b-Expressing Stable Cell Line

CD79b cDNA was PCR amplified from a cynomolgus monkey spleen cell library (James Lee, Genentech) and TA cloned into pcDNA3.1/VS-His-TOPO (Invitrogen). The open reading frame was PCR cloned into pRK2004.huFc (Genentech) for expression of soluble cyCD79b human Fc-tagged protein. The open reading frame was transferred by restriction digest into mammalian expression vector pRK.CMVP.D.nbe (Amy Shen, Genentech) for generation of a cyCD79b-expressing cell line. The pRK.CMVP.D.nbe. cyCD79b expression construct was transfected into BJAB by Nucleofection (Amaxa), using Solution T, ProgramT-16, and 5 μg DNA/× 106 cells. After selection in 1 μg/mL puromycin (Calbiochem), cells were autolocined for the top 5% expressing cells using a Coulter Epics Elite Fluorescence-Activated Cell Sorting (FACS) System. The highest expressing clone, BJAB.CMVP.D.nbe.cyCD79b.E3, was determined by FACS.

Xenograft Models

All animal studies were done in accordance with Institutional Animal Care and Use Committee-approved guidelines. Cells were washed and suspended in HBSS (Hyclone) and 2 × 107 cells were inoculated s.c. into the flank of each female C.B17/ICR severe combined immunodeficient mouse (6-8 weeks old from Charles Rivers Laboratories) in a volume of 0.2 mL. When mean tumor size reached desired volume, the mice were divided into groups of 8 to 10 with the same mean tumor size and dosed i.v. via the tail vein with ADCs or antibodies. The doses of ADC administered to mice were measured as the mass of the conjugated cytotoxic small-molecule drug. For example, mice were dosed with 100 mg antibody/kg (100 mg/kg), which was equivalent to 300 μg DM1/m2 (300 μg/m2).

In vivo Study in Cynomolgus Monkeys

Study Design. A cynomolgus monkey study was conducted at Shin Nippon Biomedical Laboratories USA in compliance with the NIH regulations concerning the use of laboratory animals. Twelve 2.6- to 5.8-year-old female monkeys (2.5-4.1 kg) were assigned to three dose groups (four per group) using a stratified randomization scheme based on individual animal body weights and CD20+ absolute lymphocyte counts. Animals were dosed i.v. on days 1 and 22 with vehicle, 30 mg/kg anti-cyCD79b (10D10), or -30 mg/kg (6,000 μg DM1/m2) anti-cyCD79b (10D10)-MCC-DM1 and were monitored twice daily for mortality, clinical abnormalities, and food consumption. Body weights were measured twice during the pre-dose phase, weekly throughout the study, and on the day of necropsy. Analyses of hematology, clinical chemistry, coagulation, toxicokinetics,
and anti-therapeutic antibody were done on blood collected during the pre-dose phase and at various time points throughout the study. All animals were euthanized and necropsied on day 43 and tissues were thoroughly evaluated by gross and microscopic examination. Urinalysis was done on urine collected by cystocentesis at necropsy.

**Flow Cytometry of Peripheral Blood and Lymphoid Tissues.** Flow cytometry and analysis of B-cell, T-cell, and NK-cell counts were done on whole peripheral blood (in-life phase of study) with the following combinations: IgG-FITC, IgG-PE, and IgG-APC; anti-CD3-FITC, anti-CD4-PE, and anti-CD8-APC; anti-CD3-FITC, anti-CD16-PE, and anti-CD20-APC; and anti-CD27-FITC, anti-CD21-PE, and anti-CD20-APC (BD Pharmingen). Blood samples were collected twice during the pre-dose phase and on days 1 (pre-dose), 2, 4, 10, 15, 22 (pre-dose), 23, 25, 29, 36, and 43. Samples were maintained at 2°C to 8°C until analyzed by flow cytometry. Whole blood (0.1 mL) was added to the immunophenotyping antibody cocktail (20 μL of each antibody) and incubated in the dark at ambient room temperature for 15 to 20 min. Leukocytes were isolated by whole-blood lysis using BD Lyse Solution (BD Pharmingen) according to the manufacturer's instructions. Cells were washed once by adding 2 mL calcium and magnesium-free PBS, resuspended in 300 μL of 0.5% formaldehyde in calcium and magnesium-free PBS, and analyzed by flow cytometry. Relative percentages of CD20+ B cells (as a percentage of total lymphocytes) were obtained from cytograms. The absolute counts were calculated from their relative lymphocyte percentages (percentage of total lymphocytes) and the absolute lymphocyte counts (from the hematology samples) according to the formula: absolute count (×10^7/μL) = (relative percentage × lymphocyte absolute count) / 100. Absolute counts were tabulated for each lymphocyte subset and further analyzed as a percentage of pre-dose baseline for each individual animal. The pre-dose baseline is defined as the average of all values collected at study specified time points before the first dose.

Tissue cells from bone marrow aspirates, spleen, and mesenteric and mandibular lymph nodes were collected at necropsy, and cell suspensions were prepared by passing through 100 mm cell strainer and washed twice with PBS/0.5% bovine serum albumin. Samples were then counted by Z2 Coulter counter and maintained at temperature range of 2°C to 8°C until analyzed by flow cytometry. Total CD20+ B cells were assessed by flow cytometry analysis similar to peripheral blood. The relative percentages (as a percentage of total lymphocytes) for each of these subsets were tabulated and analyzed as a percentage of the vehicle-treated control group (% vehicle) for each individual animal. This is calculated by taking the percent gated value for each individual animal and dividing by the average percent gated values of the vehicle-treated control group.

**Cytokine Assays.** Blood samples were taken on day 1 (pre-dose and 5 min and 6 h post-dose) and day 2 (24 h post-dose) for the evaluation of cytokine levels. Samples were collected and transferred to lithium heparin tubes and plasma was obtained by centrifugation (2,000 × g, 15 min, 2-8°C). Samples were stored at −80°C with a maximum of two freeze/thaw cycles before analysis of cytokine levels. Cytokine levels were quantified from the cynomolgus plasma samples using the MILLIPLEX Nonhuman Primate Cytokine Kit (Millipore) according to the manufacturer with some modifications. Samples were diluted 2-fold, with 0.5x serum matrix, followed by 2-fold serial dilutions up to four points. The standards were diluted with Assay Buffer to generate the following concentrations: 2,000, 666.7, 222.2, 74.1, 24.7, 8.2, 4.1, 2.1, and 1.0 pg/mL. Limit of detection was 2 pg/mL for all standards. Samples were read on a Luminex 200. The following 23 cytokines and chemokines were simultaneously quantified: interleukin (IL)-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12/23 (p40), IL-13, IL-15, IL-17, IL-18, IFN-γ, granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, monocyte chemotactic protein-1, macrophage inflammatory protein (MIP)-1α, MIP-1β, tumor necrosis factor-α, transforming growth factor-α (TGF-α), soluble CD40 ligand, and vascular endothelial growth factor.

**Immunohistochemistry.** Adjacent sections of spleen from each animal were collected, placed into a cryomold surrounded by Tissue-Tek OCT compound (Sakura Finetek), and flash-frozen in dry ice and 2-methylbutane for immunohistochemistry evaluation. Tissues were sectioned at 5 μm on glass slides and fixed in acetone. Endogenous peroxidases were quenched using a blocking solution (KPL) and endogenous biotin was blocked using an avidin/biotin blocking kit (Vector Laboratories). Slides were incubated with either 1 μg/mL anti-Ki-67 (clone MIB-1 from DAKO) or 5 μg/mL anti-CD20 (clone L26 from DAKO). Nonspecific isotype control antibodies were used in place of antibodies to CD20 or Ki-67 as negative controls. Slides were then incubated with either biotinylated 2.5 μg/mL horse anti-mouse IgG (H + L; Vector Laboratories) or 7.5 μg/mL goat anti-rabbit IgG (H + L; Vector Laboratories) followed by incubation with Vectastain ABC Elite Reagents and Pierce Metal Enhanced DAB. Slides were counterstained with Mayer’s hematoxylin for viewing by light microscopy.

**Results**

**Generation of Cell Surface Binding Species Cross-Reactive Anti-CD79 Antibodies Is Hindered by Sequence Variability in the NH2 Terminus of CD79b**

To evaluate the in vivo effects of antibodies and ADCs, our aim was to identify an antibody that binds with comparable affinity to humans as well as to an animal model CD79b. Thus, we screened all the available anti-CD79b antibodies (both commercially available and antibodies that we generated) that bound extracellular surface CD79b for the ability to bind B cells from mouse (Mus musculus), rat (Rattus norvegicus), dog (Canis lupus familiaris), cynomolgus monkey (M. fascicularis), rhesus monkey (Macaca mulatta), and baboon (Papio hamadryas). None of the six anti-huCD79b antibodies we tested cross-reacted with any of these species (data not shown). As reported previously, anti-huCD79a ADCs were not as effective as those directed against CD79b (13). Anti-huCD79a antibodies also did not

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cross-react with the nonhuman species tested (data not shown). To investigate the reason that the anti-huCD79b antibodies were not cross-reactive with other species, we cloned and sequenced cyCD79b; an alignment of human, cynomolgus monkey, and mouse CD79b is shown in Fig. 1A. Previous studies have shown that a spliced form of CD79b was recognized by two of the antibodies in our panel (19). By excluding the spliced region and regions of the extracellular domain identical between human and cynomolgus monkey CD79b as potential binding sites for the anti-huCD79b antibodies, the NH₂ terminus of CD79b was identified as the likely epitope for the anti-huCD79b antibodies (Fig. 1A). To test this directly, we synthesized two 21–amino acid peptides that corresponded to the NH₂ terminus of huCD79b and cyCD79b and tested their abilities to compete for binding of anti-huCD79b antibodies to a B-cell line (BJAB) in a FACS assay. All of the anti-huCD79b antibodies we tested were competed by the huCD79b peptide but not by the cyCD79b peptide despite only 3–amino acid differences in their sequences (Fig. 1B). These data suggest that all of our antibodies bind the NH₂ terminus of huCD79b and that sequence variability in this region is the reason we do not observe cross-reactivity in other species. It is not clear why all the surface binding antibodies bind this portion of CD79b. As all the antibodies were generated in the mouse, it is possible that the NH₂ terminus is an immunodominant epitope or that it is the only sterically available portion of CD79b when part of the BCR complex.

**Figure 1.** All FACS-reactive anti-CD79b antibodies bind the N-terminus of CD79b. **A,** alignment of human, cynomolgus monkey, and mouse CD79b amino acid sequence. The signal sequence, transmembrane (TM), Immuno Tyrosine Activation Motif (ITAM), and the peptide sequence used in competition experiments (test peptide) are indicated. **B,** FACS reactive anti-human CD79b antibodies bind the same epitope. Anti-CD79b antibodies we tested for binding to BJAB cell in the presence and absence of the indicated competitor: peptide corresponding to the N-terminus of CD79b for human and cynomolgus monkeys (illustrated, sequence difference in bold). **C,** same as in panel B except that the cells for the anti-cyCD79b (10D10) were primary cynomolgus B-cells.

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**Generation of an Antibody Directed to Cynomolgus CD79b**

Generating anti-CD79b antibodies and ADCs as therapeutics for B-cell disorders necessitates evaluation of the effects of the molecules in an animal species commonly used in drug safety studies. As our data suggested that developing...
a cross-reactive anti-huCD79b antibody would be difficult if not impossible, we concluded that we needed to develop a surrogate antibody. We examined a panel of anti-CD79b antibodies in xenograft models of non-Hodgkin’s lymphoma and concluded that the anti-huCD79b (SN8) antibody had the most potent antitumor activity as an ADC (13). Therefore, our strategy was to identify an anti-cyCD79b antibody with similar binding properties to SN8. We generated a panel of mouse monoclonal antibodies to cyCD79b and tested their binding to cynomolgus monkey B cells by flow cytometry. From the panel, we identified 10 antibodies that had high affinity to cell surface cyCD79b. As expected, the antibodies did not bind human B cells or huCD79b peptide but did bind the cyCD79b peptide (Fig. 1C; data not shown). The anti-cyCD79b (10D10) had comparable affinity to cyCD79b as anti-huCD79b (SN8) to huCD79b when measured by Scatchard analysis ($K_d = 0.3$ and $0.5$ nmol/L, respectively). Thus, we selected anti-cyCD79b (10D10) for testing of anti-CD79b antibodies and ADCs in cynomolgus monkeys. The anti-cyCD79b and anti-huCD79b mouse antibodies were subsequently cloned as human IgG1 mouse chimeric antibodies and the affinities were measured by Scatchard analysis. The chimeric antibodies anti-cyCD79b (10D10) and anti-huCD79b (SN8) had $K_d$ values of 0.6 and 0.3 nmol/L, respectively.

Although the affinities and epitopes of anti-huCD79b (SN8) and anti-cyCD79b (10D10) were comparable, we wanted to know whether the activities of their ADCs were similar. To do this, we developed a non-Hodgkin’s lymphoma cell line that expressed similar amounts of surface cynomolgus monkey and huCD79b (BJAB-cyCD79b; see Materials and Methods) so that the same cell line could be used to evaluate both ADCs. The expression levels of cynomolgus monkey and huCD79b on BJAB-cyCD79b in culture and in s.c. mouse xenograft tumors are shown in Fig. 2A. We tested the activity of anti-huCD79b (SN8)-MCC-DM1 and anti-cyCD79b (10D10)-MCC-DM1 in xenograft models of BJAB-cyCD79b and the two ADCs had comparable activities (Fig. 2B), suggesting that the biological effect of targeting CD79b by ADCs in cynomolgus monkey and humans is similar. Although a surrogate antibody by nature cannot claim to be equivalent, these data support the use of anti-cyCD79b (10D10)-MCC-DM1 for evaluation of the effects of an anti-CD79b ADC in the cynomolgus monkey.

**Anti-cyCD79b and Anti-cyCD79b-MCC-DM1 Depleted B Cells In vivo and Were Well Tolerated in Cynomolgus Monkeys**

Cynomolgus monkeys were administered two i.v. doses, 3 weeks apart (days 1 and 22), of vehicle, 30 mg/kg anti-cyCD79b (10D10; unconjugated), or ~30 mg/kg anti-cyCD79b (10D10)-MCC-DM1 (6,000 μg D M1/m2; conjugated DM1) and were monitored for changes in clinical observations, food consumption, body weight, routine clinical chemistry panels (hematology, serum chemistry, coagulation, and urinalysis), and peripheral blood lymphocyte subsets over...
All animals were necropsied on day 43 for evaluation of organ weight, macroscopic, and microscopic changes in tissues. All animals maintained body weight and were active and alert throughout the study. Toxicokinetic analysis confirmed exposure of the test articles in all animals (data not shown). Depletion of peripheral blood CD20+ B cells was detected by flow cytometric evaluation on day 2 in both groups given the test articles, with the group given anti-cyCD79b (10D10)-MCC-DM1 showing slightly greater depletion than the group given unconjugated anti-cyCD79b (10D10; Fig. 3A). Sustained depletion of CD20+ B cells throughout the study was more pronounced in the group given anti-cyCD79b (10D10)-MCC-DM1, and this finding correlated well with the depletion of B cells observed by flow cytometry in lymphoid tissues (bone marrow, lymph nodes, and spleen) taken at necropsy (Fig. 3B). There was no substantial difference in T cells or NK cells in animals dosed with anti-cyCD79b (10D10), anti-cyCD79b (10D10)-ADC, or vehicle, pointing to the specificity of the anti-CD79b antibodies for B cells (Fig. 3C and D). The initial rapid depletion of B cells was likely due to antibody-mediated clearance by the mononuclear phagocyte system. In vitro data suggest that anti-cyCD79b (10D10) can mediate antibody-dependent cell-mediated cytotoxicity (data not shown). Further, we attribute the sustained B-cell depletion of the ADC to the anti-mitotic pharmacology expected following BCR-bound ADC internalization. Evidence of this pharmacologic activity was observed on histologic sections of the spleen, tonsil, and lymph nodes that revealed marked depletion of germinal

**Figure 3.** Effect of anti-cyCD79b (10D10) and anti-cyCD79b (10D10)-MCC-DM1 on CD20+ B-cells in cynomolgus monkeys. Cynomolgus monkeys were given two IV doses of the indicated amount of anti-cyCD79b or anti-cyCD79b (10D10)-MCC-DM1, three weeks apart (n = 4 animals/group). Peripheral blood B-cells were monitored over a 43-day period and were normalized to pre-dose levels (% baseline, BL). Tissue B-cells were assessed at necropsy (on Study Day 43) and are normalized to the mean of the vehicle group. A, peripheral blood CD20+ B-cells were monitored. B-cells post-dose: statistically significant difference among the treatments, with P-value < 0.0001. Compared to Vehicle, anti-cyCD79b resulted in a 40.7 reduction, with 95% confidence interval (CI) [27.7, 53.7]. Compared to Vehicle, anti-cyCD79b-MCC-DM1 led to a 70.3 reduction, 95% CI [57.3, 83.3]. Compared to 10D10, cl10D10-ADC resulted in a 29.6 decrease, 95% CI [16.6, 42.6]. B, tissue CD20+ B-cells was monitored. A significant treatment effect was found (P-value < 0.00001). C, peripheral blood CD3+ T-cells were monitored. There were no significant differences (percentage) among the treatments, either pre- or post-dose. D, peripheral blood CD20- CD3- NK-cells of the animals were monitored. There were no significant differences (percentage) among the treatments, either pre- or post-dose.
center B cells in the group given the ADC (Fig. 4). Anti-CD20 staining results correlated well with tissue CD20+ B cells quantified by flow cytometry, as animals treated with anti-cyCD79b (10D10)-MCC-DM1 showed more pronounced decreases in CD20+ B cells compared with animals treated with anti-cyCD79b (10D10). The cell proliferation marker, anti-Ki-67, highlights the effect on proliferating B cells as near-complete absence of germinal centers in the spleen of monkeys given anti-cyCD79b (10D10)-MCC-DM1.

Other than peripheral and lymphoid tissue B-cell depletion, changes identified in the monkeys given unconjugated anti-cyCD79b (10D10) were limited to decreased thymic weight and thymic lymphoid depletion (response to physiologic “stress”) and hepatic sinusoidal cell hypertrophy and hyperplasia [response to exogenous protein (IgG) administration].

In monkeys administered anti-cyCD79b (10D10)-MCC-DM1, hematology revealed reversible decreases in platelets following each dose (Fig. 5). Decreases in RBC mass (RBC counts, hematocrit, and hemoglobin) were observed in all groups following dosing and collection of blood for toxicokinetic and flow cytometric evaluations; however, sustained but reversible decreases in RBC mass were seen in monkeys given the ADC (Fig. 5; data not shown).

Notable changes in clinical chemistry in monkeys given anti-cyCD79b (10D10)-MCC-DM1 included reversible elevations in liver enzymes (aspartate transferase, alkaline phosphatase, and alanine transferase) as well as decreases in albumin and increases in globulins and fibrinogen (Fig. 5; data not shown). Histopathologic evaluation of the liver revealed minimal hydropic degeneration of hepatocytes, as well as hypertrophy and hyperplasia of sinusoidal cells.

Thymic weight decreases and thymic lymphoid depletion was also noted and the effect was more pronounced in the ADC-dosed group than in the unconjugated antibody-dosed group. In addition, minimal axonal degeneration of the sciatic nerve was seen in the anti-cyCD79b (10D10)-MCC-DM1 group, a finding not unexpected for tubulin-inhibitory drugs.

Given that the CD79b is a signaling component of the BCR, anti-CD79b antibodies have the potential to induce BCR signaling that may result in the production of cytokines; in particular, IL-1β, IL-6, IL-10, TGF-α, granulocyte-macrophage colony-stimulating factor, MIP-1α, and MIP-1β have been shown to be produced by B cells on stimulation of the BCR (20–22). Therefore, we evaluated a panel of 23 cytokines in all monkeys for evidence of systemic cytokine release (see Materials and Methods). Granulocyte colony-stimulating factor, INF-γ, IL-1β, IL-2, IL-4, IL-5, IL-10, IL-13, IL-17, TGF-α, vascular endothelial growth factor, and IL-18 were all below the lower limit of detection. IL-12/23 (p40), IL-15, IL-1ra, IL-6, IL-8, MIP-1α, MIP-1β, monocyte chemotactic protein-1, soluble CD40 ligand, granulocyte-macrophage colony-stimulating factor, and TGF-α were detected in some of the animals (Fig. 6; data not shown). However, no increases in any of the detected cytokines over the vehicle control group were observed in the anti-cyCD79b or anti-cyCD79b-MCC-DM1 groups. In some cases, cynomolgus monkeys are not predictive of cytokine release in humans; however, this appears to be a property of the target and not a general property of cynomolgus monkeys (23). In vitro cytokine release data...
with the humanized anti-huCD79b (SN8) in human peripheral blood mononuclear cells was also done and showed increases in IP-10 and IL-1α above the negative control antibody trastuzumab; however, in both cases, the increase was less than that observed for anti-CD3 (OKT3; Supplementary Fig. S1). All other cytokines evaluated (eotaxin, granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, IFN-α2, INF-γ, IL-1α, IL-1β, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p40), IL-12 p70, IL-13, IL-15, IL-17, IP-10, monocyte chemotactic protein-1, MIP-1α, MIP-1β, soluble CD40 ligand, TGF-α, tumor necrosis factor-α, TNF-β, and vascular endothelial growth factor) were not elevated above the negative control antibody. These data suggest that the risk of systemic cytokine release following administration of an anti-CD79b antibody in patients is low.

From this study, we concluded that targeting cyCD79b with an antibody or ADC was well tolerated in cynomolgus monkeys at ~30 mg/kg (6,000 μg DM1/m²). Furthermore, this study provided evidence of pharmacologic activity, that is, depletion of peripheral and tissue B cells and, in
particular, the depletion of dividing B cells in germinal centers by the ADC.

Discussion

As antibodies to huCD79b did not cross-react with other species, we developed a surrogate antibody to cynomolgus monkey CD79b that had a similar epitope and affinity as the potential human therapeutic antibody, anti-huCD79b (SN8), to evaluate the effects of an anti-CD79b antibody and an anti-CD79b ADC in nonhuman primates. The anti-cynomolgus antibody anti-cyCD79b (10D10) and the ADC anti-cyCD79b (10D10)-MCC-DM1 were well tolerated and resulted in reduced peripheral and tissue B cells.

Figure 6. Effect of anti-cyCD79b (10D10) and anti-cyCD79b (10D10)-MCC-DM1 on cytokine levels in cynomolgus monkeys. Plasma cytokine levels were quantified for 23 cytokines to determine if anti-CD79b antibodies induced B-cell receptor signaling and systemic cytokine release. Data is shown for each animal (4/group) at 4 timepoints (pre-dose, and 5 min, 6 hr, and 24 hr post-dose) for IL-1ra, IL-6, IL-8, IL-12/23 (p40), IL-15, GM-CSF, MCP-1, MIP-1α, MIP-1β, sCD40L, and TGF-α. Data not shown for IL-1β, IL-2, IL-4, IL-5, IL-10, IL-13, IL-17, IL-18, IFN-γ, G-CSF, TNF-α, and VEGF (signal < 2 pg/ml limit of detection).
As reduction of B cells has been shown to be an effective means of treating certain autoimmune diseases, our data suggest that CD79b is a promising target for B-cell disorders. For example, the anti-CD22 therapeutic antibody epratuzumab is currently being evaluated in clinical trials for systemic lupus erythematosus and primary Sjögren’s syndrome (24). The maximal B-cell depletion following two doses of the unconjugated anti-cyCD79b antibody in cynomolgus monkeys was 65%, which is comparable with the 50% depletion observed in humans dosed with epratuzumab (anti-CD22; ref. 24). These results suggest that an anti-CD79b antibody could be effective in treating autoimmune diseases.

The ADC anti-cyCD79b-MCC-DM1 resulted in even greater B-cell depletion (94%) in cynomolgus monkeys. Additionally, our observation that germinal center B cells were depleted by anti-cyCD79b-MCC-DM1 is encouraging from a therapeutic perspective, as anti-CD79b ADCs may be expected to target proliferating non-Hodgkin’s lymphoma cells in a manner similar to dividing B cells in germinal centers. These data, along with the efficacy seen in xenograft models (Fig. 2B; ref. 13), suggest that anti-CD79b-MCC-DM1, if clinically well tolerated, could be particularly effective against B-cell neoplastic disorders such as non-Hodgkin’s lymphoma. Recent data suggest the MCC-DM1 ADCs have promise clinically, as trastuzumab (Herceptin)-MCC-DM1 antibodies may be promising therapeutic agents in human B cells. Our data suggest that targeting CD79b with an ADC composed of the same linker-drug would be similarly well-tolerated.

In summary, our data suggest that anti-CD79b and anti-CD79b-MCC-DM1 antibodies may be promising therapeutics for the treatment of autoimmune diseases and B-cell malignancies such as non-Hodgkin’s lymphoma.

Disclosure of Potential Conflicts of Interest

All authors were employees of Genentech, Inc., during the conduct of this work.

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References

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