Therapeutic potential of an anti-CD79b antibody–drug conjugate, anti–CD79b-vc-MMAE, for the treatment of non-Hodgkin lymphoma

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Here we describe the generation of an antibody–drug conjugate (ADC) consisting of a humanized anti-CD79b antibody that is conjugated to monomethylauristatin E (MMAE) through engineered cysteines (THIOMABs) by a protease cleavable linker. By using flow cytometry, we detected the surface expression of CD79b in almost all non-Hodgkin lymphoma (NHL) and chronic lymphocytic leukemia patients, suggesting that anti–CD79b-vcMMAE could be widely used in these malignancies. By using NHL cell lines to simulate a patient population we discovered that a minimal cell-surface expression level of CD79b was required for in vitro activity. Within the subpopulation of cell lines above this minimal threshold, we found that sensitivity to free MMAE, mutation of cancer genes, and cell doubling time were poorly correlated with in vitro activity; however, the expression level of BCL-XL was correlated with reduced sensitivity to anti–CD79b-vcMMAE. This observation was supported by in vivo data showing that a Bcl-2 family inhibitor, ABT-263, strikingly enhanced the activity of anti–CD79b-vcMMAE. Furthermore, anti–CD79b-vcMMAE was significantly more effective than a standard-of-care regimen, R-CHOP (ie, rituximab with a single intravenous injection of 30 mg/kg cyclophosphamide, 2.475 mg/kg doxorubicin, 0.375 mg/kg vincristine, and oral dosing of 0.15 mg/kg prednisone once a day for 5 days), in 3 xenograft models of NHL. Together, these data suggest that anti–CD79b-vcMMAE could be broadly efficacious for the treatment of NHL. (Blood. 2009;114:2721-2729)

Introduction

B-cell non-Hodgkin lymphomas (NHLs) are clinically heterogeneous, varying widely in genetic drivers of transformation and clinical outcome.1 Standard treatment for most subtypes of NHL usually involves combination chemotherapy and/or rituximab anti-CD20 monoclonal antibody therapy, and in some cases such therapies can be curative. However, most NHLs are not cured and result in 19 660 mortalities per year in the United States,2 thus illustrating the need for more effective therapies to improve patient outcomes.

One attractive approach for the treatment of NHL is the use of antibody–drug conjugates (ADCs), cytotoxic drugs covalently linked to antibodies through specialized chemical linkers. ADCs provide a means to improve the potency of chemotherapy by increasing the accumulation of cytotoxic drug within neoplastic cells while reducing the nonspecific cytotoxic effects of systemic drug administration.3,4 The success of gemtuzumab ozogamicin (Mylotarg; Wyeth), an anti-CD33 calicheamicin ADC, in the treatment of acute myeloid leukemia demonstrates the potential for this technology in the treatment of hematologic malignancies.5 Furthermore, the characteristics of NHL strongly support it as an indication for ADCs. NHL is responsive to chemotherapy, unconjugated antibody therapies, and radioconjugate therapies, suggesting that these tumors are accessible to antibody-based therapies and would ultimately be responsive to cytotoxic drugs delivered by an ADC. It is also a highly attractive feature of ADCs that specific targeting of chemotherapeutic agents to tumor cells may blunt the undesirable effects of systemic administration of chemotherapy.

Since the development of gemtuzumab ozogamicin, several second-generation ADC linker-drug technologies have been developed and are showing promising results in early clinical trials.3 For example, SGN-35, an anti-CD30 ADC conjugated to the microtubule inhibitor monomethylauristatin E (MMAE) linked to antibody cysteines by a maleimidocaproyl-valine-citrulline-p-aminobenzyloxycarbonyl linker that is designed to be cleaved by cathepsins (MC-vc-PAB-MMAE), has shown promising safety and efficacy in a phase 1 trial for the treatment of Hodgkin lymphoma.6 More recently Junutula et al7 described a promising new THIOMAB technology for ADCs. Antibodies were engineered with cysteine substitutions at positions on light or heavy chains that provide reactive thiol groups and do not affect antibody production or function. These THIOMABs allow the homogenous conjugation of 2 drugs per antibody without purification from a heterogeneous mixture, which would be impractical for large-scale manufacture. When the MC-vc-PAB-MMAE linker drug was conjugated via engineered cysteines to an antibody against the ovarian cancer antigen MUC16, the resultant ADCs had similar activity in xenograft models with the same amount of conventionally conjugated antibody that has a greater drug load. More importantly, these ADCs could be tolerated at substantially greater doses on the basis of both the amount of MMAE delivered and on the amount of conjugated antibody than their conventionally conjugated counterparts.7
Our previous work demonstrated that CD79, the signaling component of the B-cell receptor, is a promising ADC target for the treatment of NHL. CD79 has the appropriate expression pattern, being expressed only on B cells and in most NHLs. This narrow expression pattern allows the antibody to be targeted to the cancer with minimal targeting to normal tissue. The targeting to normal B cells should not be a major concern because experience with rituximab suggests that depletion of normal B cells is not a major safety issue. In addition to its signaling functions, when the B-cell receptor is cross-linked, it is targeted to the major histocompatibility complex class II compartment, a lysosome-like compartment, as part of class II antigen presentation by B cells. This feature of CD79 biology makes it a particularly attractive target for the use of ADCs because antibodies against CD79 are delivered to these lysosomal compartments, which are known to contain protease that can release the cytotoxic drug. Here we describe the generation of a THIOMAB drug conjugate (TDC) targeted to CD79b and demonstrate the potential as treatment for a broad range of NHL subtypes.

**Methods**

**Proteins and antibodies**

The extracellular domain of human CD79b (CD79b EC) was expressed in Chinese hamster ovary cells alone or as an Fc fusion (CD79b-Fc) and purified by as previously described. A 16-amino acid peptide (AR-SEDTRYRPNPKGSACK) containing the epitope for SN8 was synthesized by conventional means.

A hybridoma expressing the murine antibody anti-CD79b SN8 (1) was obtained from Ben Seon (Roswell Park Cancer Institute). The methods of cloning of SN8, humanization of SN8, antibody production, and affinity measurements are given in the supplemental Methods section (available on the Blood website; see the Supplemental Materials link at the top of the online article).

**Cell lines and viability assays**

The NHL cell lines DOHH2, Granta-519, Karpas-1106P, MHH-PREB-1, NU-DUL-1, NU-DUL-1, Ramos, RC-K8, REC-1, SC-1, SU-DHL-1, SU-DHL-4, SU-DHL-5, SU-DHL-6, SU-DHL-8, SU-DHL-10, SU-DHL-15, U-698-M, WSU-DLCL2 and WSU-NHL DB, Farage, HT, MC116, Pfeiffer, Toledo A4/Fukada, SCC-3, and TK were maintained in RPMI 1640 supplemented with 10% fetal bovine serum (Sigma-Aldrich) and 2 mol/L L-glutamine. The in vitro efficacy of CD79b-vcMMAE was measured by CellTiter-Glo Luminescent Cell Viability Assay (Promega Corp). Cells were tested in quadruplicate at 1 to 5 × 10^4 per well in 384-well plates in RPMI containing 10% fetal bovine serum overnight before treated with anti-CD79b TDC or the control anti-gD-drug conjugate. The concentration of anti-CD79b-TDC resulting in the 50% inhibition of cell viability was calculated from a 4-variable curve analysis and was determined from a minimum of 3 independent experiments. Details of cell lines and assays are in the supplemental Methods section.

**Microarray data generation and analysis**

Gene expression analysis of NHL cell lines was conducted by the use of total cellular RNA extracted with mirVanaTM miRNA Isolation Kit (Ambion) and on the Affymetrix HGU133Plus_2.0 or Gene 1.0ST platform (Affymetrix). Preparation of complementary RNA, array hybridizations, and subsequent data analysis were performed according to the manufacturer’s instruction. Methods of gene expression analysis are given in the supplemental Methods. All microarray data have been deposited with Gene Expression Omnibus and are accessible through GEO Series accession number GSE15329 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE15329).

**Somatic mutation analysis**

Cancer-related genes were sequenced in the NHL cell lines by the use of Sanger sequencing technology. The identified point mutations and small indels were cleaned up by removing known single nucleotide polymorphisms in the Single Nucleotide Polymorphism database (http://www.ncbi.nlm.nih.gov/projects/SNP/) and synonymous mutations except for the ones located at the exon junctions. The associations between the mutation status of individual genes and anti-CD79b-vcMMAE IC50 values were tested by the use of Wilcoxon rank sum test. All data analyses were conducted using R (http://www.r-project.org).

**Xenograft experiments**

All animal studies were performed in compliance with National Institutes of Health guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee at Genentech Inc. Cells for implantation were washed and suspended in Hanks balanced salt solution (HyClone) and inoculated subcutaneously into the flanks of female CB17 ICR SCID mice 7 to 16 weeks of age (Charles River Laboratories) in a volume of 0.2 mL/mouse. When mean tumor size reached desired volume, the mice were divided into groups of 8 to 10 mice with the same mean tumor size and dosed intravenously via the tail vein with ADCs or antibodies.

Rituximab was dosed at 30 mg/kg intraperitoneally, which is above the maximum efficacious dose. CHOP (a single intravenous injection of 30 mg/kg cyclophosphamide, 2.475 mg/kg doxorubicin, 0.375 mg/kg vincristine, and oral dosing of 0.15 mg/kg prednisone once a day for 5 days) was dosed to a point where we observed 5% weight loss in the animals. Thus, this should be at or near the maximum tolerated dose. A single dose of rituximab and anti-CD79b-vcMMAE and a single treatment of CHOP and R-CHOP were used to allow direct comparisons of the therapies.

For the xenograft experiment combining ABT-263 and anti-CD79b-vcMMAE, anti-CD79b-vcMMAE was dosed once intravenously at 1.5 mg/kg, and ABT-263 was dosed orally daily for 21 days at 100 mg/kg.

**CD79b expression levels**

The Leeds Research Ethics Committee approved the use of patient samples for the flow cytometry studies. CD79b expression in patient samples by flow cytometry was determined by the use of the anti-CD79b (CB3-1) antibody. All geometric mean fluorescence intensity (geoMFI) values for CD79b were normalized by subtraction of the CD3-PE fluorescence values. The data are shown as log2 geoMFI. The expression data derived from the NHL cell lines was performed by the use of anti-CD79b (SN8)-PE (BD Biosciences), the parent antibody of our humanized ADC. Both SN8 and CB3-1 had the same conjugate (phycoerythin) used in the immunostaining; the difference in their signals was normalized by measuring the geoMFI of selected cell lines with both antibodies and calculating the ratio of the SN8-PE and CB3-1-PE signals. Details of fluorescence-activated cell sorting and Immunohistochemistry methods and quantification are given in the supplemental Methods section.

**Results**

**Generation of an anti-CD79b ADC suitable for the treatment of NHL in humans**

We had previously identified the anti-CD79b(SN8) antibody that was particularly effective in xenograft models for the use as an ADC in the treatment of NHL. As a first step toward generating an anti-CD79b ADC suitable for the treatment of NHL, we humanized anti-CD79b(SN8). The human acceptor framework used for humanization of anti-CD79b(SN8) was based on the consensus human κ VL domain and the human subgroup III consensus VH domain. The VL and VH domains of murine anti-CD79b(SN8) were aligned with the human κ and subgroup III
domains; each complementarity-determining region (CDR) was identified and grafted into the human acceptor framework to generate a CDR graft that could be displayed as an Fab on phage (supplemental Figure 1). Phage displaying the SN8 CDR graft bound to immobilized CD79bεcd; however, when the SN8 CDR graft sequence was expressed as an IgG, fluorescence-activated cell sorting analysis of its affinity for CD79becd indicated that binding affinity had been reduced by more than 100-fold (data not shown), and Biacore analysis indicated a loss of more than 250-fold (Table 1).

To increase the affinity of the humanized antibody, CDRs within the anti-CD79b(SN8) CDR graft were randomized on phage and selected for binding to immobilized CD79b. Selected clones were expressed as IgG for analysis by Biacore, leading to the identification of several affinity-improved clones; however, none of the variants had an affinity similar to murine anti-CD79b(SN8), leading us to explore framework changes to improve antigen binding. A variant containing several “vernier” positions16 was constructed and found to possess similar binding affinity to CD79b as chimeric anti-CD79b(SN8) (Table 1). IgG variants were designed to identify the minimum set of framework positions needed to maintain high affinity (Table 1). Framework positions 47 in VL and 75 and 80 in VH were found dispensable as evidenced by variant 17. Addition of changes in H3 identified by phage affinity maturation led to an additional 2-fold improvement (variant 18). To avoid potential manufacturing issues, a potential iso-aspartic acid–forming site (Asp–Gly) in CDR-L1 of the humanized SN8 variants was eliminated by converting D28 to Glu (D28E, variant 28); other substitutions also were tolerated (Table 1). Binding affinity of anti-CD79b(SN8v28) was 0.26 ± 0.02 nmol/L, which was approximately the same as the murine SN8 (0.63 ± 0.14 nmol/L) by Scatchard analysis on CD79b expressing cell lines (data not shown).

Recent phase 1 data suggested that a standard format MC-vc-PAB-MMAE ADC targeted to CD30 (SGN-35) for the treatment of Hodgkin lymphoma is tolerated up to 1.88 mg/kg and has shown early signs of efficacy.6 Given the potential of the THIOMAB format to increase the tolerability of MC-vc-PAB-MMAE ADCs and our previous data showing that anti-CD79b ADCs can be highly effective in xenograft models of lymphoma, we predicted that anti-CD79b(SN8v28)THIOMAB-MC-vc-PAB-MMAE (referred to as anti–CD79b-vcMMAE henceforth) would be an

### Table 1. Biacore analysis of SN8 framework variants

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wt indicates wild type; N, asparagine; A, alanine; S, serine; E, glutamine; and NDB, no detectable binding.
excellent candidate ADC for the treatment of NHL. We therefore made an HC-A114C THIOMAB version conjugated to MC-vcMMAE and tested the resultant TDC in a BJAB xenograft model of NHL. A single dose of 2 mg/kg ADC (18 μg/kg conjugated MMAE) resulted in sustained complete tumor remission in all 10 animals tested (Figure 1). This activity was greater than that of other anti-CD79b(SN8) conjugates we have previously tested.13

Minimal expression of CD79b on the cell surface is required for sensitivity of cell lines to anti–CD79b-vcMMAE in vitro

To reveal parameters that could determine sensitivity to anti–CD79b-vcMMAE, we assessed cell viability in a large panel of 34 NHL cell lines (supplemental Table 1). The degree of sensitivity to anti–CD79b-vcMMAE and an isotype control anti-gD-vcMMAE was determined from a dose–response curve of 0 to 10 μg/mL, and IC50 values were calculated (supplemental Table 1). To ensure that the IC50 values calculated were specific to anti–CD79b-vcMMAE, we compared IC50 values to that of the isotype control anti-gD-vcMMAE. Very few cell lines achieved an IC50 with the anti-gD-MMAE (8 of 34), and most of these were only achieved at greater concentrations in the titration range. Nevertheless, in these cases there was a substantial and statistically significant difference between the activity of anti–CD79b-vcMMAE and anti-gD-vcMMAE. Therefore, the data generated are indicative of specific anti–CD79b-vcMMAE activity. These data first demonstrate that the anti–CD79b-vcMMAE has very potent and broad activity across a large panel of NHL cell lines in vitro and, second, because there is a dynamic range of IC50 values, we can assess the molecular parameters that may dictate sensitivity and/or resistance to anti–CD79b-vcMMAE.

Because expression levels of CD79b on the cell surface are likely to be an important parameter that may dictate response to anti–CD79b-vcMMAE, we used the methodology of flow cytometry across the NHL cell-line panel before drug treatment to assay this parameter. We then assessed whether there was a correlation with the IC50 values obtained with anti–CD79b-vcMMAE (Figure 2A-B). These data revealed a substantial dynamic range in CD79b expression on the cell surface and that a threshold effect was observed in that below a specific level of antigen (< 6.82 geoMFI units) on the cell surface resulted in cells being insensitive to the maximum amount of anti–CD79b-vcMMAE tested (10 μg/mL). Notably, only 15.4% (2 of 13) of the resistant cell lines maintained CD79b expression on the cell surface, suggesting that the mechanism of primary insensitivity is largely attributable to the absence of CD79b cell-surface expression (Figure 2A-B).

Given that the expression of CD79b is required to elicit in vitro activity and that absence of expression is the primary mechanism for the absence of a response in vitro, we wished to determine the prevalence of expression of CD79b on the cell surface from primary human lymphoma and chronic lymphocytic leukemia (CLL) samples to estimate a patient population that may gain benefit. To this end, patient samples were subjected to flow cytometry. The use of a threshold of more than 2.1 geo MFI units as determined from the cell-line panel as expressing antigen (see “Methods” for details) revealed that CD79b was detected in nearly all cases of CLL, marginal zone lymphoma, hairy cell leukemia, diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), and mantle cell lymphoma (MCL; Figure 2C). Furthermore, CD79b expression also was detected in almost all cases of FL and DLBCL that had relapsed from previous chemotherapy regimens, highlighting the clinical relevance of this target and potential therapeutic utility of anti–CD79b-vcMMAE (Figure 2D left and right panels, respectively). To speculate on the potential prevalence of the anti–CD79b-vcMMAE responding population within these patient populations we used the same threshold as that determined from our in vitro data whereby an expression of more than 6.82 is required to elicit activity. As such, 90% of DLBCL (53 of 59), 97% of FL (56 of 58), 23% of CLL (17 of 75), 95% of marginal zone lymphoma (57 of 60), 100% of hairy cell leukemia (20 of 20), and 95% of MCL (19 of 20) would express sufficient CD79b on the cell surface to elicit a response to anti–CD79b-vcMMAE.

Intriguingly, when the CLL cases were divided in peripheral CLL cells and bone marrow–derived CLL cells, a greater proportion of bone marrow–derived CLL cases expressed CD79b more than the 6.82 threshold (15.4% vs 26.5%, respectively). It is therefore possible that anti–CD79b-vcMMAE may be able to clear the bone marrow subpopulation of CLL cells, which is believed to be the precursor or stem cell niche of CLL. Furthermore, 87% of FL (20 of 23) and 76.5% of DLBCL (13 of 17) cases after relapse from chemotherapy-containing regimens express sufficient CD79b required for anti–CD79b-vcMMAE activity. Together, these data confirm that if the threshold amount of CD79b required for in vitro sensitivity of the cell lines translates into the clinic, a significant percentage of lymphoma and CLL cases would express sufficient levels of CD79b to respond to anti–CD79b-vcMMAE.

With archival tissue blocks being the primary source of tissue for diagnostics assays in NHL and because CD79b surface expression is a prerequisite for response to anti–CD79b-vcMMAE in preclinical models, we developed an immunohistochemistry (IHC) assay for CD79b that was robust for the testing of formalin-fixed, paraffin-embedded clinical material. Initially, we generated a tissue microarray from formalin-fixed, paraffin-embedded cell-line pellets; assessed IHC scoring quantitatively and qualitatively; and correlated it with anti–CD79b-vcMMAE IC50 values. The IHC data indicated that there was indeed a broad range of expression levels of CD79b and that a subpopulation of the resistant cell lines did not express the antigen whereas another subpopulation of resistant cells did have detectable levels of CD79b.
and bars indicate CD79b cell surface–negative and –positive cell lines, respectively. (B) Cell-surface expression is the primary predictor of response in vitro. (C) CD79b is broadly expressed across lymphomas and CLL. HCL indicates hairy cell leukemia; MZL, marginal zone lymphoma. (D) CD79b expression is broadly across DLBCL molecular subtypes as determined by gene expression microarrays: germinal center B cell–like (GCB) DLBCL, activated B cell–like (ABC) DLBCL, and type 3, which has an intermediate profile between the GCB and ABC subtypes.17 Notably, CD79b protein expression was distributed throughout the broad expression pattern in DLBCL. There was a dynamic range of expression of CD79b by IHC similar to that observed in the cell lines and flow cytometry of primary lymphoma and CLL samples. We next assessed the distribution of CD79b across DLBCL molecular subtypes as determined by gene expression microarrays according to the work by Wright et al17 (Figure 2E). DLBCL has 3 major subtypes as determined by gene expression microarrays: germinat center B cell–like (GCB) DLBCL, activated B cell–like (ABC) DLBCL, and type 3, which has an intermediate profile between the GCB and ABC subtypes.17 Notably, CD79b protein expression was distributed broadly across all 3 molecular subtypes as assessed by the germinat center index, where a positive score reflects a GCB subtype, a highly negative score reflects an ABC subtype, and scores centering on 0 to −80 reflects type III. In addition, when the germinat center index values were calculated for the NHL cell lines and correlated with the anti–CD79b-vcMMAE IC50 values, it was apparent that anti–CD79b-vcMMAE has activity across all of these molecular subtypes (supplemental Figure 5), including the poor prognostic group of ABC DLBCL.

Further defining molecular parameters of sensitivity

Although the presence of cell-surface CD79b is a prerequisite for a response, there exists a wide range of sensitivities to anti–CD79b-vcMMAE within the cell surface–positive subgroup, as defined by an anti–CD79b-vcMMAE index greater than 2.1 (Figure 2B). There was indeed a significant correlation with levels of cell-surface CD79b within this cell surface–positive group and sensitivity to anti–CD79b-vcMMAE (supplemental Figure 6); however, it is evident that this relationship is nonlinear. More specifically, cell lines exist that displayed among the highest sensitivities to anti–CD79b-vcMMAE yet had the least amount of antigen on the cell surface, such as SU-DHL-5 (geo MFI = 9.34 and IC50 = 0.25 μg/mL; supplemental Table 1). In contrast, there were cell lines that had the greatest levels of CD79b on the cell surface and elicited a less-sensitive phenotype within the CD79b-expressing subgroup, eg, WSU-NHL (geo MFI = 12.13 and IC50 = 6.39 μg/mL; supplemental Table 1). Such data provided the impetus for exploring other potential factors that may be important for regulating the response to anti–CD79b-vcMMAE.

A conceivable hypothesis is that the activity of a microtubule-targeted agent, such as MMAE, may depend on the number of times that a cell enters the G2/M phase of the cell cycle. To test this hypothesis, we assessed the cell doubling time for all of the cell lines in their log phase of growth and performed a Spearman correlation with anti–CD79b-vcMMAE in vitro activity (Figure 3A). Intriguingly, no correlation with sensitivity and cell doubling...
time was detected (P = .127). In fact, some of the most sensitive cell lines have among the longest cell doubling times within the cell line panel, such as DB (doubling time = 36.06 hours; supplemental Table 1). Furthermore, we did not observe a correlation with cell doubling time and sensitivity to unconjugated MMAE (supplemental Figure 7) or a correlation with anti–CD79b-vcMMAE activity and unconjugated MMAE (supplemental Figure 8). Overall, these data suggest that the proliferation rate of lymphoma or leukemic cells is unlikely to perturb or predict benefit from anti–CD79b-vcMMAE.

We next wished to comprehend whether somatic mutations in candidate key oncogenes or tumor suppressors would play any role in regulating response to anti–CD79b-vcMMAE in vitro. Because somatic mutations of the tumor suppressor TP53 occur in approximately 20% of DLBCL cases \(^{18,19}\) and correlate with poor patient prognosis in patients treated with an anthracycline-containing regimen,\(^{19}\) we wished to determine first whether anti–CD79b-vcMMAE was effective in a TP53 mutant context and second whether mutation of TP53 had any contribution to the range of drug sensitivity observed. To this end, we sequenced TP53 for mutations from the cell-line panel and performed a Wilcoxon rank sum test by using the IC\(_{50}\) values and mutational status. Strikingly, the activity of anti–CD79b-vcMMAE was potent in p53 mutant cell lines in addition to p53 wild type (Figure 3B), and there was no statistically significant association with TP53 status and anti–CD79b-vcMMAE activity (P = .22). We also tested whether other known somatic mutations in NHL may play a role in determining sensitivity to anti–CD79b-vcMMAE (Figure 3C and supplemental Figure 9A). Where it made biologic sense, we combined mutations to represent one module for significance association, eg, KRAS and BRAF. Similarly to the results with TP53, no significant association was detected with any of the tested somatic mutations, and similar conclusions were drawn when testing this association across all cell lines, including CD79b surface–positive and –negative (supplemental Figure 9B). These data negate the possibility that common somatic mutations may perturb anti–CD79b-vcMMAE activity and that they are unlikely to play a role in predicting response.

Because some of the obvious candidate parameters failed to correlate with the relative sensitivity of the CD79b cell surface–positive cell lines, we took an unbiased approach and used gene expression microarrays derived from the cell lines at baseline that express CD79b on the cell surface to identify genes that correlate with the IC\(_{50}\) values of anti–CD79-b-vcMMAE using a Spearman rank correlation. Remarkably, only 0.79% (160 of 20,191) genes passed the statistical significance threshold of P less than or equal to .05 (supplemental Table 2). It is noteworthy that CD79b mRNA did not correlate with sensitivity to anti–CD79b-vcMMAE (P = .60). However, our surveying of the top differentially expressed genes identified that BCL-XL (BCL2L1) expression correlated significantly with reduced sensitivity to anti–CD79b-vcMMAE (Figure 4A). BCL-XL is a member of the BCL2 antiapoptotic family of proteins that function by preventing disruption of the mitochondria potential and cytochrome c release. Furthermore, BCL-XL has left its hallmark in clinical studies, where it has previously been associated with chemotherapy resistance\(^{20,21}\) and poor prognosis in FL.\(^{22}\) It is therefore tempting to

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**Figure 3.** CD79b-vcMMAE efficacy is not blunted by somatic mutations or cell doubling time. (A) In vitro doubling time does not correlate with anti–CD79b-vcMMAE activity. (B) Anti–CD79b-vcMMAE has potent in vitro activity in both TP53 mutant and wild-type cell lines. (C) Anti–CD79b-vcMMAE in vitro activity is not perturbed by somatic mutations in common oncogenes.

**Figure 4.** Gene expression profiling reveals that BCL-XL correlates with reduced sensitivity CD79b-vcMMAE that can be overcome with ABT-263. (A) BCL-XL (BCL2L1) correlates with reduced anti–CD79b-vcMMAE activity. Red arrow highlights Granta-519 cell line. (B) ABT-263 can add to the activity of anti–CD79b-vcMMAE in a NHL model, Granta-519, with high BCL-XL expression.
speculate that BCL-XL levels may contribute to the reduced sensitivity of anti–CD79b-vcMMAE, given the a priori knowledge of BCL-XL function in lymphoma and response to chemotherapy. To test the hypothesis that BCL-XL may blunt anti–CD79b-vcMMAE activity, we chose a xenograft model as a higher-bar approach compared with in vitro assays and used a subnanomolar-specific inhibitor of BCL-XL and BCL2 known as ABT-263.23 ABT-263 is an orally available BCL-XL and BCL2 inhibitor that enhances mitochondrial-dependent cell death by disrupting the interaction of the antiapoptotic proteins BCL2 and BCL-XL and the proapoptotic protein Bad. In essence, this releases the apoptotic brakes of the tumor cell and renders them more susceptible to apoptosis. Therefore, a Granta-519 xenograft model that expresses high levels of BCL-XL was treated with a dose of anti–CD79b-vcMMAE that resulted in tumor stasis, and the effect of adding ABT-263 was assessed (Figure 4B). Notably, the addition of ABT-263 resulted in 4 partial remission and 4 complete remission of the 8 mice tested compared with 1 partial remission and 0 complete remissions of the 8 mice tested with anti–CD79b-vcMMAE. In sharp contrast, no efficacy was observed with ABT-263 as a single agent at the dose tested. These data, therefore, highlight that inhibiting the BCL2 family members can enhance sensitivity to anti–CD79b-vcMMAE.

Comparison of the activity of anti–CD79b-vcMMAE standard of care in vivo

Thus far, these data suggest that anti–CD79b-vcMMAE is very effective in vivo and in vitro. We were therefore interested to compare anti–CD79b-vcMMAE efficacy to that of standard-of-care regimens in xenograft models (Figure 5). Standard of care for most lymphomas is rituximab (R) plus cyclophosphamide doxorubicin, vincristine, and prednisolone (CHOP). We selected 3 of the cell lines that were sensitive in vitro to anti–CD79b-vcMMAE derived from different subtypes of lymphoma and that had growth characteristics favorable for xenografts to generate large cohorts of mice and statistically meaningful data: Ramos (Burkitt lymphoma; Figure 5A), Granta-519 (MCL; Figure 5B) and WSU-DLCL2 (DLBCL; Figure 5C). We compared the activity of anti–CD79b-vcMMAE with a single cycle of CHOP, rituximab, or R-CHOP to a single treatment with anti–CD79b-vcMMAE at 5 or 7 mg/kg. Only the mice treated with CHOP lost body weight (0%-5% at day 5), whereas mice treated with vehicle, control ADC, rituximab, or anti–CD79b-vcMMAE gained 2% to 4% body weight. The administration of R-CHOP in all 3 xenograft models resulted in its ability to slow tumor growth or decrease tumor volumes, whereas the anti–CD79b-vcMMAE, strikingly, resulted in sustained complete tumor remission in all 3 of the models tested. These data hint at the potential for anti–CD79b-vcMMAE to be a potent therapy for the treatment of NHL.

Discussion

Here we describe the development and activity of anti–CD79b-vcMMAE, an ADC targeted to CD79b in preclinical NHL models. We show that anti–CD79b-vcMMAE has profound activity across a broad range of lymphoma cell lines and molecular subtypes. Of particular interest is the fact that CD79b expression in DLBCL cases and activity in cell lines also occurs in the poor prognosis ABC-like subgroup of DLBCL, which remains a poor prognostic group even in the clinical context of R-CHOP.24 It is therefore tempting to speculate that such a poor prognostic group of patients may gain benefit from anti–CD79b-vcMMAE as a single agent or in combination with R-CHOP. This speculation can be further supported by our data that anti–CD79b-vcMMAE was more
efficacious than R-CHOP in 3 different xenograft models. Furthermore, the fact that we see sufficient CD79b cell-surface expression in FL and DLBCL cases that have relapsed from previous chemotherapy-containing regimens is very encouraging and permits the opportunity to test anti–CD79b-vcMMAE in patients who have very few treatment options available.

Another clinically poor prognosis subgroup in CLL, FL, and DLBCL is mutation and/or loss of TP53. A loss of function of TP53 results in a very poor clinical outcome across many lymphomas, and current standard-of-care therapeutics have little benefit in these populations. It was therefore an important finding that anti–CD79b-vcMMAE displayed activity in cell lines that were TP53 mutant as well as wild type. These data provide further evidence for potential clinical utility. In addition, our observations that anti–CD79b-vcMMAE is not perturbed in cell lines that display oncogenic or tumor suppressor mutations is very encouraging because cancers acquire novel mutations through selection in response to environmental stress and/or treatment regimens. These data are highly suggestive that anti–CD79b-vcMMAE could be effective as a salvage therapy because patients have undergone multiple lines of treatment and presumably a concomitant change in spectrum of mutations.

It has been suggested that microtubule-targeting agents may depend on the proliferation rate of the target cell to elicit antiproliferative activity and could potentially blunt their ability to have activity in a more indolent disease state. Intriguingly, no correlation with sensitivity and cell doubling time was detected, which is highly suggestive that anti–CD79b-vcMMAE may have the potential to elicit activity in more indolent disease subtypes such as CLL and/or follicular lymphoma. However, it is conceivable that cell lines may have a much more rapid proliferation rate in vitro compared with tumor cells in vivo in their microenvironment, and hence this correlation may only apply below a certain threshold that is not represented in our cell line panel.

To maximize patient benefit to targeted therapies and to identify biomarkers that could enable the selection of a patient population that will gain most benefit from anti–CD79b-vcMMAE would certainly be a desirable goal. We found that a minimal expression threshold of CD79b was required for sensitivity to anti–CD79b-vcMMAE in our cell lines and from flow cytometry data on primary patient tumors. These data suggest that a significant number of cases will likely express sufficient CD79b to be responsive to anti–CD79b-vcMMAE therapy. However, because fresh tissue is not always available for flow cytometry assessment of CD79b expression in lymphoma cases at the time of patient enrollment on a clinical trial, we developed an IHC assay that can be readily used on the archival tissue block that should be available for most patients since such a specimen is required to confirm diagnosis.

As part of the search for other parameters that could be modulating anti–CD79b-vcMMAE activity, identification of BCL-XL as a correlate with reduced sensitivity is of particular interest because it had been previously described as a resistance mechanism to chemotherapy and poor prognosis in FL. In the cohort of FL cases analyzed for BCL-XL mRNA expression, high levels of expression correlated with a decrease in overall survival. It is therefore not surprising that BCL-XL is an attractive target for clinical candidates currently in development for the treatment of NHL. One of these clinical candidates is ABT-263, and we found it could substantially increase the activity of anti–CD79b-vcMMAE in vivo in a cell line that was less sensitive to anti–CD79b-vcMMAE and harbored high expression levels of BCL-XL. It is particularly encouraging that a potential source of primary resistance to anti–CD79b-vcMMAE could be overcome with a combination strategy including ABT-263, thereby formulating the possibility of a therapeutic combination of anti–CD79b-vMMMAE and ABT-263 in the clinic that could result in durable remissions.

Overall, we provide substantial evidence that warrants anti–CD79b-vcMMAE testing in the clinic with the ultimate goal of achieving durable and meaningful clinical responses in patients who are refractory to all current standard of care regimens and improving on existing therapies.

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Authorship

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References


Therapeutic potential of an anti-CD79b antibody–drug conjugate, anti–CD79b-vc-MMAE, for the treatment of non-Hodgkin lymphoma