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RESEARCH ARTICLE

Targeting MCL-1 and BCL-2 with polatuzumab vedotin and venetoclax overcomes treatment resistance in R/R non-Hodgkin lymphoma: Results from preclinical models and a Phase Ib study

Elisabeth A. Lasater[1] | Dhara N. Amin[2,3] | Rajat Bannerji[4] | Raghuveer Singh Mali[1] | Kathy Barrett[5] | Ryan N. Rys[6,7] | Jason Oeh[1] | Eva Lin[2] | Tim Sterne-Weiler[8] | Ellen Rei Ingalla[1] | MaryAnn Go[1] | Shang-Fan Yu[1] | Maxwell M. Krem[9] | Chris Arthur[10] | Uwe Hahn[11] | Anna Johnston[12] | Vinit Karur[13] | Nadia Khan[14] | Paula Marlton[15] | Tycel Phillips[16] | Giuseppe Gritti[17] | John F. Seymour[18] | Monica Tani[19] | Sam Yuen[20] | Scott Martin[2] | Matthew T. Chang[8] | Christopher M. Rose[21] | Victoria C. Pham[21] | Andrew G. Polson[1] | YiMeng Chang[22] | Claudia Wever[6,7] | Nathalie A. Johnson[6,7] | Yanwen Jiang[5] | Jamie Hirata[23] | Deepak Sampath[1] | Lisa Musick[23] | Christopher R. Flowers[24] | Ingrid E. Wertz[2,3]

Correspondence
Christopher R. Flowers, Department of Lymphoma and Myeloma, UT MD Anderson Cancer Center, Houston, TX, USA.
Email: crflowers@mdanderson.org

Ingrid E. Wertz, Lyterian Therapeutics, 1 Letterman Drive, Suite DM-900, San Francisco, CA, 94129, USA.
Email: ingrid@lyterian.com

Present address
Dhara N. Amin, Ambagon Therapeutics, San Francisco, California, USA.

Rajat Bannerji, Ipsen, Paris, France.

Raghuveer Singh Mali, AbbVie, South San Francisco, California, USA.

Ellen Rei Ingalla, Calico Life Sciences, South San Francisco, California, USA.

Maxwell M. Krem, Kansas City VA Medical Center, Kansas City, Missouri, USA.

Abstract

The treatment of patients with relapsed or refractory lymphoid neoplasms represents a significant clinical challenge. Here, we identify the pro-survival BCL-2 protein family member MCL-1 as a resistance factor for the BCL-2 inhibitor venetoclax in non-Hodgkin lymphoma (NHL) cell lines and primary NHL samples. Mechanistically, we show that the antibody-drug conjugate polatuzumab vedotin promotes MCL-1 degradation via the ubiquitin/proteasome system. This targeted MCL-1 antagonism, when combined with venetoclax and the anti-CD20 antibodies obinutuzumab or rituximab, results in tumor regressions in preclinical NHL models, which are sustained even off-treatment. In a Phase Ib clinical trial (NCT02611323) of heavily pre-treated patients with relapsed or refractory NHL, 25/33 (76%) patients with follicular lymphoma and 5/17 (29%) patients with diffuse large B-cell lymphoma achieved complete or partial responses with an acceptable safety profile when treated with the recommended

Elisabeth A. Lasater, Dhara N. Amin, and Rajat Bannerji contributed equally to this study.

For affiliations refer to page 460

Vinit Karur, New York Presbyterian, New York, New York, USA.

Matthew T. Chang, Loxo Oncology at Lilly, San Francisco, California, USA.

Claudia Wever, AstraZeneca, Cambridge, UK.

Deepak Sampath, Ultragenyx, San Francisco, California, USA.

Ingrid E. Wertz, Lyterian Therapeutics, San Francisco, California, USA.

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1 | INTRODUCTION

Non-Hodgkin lymphoma (NHL) encompasses a range of complex hematologic malignancies. More than 60 NHL subtypes have been identified and are broadly categorized as indolent or aggressive.[1] Follicular lymphoma (FL) is the most common indolent subtype. Advanced-stage FL is not curable with standard treatments and has a pattern of recurrent relapses. Diffuse large B-cell lymphoma (DLBCL) is the most common variant of NHL,[2] and is potentially curable with anthracycline-based chemoimmunotherapy, but 40% of patients experience relapse and may become refractory to treatment.[3] Thus, there is an unmet medical need to identify therapeutic regimens for B-cell NHLs that have improved efficacy and safety,[4] particularly for patients with disease that is no longer responsive to standard chemotherapy-based regimens.

Venetoclax is a selective BCL-2 inhibitor approved for the treatment of patients with chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma,[5,6] and for the treatment of acute myeloid leukemia in patients aged 75 years or older.[7,8] Patients with CLL and mantle cell lymphoma (MCL) show high response rates to single-agent venetoclax[9] or the combination of venetoclax with one of the anti-CD20 antibodies rituximab or obinutuzumab.[10,11] However, venetoclax efficacy is limited in other lymphoid neoplasms. Specifically, FL and DLBCL are characterized by BCL-2 overexpression and increased dependence on BCL-2, and showed 38% and 18% response rates to venetoclax monotherapy, respectively.[9] Tumor cell expression of other pro-survival BCL-2 family members, insufficient function of BCL-2 family activators that antagonize the pro-survival members, or defects in the pro-apoptotic effectors BAX or BAK[12] may limit broader venetoclax efficacy. Our study goals were to comprehensively investigate the mechanisms of venetoclax resistance in NHL cell lines and primary patient samples and, based on these findings, to design and evaluate the clinical safety of a therapeutic regimen to overcome such resistance.

2 | METHODS

For details of the methods used, please see the Supplementary Information (Data S1).

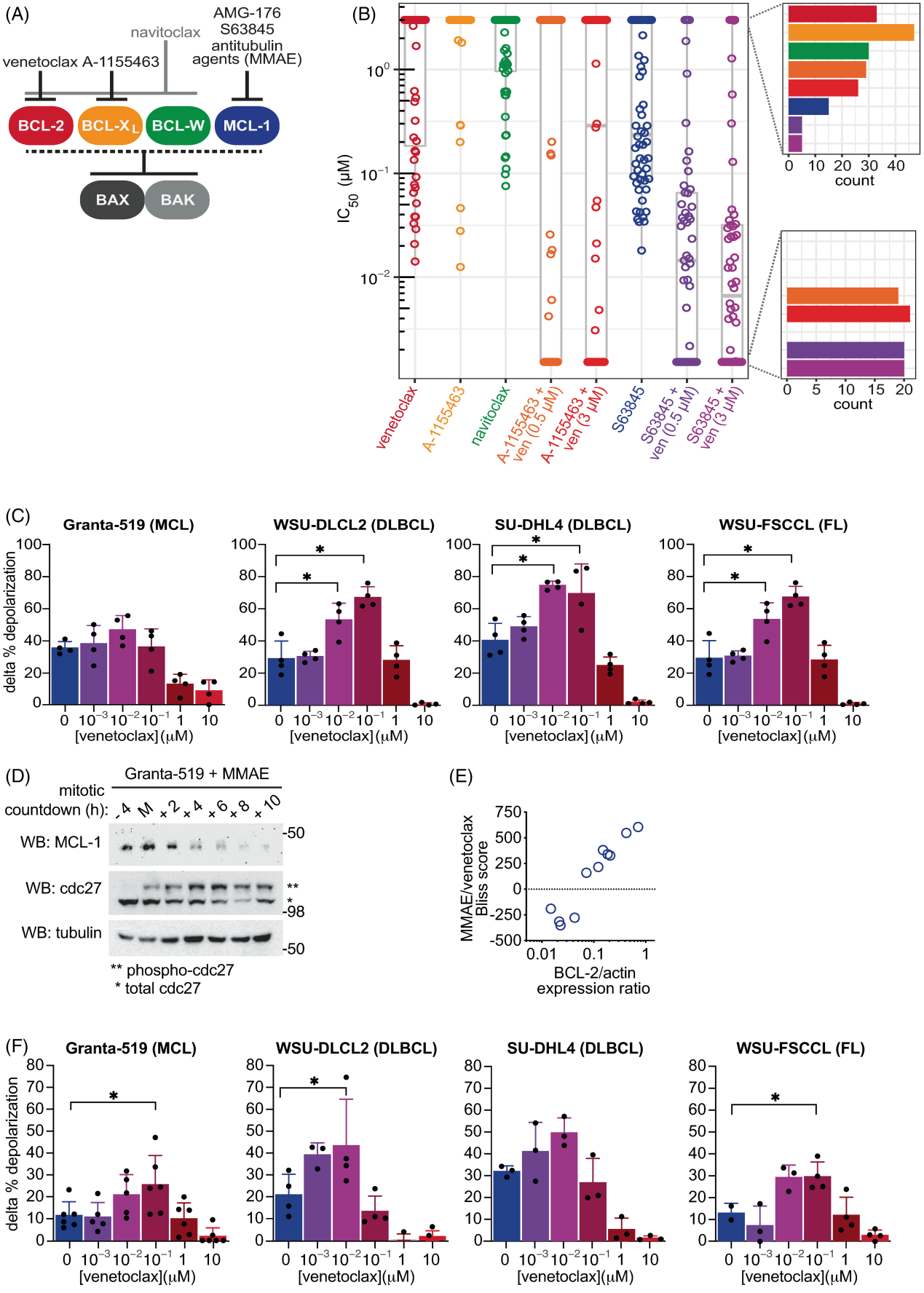
3 | RESULTS

3.1 | MCL-1 and BCL-X_L are venetoclax resistance factors in NHL cell lines

To investigate which pro-survival BCL-2 family members limit venetoclax activity, a panel of 55 NHL cell lines was profiled for sensitivity to small molecule inhibitors (BCL-2 Homology-3 [BH3] mimetics) that antagonize the pro-survival BCL-2 family member function. These included venetoclax, the selective BCL-X_L inhibitor A-1155463,[13] the BCL-2/BCL-X_L/BCL-W inhibitor navitoclax,[14] and the MCL-1 inhibitor S63845[15] (Figure 1A). The NHL cell lines showed modest or no sensitivity to any of these single agents (Figure 1B; Table S1). Antagonizing BCL-X_L with A-1155463, and to a greater extent, antagonizing MCL-1 with S63845, enhanced venetoclax sensitivity in many NHL cell lines, suggesting that BCL-X_L and MCL-1 expression may contribute to venetoclax resistance (Figure 1B; Table S1). Interestingly, navitoclax did not enhance cell death more than the combination of A-1155463 and venetoclax, suggesting that BCL-W is not a prominent regulator of venetoclax resistance in NHL cell lines (Figure 1B; Table S1).

To further investigate regulation of NHL cell viability by BCL-2 family proteins, we analyzed CRISPR-mediated knockout (KO) data reported within the DepMap database (https://depmap.org/portal/). Using a CERES score of −0.5 or less as an indicator of impaired cell fitness, the KO data in the reported NHL cell lines corroborate the small molecule antagonist data (Figure 1A). Specifically, both data sets indicated that BCL-X_L, MCL-1, and, to a lesser extent, BCL-2 regulate NHL cell line viability (Figure 1B; Table S1, Figure S1A), whereas BCL-W and A1, other pro-survival BCL-2 family proteins, do not (Figure S1A). By comparing viability data in response to pro-survival BCL-2 family KO versus treatment with BCL-2 family antagonists, we found that the cell lines sensitive to BCL-X_L KO are not sensitive to BCL-X_L inhibition by either A-1155463 or navitoclax, whereas most cell lines that are sensitive to BCL-2 or MCL-1 KO are also sensitive to inhibition by venetoclax and navitoclax, or S63845, respectively (Figure 1A; Figure S1B). This finding suggests that BCL-X_L KO does not accurately reflect BCL-X_L antagonism, thus pointing to a scaffolding role of the BCL-X_L protein that will be investigated in future studies.

Next, we performed BH3 profiling[16] using small-molecule BH3 inhibitors to functionally characterize BCL-2 family protein regulation



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of mitochondrial outer membrane potential in NHL cell lines (Figure 1A). Treatment of cells with venetoclax, either in combination with S63845 or with A-1155463, confirmed dependence on both BCL-2 and MCL-1 for maintaining membrane polarization (Figure 1C), with a modest contribution from BCL-X_L (Figure S1C). Thus, cell viability in response to BCL-2 family inhibitors, analysis of cell fitness in response to KO of pro-survival BCL-2 family protein expression, and BH3 profiling data collectively indicate that for most NHL cell lines evaluated, MCL-1, and to a lesser extent BCL-X_L, foster venetoclax resistance.

3.2 | MCL-1 is depleted in response to treatment with anti-tubulin agents

A-1155463 and navitoclax effectively inhibit BCL-X_L, however, preclinical and clinical studies have demonstrated that BCL-X_L inhibition results in dose-limiting thrombocytopenia.[17,18] We therefore focused on antagonizing MCL-1 to overcome venetoclax resistance and enable an improved safety profile relative to BCL-X_L antagonism. Since the safety and efficacy of MCL-1-selective antagonists are under active investigation in clinical trials,[19,20] we considered an alternative MCL-1-targeting strategy. We and others have reported that the anti-tubulin chemotherapeutics paclitaxel and vincristine promote MCL-1 degradation, which contributes to treatment-induced cell death.[21,22] The anti-tubulin agent monomethyl auristatin-E (MMAE) also promoted mitotic arrest and decreased MCL-1 protein levels in NHL cell lines (Figure 1D; Figure S2A). Next, we investigated whether MMAE sensitized NHL cell lines to venetoclax and whether BCL-2 protein expression correlated with sensitivity to venetoclax and MMAE co-treatment. Eleven NHL cell lines with a range of BCL-2 protein expression (Figure S2B) were treated with a matrix of MMAE and venetoclax concentrations (Table S2), and corresponding viability data were evaluated by Bliss analysis, where higher Bliss scores indicate stronger synergistic responses.[23] MMAE sensitized NHL cell lines to venetoclax (Table S2) and co-treatment reduced NHL cell line viability in a BCL-2-dependent manner (Figure 1E). BH3 profiling studies revealed that venetoclax and MMAE co-treatment enhanced mitochondrial outer membrane depolarization (Figure 1F), mechanistically linking MMAE-induced MCL-1 depletion with sensitization to venetoclax treatment. Thus, venetoclax and MMAE co-treatment enhance NHL cell death via simultaneous inhibition of BCL-2 and depletion of MCL-1.

Polatuzumab vedotin, a CD79b-directed antibody-drug conjugate that delivers MMAE to CD79b-expressing cells, has been approved for the treatment of relapsed/refractory (R/R) DLBCL[24,25] and has demonstrated activity in previously untreated DLBCL and R/R FL.[26,27] As with free MMAE, polatuzumab vedotin promoted MCL-1 protein depletion over time (Figure S3A), which was dependent on MMAE and not induced by free anti-CD79b antibody (Figure S3B). Minimal changes were seen in pro-apoptotic protein levels, other than NOXA depletion in WSU-DLCL2 cells, possibly as a consequence of MCL-1 depletion (Figure S3A).[28] Because NOXA is a pro-apoptotic protein, reduced expression is not expected to contribute to polatuzumab vedotin-induced cell death.[12] Protein expression of other pro-survival BCL-2 family members did not change significantly; however, higher molecular weight BCL-2 and BCL-X_L species were evident upon the initiation of mitotic arrest (Figure S2A; Figure S3A). Phosphorylation of BCL-2 residues S69, S70, and S87 and BCL-X_L residue S62 has been reported to modulate cell death responses to anti-tubulin agents.[29] Therefore, we quantified BCL-2 and BCL-X_L phosphorylation at these sites following polatuzumab vedotin

FIGURE 1 MCL-1 is a venetoclax resistance factor in NHL cell lines. (A) A schematic of BCL-2 family protein interactions and regulation by therapeutic agents. BAX and BAK promote cell death if they are not neutralized by BCL-2, BCL-X_L, BCL-W, or MCL-1. Venetoclax is a BCL-2-selective antagonist, navitoclax antagonizes BCL-2, BCL-X_L, and BCL-W, AMG-176 and S63845 are MCL-1 inhibitors, and anti-tubulin agents promote MCL-1 degradation. (B) IC_{50} values of NHL cell lines profiled with the indicated BCL-2 family antagonists. For the combination treatments, the indicated data points represent the S63845 IC_{50} values for each cell line in combination with venetoclax or navitoclax fixed at 3 μM. The inset bar plots represent counts of the number of data points with IC_{50} at the maximum or minimum concentration screened in each condition while maintaining their order and color (See Table S1 for corresponding cell viability data). (C) Delta percent depolarization of NHL cell lines following co-treatment with S63845 and venetoclax. Cells were pre-treated with 1 μM S63845 (0.5 μM for WSU-FSCCL cell line) for 4–5 h followed by incubation with increasing concentrations of venetoclax as indicated and mitochondrial depolarization was measured. Delta percent depolarization = (percent depolarization of S63845 + venetoclax) − (percent depolarization of venetoclax alone) at indicated venetoclax concentration. Data represents average ± standard deviation. $N \geq 4$. *p = .0006, .0003 and .0016 for S63845 + 0.01 μM venetoclax versus S63845 alone for WSU-DLCL2, SU-DHL4 and WSU-FSCCL, respectively; **p = .0084, .0015, and <.0001 for S63845 + 0.1 μM venetoclax versus S63845 alone for WSU-DLCL2, SU-DHL4 and WSU-FSCCL respectively, by one-way ANOVA with Dunnett's posttest. (D) Western blot analysis of whole cell lysates from Granta-519 cells treated with 10 nM MMAE. MMAE promotes mitotic arrest (M), as indicated by cdc27 phosphorylation and subsequent MCL-1 degradation. (−4 = 4 h prior to mitotic arrest, +2 = 2 h after the onset of mitotic arrest, and so on). (E) Positive Bliss scores calculated from MMAE/venetoclax combination studies in NHL cell lines are positively correlated with BCL-2 expression levels (Pearson $r = 0.795$, $p = .0035$). (See Table S2 for corresponding cell viability and Bliss score source data.) (F) Delta percent depolarization of NHL cell lines following co-treatment with MMAE and venetoclax. Cells were pre-treated with 10 nM MMAE (2 nM for WSU-FSCCL cell line) for 16–18 h followed by incubation with increasing concentrations of venetoclax as indicated and mitochondrial depolarization measured. Delta percent depolarization was calculated as described for Figure 1C. Data represents average ± standard deviation. $N = 3$–4. *$p = .0241$ for MMAE + 0.01 μM venetoclax versus MMAE alone for WSU-DLCL2; **$p = .0278$ for MMAE + 0.1 μM venetoclax versus MMAE alone for Granta-519 by one-way ANOVA with Dunnett's post-test. ANOVA, analysis of variance; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; h, hours; MCL, mantle cell lymphoma; MMAE, monomethyl auristatin-E; NHL, non-Hodgkin lymphoma; WB, Western blot.

treatment in Granta-519 cells using high-sensitivity parallel reaction monitoring mass spectrometry (Figure S3C). BCL-2 S87 phosphorylation increased from approximately 0.5% to 1% with polatuzumab vedotin treatment (Figure S3D). Phosphorylation of BCL-2 residues S69 and S70 and BCL-X_L residue S62 was not detected either with or without treatment. Thus, BCL-2 S69, S70, and S87 phosphorylation and BCL-X_L S62 phosphorylation are unlikely to contribute significantly to polatuzumab vedotin-induced cell death in this system.

3.3 | Polatuzumab vedotin promotes cell death that is dependent on BCL-2 family proteins

Similar to free MMAE, polatuzumab vedotin reduced NHL cell viability in combination with venetoclax or navitoclax (Figure 2A; Table S2). Decreased viability by either combination was characterized by increased caspase-3/-7 activation (Figure S4A) and earlier poly-ADP ribosylase (PARP) cleavage (Figure S4B), both of which are hallmarks of apoptotic cell death.[12] Furthermore, the combination activated caspases in Granta-519 and WSU-DLCL2 wild-type cells, but not in BAX/BAK KO cells (Figure 2B; Figure S4C), confirming cell death dependence on BCL-2 family proteins.[12] As a control, we confirmed that BAX/BAK KO cell lines remained sensitive to staurosporine, a broad kinase inhibitor that promotes non-apoptotic cell death (Figure S4D). Caspase activity induced by the combination of venetoclax and MMAE in HCT-116 colonic adenocarcinoma cells and murine embryonic fibroblast cells was also BAX/BAK dependent (Figure S4E), suggesting a more universal and conserved cell death mechanism beyond NHL cell lineages. Our collective data indicate that polatuzumab vedotin depletes MCL-1 and, in combination with venetoclax, activates the intrinsic, BAX/BAK-dependent apoptotic pathway in NHL cells, rather than a more general cell toxicity response.

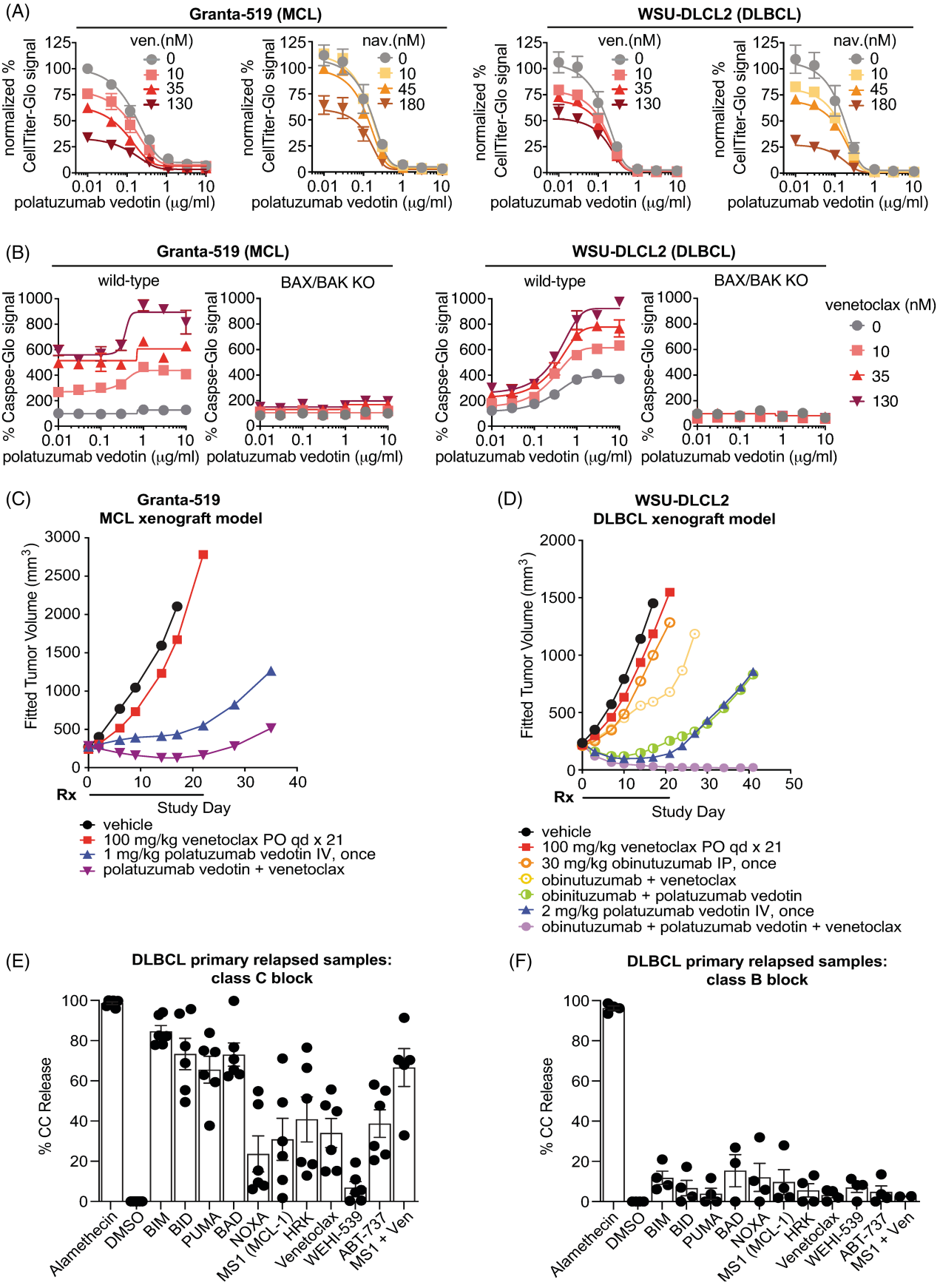
Next, we compared treatment with polatuzumab vedotin to treatment with the MCL-1 antagonists S63845[30] and AMG 176,[15] in combination with venetoclax in CD79b-expressing NHL cell lines. The top inhibitor concentrations were chosen to maintain on-target specificity[15,30] or CD79b target antigen saturation.[31] Venetoclax combined with either MCL-1 inhibitor decreased cell viability, which was comparable with the combination of venetoclax and polatuzumab vedotin (Figure S5A). We then evaluated the effect of over-expressing (O/E) MCL-1 in regulating the response of NHL cell lines to venetoclax and polatuzumab vedotin co-treatment (Figure S5B). All four MCL-1 O/E cell lines were more resistant to venetoclax alone (Table S3), and we observed decreased cell death with increased Bliss scores upon polatuzumab vedotin co-treatment[23] (Table S3). To further evaluate the role of MCL-1 in regulating the polatuzumab vedotin and venetoclax co-treatment responses, we engineered MCL-1 KO cell lines (Figure S5C). All four MCL-1 KO cell lines were less sensitive to polatuzumab vedotin relative to the wild-type cell lines (Table S4), presumably because the MCL-1 KO cells shift dependence from MCL-1 to other BCL-2 family members for survival. The MCL-1 KO cell lines RI-1 and RL (DLBCL) and SC-1 (FL) showed enhanced sensitivity and decreased Bliss scores relative to the respective wild-type cell lines upon venetoclax and polatuzumab vedotin co-treatment, indicating a shift to BCL-2 dependence. Interestingly, the Granta-519 (MCL) MCL-1 KO cell line was more resistant to the venetoclax and polatuzumab vedotin combination relative to the wild-type cell line (Table S4). This finding suggested that the Granta-519 MCL-1 KO cell line shifted dependence to a BCL-2 family member other than BCL-2. Indeed, the BCL-X_L-selective inhibitor A-1155463 enhanced cell death in combination with polatuzumab vedotin and had a lower Bliss score relative to the wild-type line (Table S4). Thus, MCL-1 depletion, either induced by polatuzumab vedotin treatment or engineered via genetic knockout, sensitizes NHL cell lines to venetoclax-induced cell death, unless adaptation to MCL-1 depletion imparts resistance by shifting dependence to another BCL-2 family member such as BCL-X_L.

3.4 | Polatuzumab vedotin promotes MCL-1 degradation via the ubiquitin/proteasome system

We next characterized the cellular machinery responsible for decreasing MCL-1 levels in response to polatuzumab vedotin treatment. MCL-1 transcription was not altered during mitotic arrest in WSU-DLCL2 cells (Figure S6A), consistent with previous reports.[21,22] This implicates a role for the ubiquitin/proteasome system, a primary conduit for regulated protein degradation in eukaryotic cells.[32] Co-treatment with proteasome and ubiquitin-activating enzyme E1 (UAE1) inhibitors, but not caspase inhibitors, blocked MCL-1 degradation (Figure S6B,C), indicating that MCL-1 is degraded by the ubiquitin/proteasome system. The ubiquitin ligase SCF^{FBW7} is reported to promote MCL-1 degradation in solid tumor cell lines in response to anti-tubulin agents.[21] Knockdown of FBW7, the substrate-binding subunit of the SCF ubiquitin ligase complex, attenuated MCL-1 degradation following polatuzumab vedotin treatment in NHL cell lines (Figure S7A,B). Furthermore, FBW7 shRNA-treated cells were more resistant to caspase activation induced by polatuzumab vedotin in combination with venetoclax, relative to control shRNA-treated cells (Figure S7C). Collectively, these studies reveal that MCL-1 degradation by the ubiquitin/proteasome system contributes to polatuzumab vedotin-induced apoptosis (Figure S7D).

3.5 | Durable efficacy is achieved in NHL xenograft models with a combination regimen of venetoclax, polatuzumab vedotin, and obinutuzumab

In vivo, the combination of venetoclax with polatuzumab vedotin was more efficacious relative to the single-agent treatments in the Granta-519 MCL xenograft model (Figure 2C). Consistent with in vitro mechanistic studies, MCL-1 protein levels progressively decreased and caspase-3 cleavage increased with longer treatment



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times in tumors (Figure S8A). The MCL-1 antagonist AMG 176 had limited efficacy, and combining venetoclax with AMG 176 modestly enhanced tumor growth inhibition relative to each single agent (Figure S8B). Consistent with the nearly complete MCL-1 degradation induced by polatuzumab vedotin in the Granta-519 model (Figure S8A), AMG 176 combined with polatuzumab vedotin demonstrated no improvement in efficacy over polatuzumab vedotin monotherapy; thus, polatuzumab vedotin co-treatment with venetoclax was the most effective combination in the Granta-519 MCL xenograft model (Figure S8B).

We next evaluated the WSU-DLCL2 DLBCL xenograft model, given the modest clinical responses of DLBCL to venetoclax monotherapy.[9] Notably, MCL-1 degradation in WSU-DLCL2 tumors treated with polatuzumab vedotin and venetoclax was less pronounced than in Granta-519 xenografts (Figure S8A, C). When assessing anti-tumor efficacy, venetoclax and polatuzumab vedotin monotherapies showed minimal responses in vivo, consistent with clinical data[9,26] (Figure S8D). Venetoclax and polatuzumab vedotin co-treatment was more efficacious than either monotherapy in the WSU-DLCL2 model, however, the combination was less efficacious in WSU-DLCL2 DLBCL xenografts compared with Granta-519 MCL xenograft responses (Figure 2C; Figure S8D). Combining polatuzumab vedotin with AMG 176 modestly enhanced efficacy compared with AMG 176 or polatuzumab vedotin monotherapy in this model (Figure S8E). This effect may be due to AMG 176 antagonizing remaining MCL-1 not degraded by polatuzumab vedotin treatment in the WSU-DLCL2 xenograft model (Figure S8C,E). Co-treatment of venetoclax with AMG 176 resulted in tumor growth inhibition comparable with the venetoclax and polatuzumab vedotin combination (Figure S8E). Taken together, these in vivo efficacy studies indicate that sustained anti-tumor activity can be achieved by venetoclax and polatuzumab vedotin co-treatment, which compares favorably with the combination of venetoclax and clinical MCL-1 inhibitors.

Nevertheless, sustained tumor regressions were not achieved in the WSU-DLCL2 DLBCL xenograft model with either treatment regimen. To improve efficacy in this refractory model, we evaluated treatment options that: (1) are approved for the treatment of B-cell lymphomas; (2) have the potential to overcome apoptotic blocks that remain after treatment with venetoclax and polatuzumab vedotin co-treatment; and (3) are predicted to maintain an acceptable combination safety profile. The anti-CD20 antibodies rituximab and obinutuzumab fulfill these criteria; more specifically, they promote antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and apoptosis in vivo, cell death mechanisms that are complementary to the apoptosis induced by BCL-2 family inhibition and may thus overcome apoptotic blocks to venetoclax and polatuzumab vedotin co-treatment.[33] Furthermore, combining either rituximab or obinutuzumab, with polatuzumab vedotin enhances efficacy in patients with FL and DLBCL.[24,27,34,35] Obinutuzumab, venetoclax and polatuzumab vedotin co-treatment was more efficacious than any of the respective single agents or doublets, resulting in WSU-DLCL2 xenograft regression (Figure 2D). Regressions were sustained off-treatment, a notable result given the reported resistance of the WSU-DLCL2 model to anti-CD20 treatments.[36] Thus, we identify an efficacious, clinically feasible therapeutic regimen that is effective in treating a model representing NHL subtypes where an unmet medical need exists. For all in vivo studies, treatments were well tolerated based on minimal changes in animal body weights and vitality (data not shown).

3.6 | MCL-1 contributes to venetoclax resistance in primary R/R DLBCL samples ex vivo

Next, we analyzed cryopreserved, primary R/R DLBCL patient samples ($n = 9$) to evaluate whether the cell death regulatory pathways that we characterized in NHL cell lines and xenograft models are represented in patient-derived malignancies. We used flow cytometry-based BH3 profiling (iBH3)[16] to assess the apoptotic response of primary samples to a panel of BH3 mimetics and pro-apoptotic activators (Figure S9A,B,C). iBH3 profiling enabled the classification of samples based on the type of apoptotic blocks: class A blocks result from insufficient function of activator proteins that antagonize the pro-survival BCL-2 family proteins; class B blocks are resistant to apoptosis due to defects in the pro-apoptotic effectors BAX/BAK; and class C blocks are due to pro-survival proteins, such as BCL-2, inhibiting cell death (Figure 1A).[37,38] The primary R/R DLBCL samples analyzed displayed both class C ($n = 5$) and class B ($n = 4$) apoptotic blocks (Figures 2E,F). Class C samples showed moderate responses to singular BCL-2 and MCL-1 inhibition by venetoclax or

FIGURE 2 Polatuzumab vedotin/MMAE promotes MCL-1 degradation and sensitizes NHL cell lines to venetoclax. (A) Normalized percent CellTiter-Glo signal following 72 h of treatment with increasing concentrations of polatuzumab vedotin and venetoclax as indicated. Data points are mean values calculated from triplicates, error bars are standard deviation. (B) Percent Caspase-Glo signal in Granta-519 and WSU-DLCL2 wild-type and BAX/BAK KO cell lines. Cells were co-treated with increasing concentrations of polatuzumab vedotin and venetoclax for 24 h as indicated. (C) Fitted tumor volumes from mice bearing Granta-519 xenograft tumors, treated as indicated, are plotted relative to treatment time. (D) Fitted tumor volumes from mice bearing WSU-DLCL2 xenograft tumors, treated as indicated, are plotted relative to treatment time. (E) and (F) Percent CC release in primary R/R DLBCL samples following treatment with BH3 peptides or small molecules as indicated. Data represent average response with individual samples shown, error bars represent standard error of the mean. Alamethicin and DMSO act as positive and negative controls, respectively. Class C apoptotic block R/R DLBCL samples show good responses to apoptotic stimuli, notably BCL-2 and MCL-1 inhibition (E). However, nearly half of R/R DLBCL samples show no response to any apoptotic stimuli, defined as class B apoptotic block (F). DLBCL, diffuse large B-cell lymphoma; IP, intraperitoneal; IV, intravenous; KO, knockout; MCL, mantle cell lymphoma; MMAE, monomethyl auristatin-E; nav, navitoclax; NHL, non-Hodgkin lymphoma; PO, oral administration; qd, once daily; R/R, relapsed/refractory; ven, venetoclax.

MS1, a BH3 peptide with a high affinity for MCL-1 (Figure 2E). These samples showed enhanced cytochrome c release when both MCL-1 and BCL-2 were antagonized, indicating the potential efficacy of co-targeting MCL-1 in combination with venetoclax in R/R DLBCL. The remaining R/R DLBCL samples ($n = 4$) displayed class B profiles, showing resistance to incubation with any BH3 mimetic (Figure 2F). These data mirror conclusions from our previous study that profiled pre-treatment indolent FL and DLBCL primary samples.[37]

In sum, our in vitro pharmacologic and genetic viability profiling using NHL cell lines, in vivo xenograft studies, and BH3 profiling studies evaluating R/R primary DLBCL samples collectively predict that some NHL malignancies are sensitive to combined inhibition of BCL-2 and MCL-1, which can be achieved by combination treatment with polatuzumab vedotin and venetoclax. However, a subset of NHL patients will have tumors that are not fully sensitive to BCL-2 and MCL-1 inhibition and will also require therapeutic agents that trigger cell death independent of the mitochondrial pathway, such as the anti-CD20 antibodies obinutuzumab or rituximab.

3.7 | A treatment regimen of polatuzumab vedotin with venetoclax and anti-CD20 antibodies is safe and effective in R/R FL and DLBCL

3.7.1 | Phase Ib clinical trial rationale and study aims

Based on our strong mechanistic rationale, preclinical efficacy data, and profiling of primary R/R NHL patient samples that demonstrate proof of concept, we evaluated the combination regimen of venetoclax and polatuzumab vedotin in a Phase Ib/II clinical trial with obinutuzumab or rituximab in subsets of NHL patients with R/R FL or R/R DLBCL, respectively. Here, we report the results of the completed Phase Ib study (NCT02611323), the aims of which were to investigate safety and tolerability, establish the recommended Phase II doses (RP2D), and assess the preliminary efficacy of polatuzumab vedotin and venetoclax in combination with a fixed dose of an anti-CD20 agent.

3.7.2 | Patient demographics

Data cutoff was July 10, 2019 for the FL patient cohorts and January 30, 2020 for the DLBCL patient cohorts. Thirty-three patients with R/R FL and 17 patients with R/R DLBCL were enrolled at 25 sites in North America, Italy, and Australia. Patient demographics and baseline characteristics are shown in Table S5. The median age of patients in both the FL and DLBCL dose-escalation cohorts was 61 years, and the majority had an Eastern Cooperative Oncology Group performance score of 0 or 1. The median number of prior lines of anti-lymphoma therapy was three for patients with FL and two for patients with DLBCL (range 1–7 for both subtypes). Most patients had disease that was refractory to the last treatment (60.6% FL; 94.1% DLBCL), and eight patients with FL (24.2%) had experienced progression of disease within 24 months of their initial lymphoma treatment, which is a well-established prognostic factor associated with inferior survival.[39]

3.7.3 | Dose-limiting toxicities (DLT) and RP2D

Two patients in FL cohort 1 (polatuzumab vedotin 1.4 mg/kg and venetoclax 400 mg) experienced DLTs: one with grade 3 laboratory tumor lysis syndrome (TLS) and one with grade 3 aspartate transaminase/alanine transaminase elevation. Neither case resulted in clinical sequelae, and both patients recovered with supportive care and a temporary interruption of all study drugs. In addition, both patients were able to restart all treatment and complete induction therapy. Based on the predictability and reversibility of these events, the DLT criteria were amended to allow asymptomatic laboratory TLS and increased liver function tests up to eight times the upper limit of normal, resolving within 7 days. Cohort 1a was added to include polatuzumab vedotin at 1.4 mg/kg and a lower dose of venetoclax at 200 mg. Following cohort 1a clearing, an additional three patients were enrolled into cohort 1 (Figure 3A); no DLTs were reported in this cohort. Subsequently, one patient in cohort 4 (polatuzumab vedotin 1.8 mg/kg and venetoclax 600 mg) experienced a DLT of neutropenic sepsis. Cohort 4 was expanded to include an additional three patients, none of whom experienced DLTs; thus, cohort 4 cleared and cohort 6 was opened (Figure 3A). An additional three patients were enrolled in cohort 6 (Figure 3A) to confirm tolerability at this dose level. The maximum tolerated dose (MTD) of polatuzumab vedotin in combination with venetoclax and obinutuzumab was not reached and the RP2D for this combination in patients with FL was identified as polatuzumab vedotin 1.8 mg/kg and venetoclax 800 mg, with the standard dose of obinutuzumab 1000 mg.

There were no DLTs reported during the DLBCL dose-escalation phase, which included three cohorts escalating the dose of venetoclax (cohort A, 400 mg; cohort B, 600 mg; cohort C, 800 mg) with fixed doses of polatuzumab vedotin 1.8 mg/kg and rituximab 375 mg/m^2 (Figure 3B). The MTD was not reached, and the RP2D for this combination in patients with DLBCL was identified as polatuzumab vedotin 1.8 mg/kg and venetoclax 800 mg, with the standard fixed dose of rituximab 375 mg/m^2.

3.7.4 | Safety

The most common all-grade adverse events (AEs) in the FL and DLBCL treatment arms together ($n = 50$) were diarrhea (31/50; 62%), neutropenia (25/50; 50%), and fatigue (20/50; 40%). Table S6 and Table S7 provide details on all-grade AEs per dose escalation cohort. Grade 3/4 AEs were reported in 21 (64%) patients with FL and 15 (88%) patients with DLBCL (Table S8). The most common grade 3/4 AEs were neutropenia, thrombocytopenia, and infections, reported in 42%, 21%, and 21%, respectively, in patients with FL and 59%, 5.9%, and 18%, in patients with DLBCL (Table S8; Table S9; Table S10). Patients who experienced neutropenia received supportive care with granulocyte colony-stimulating factors (e.g., filgrastim).

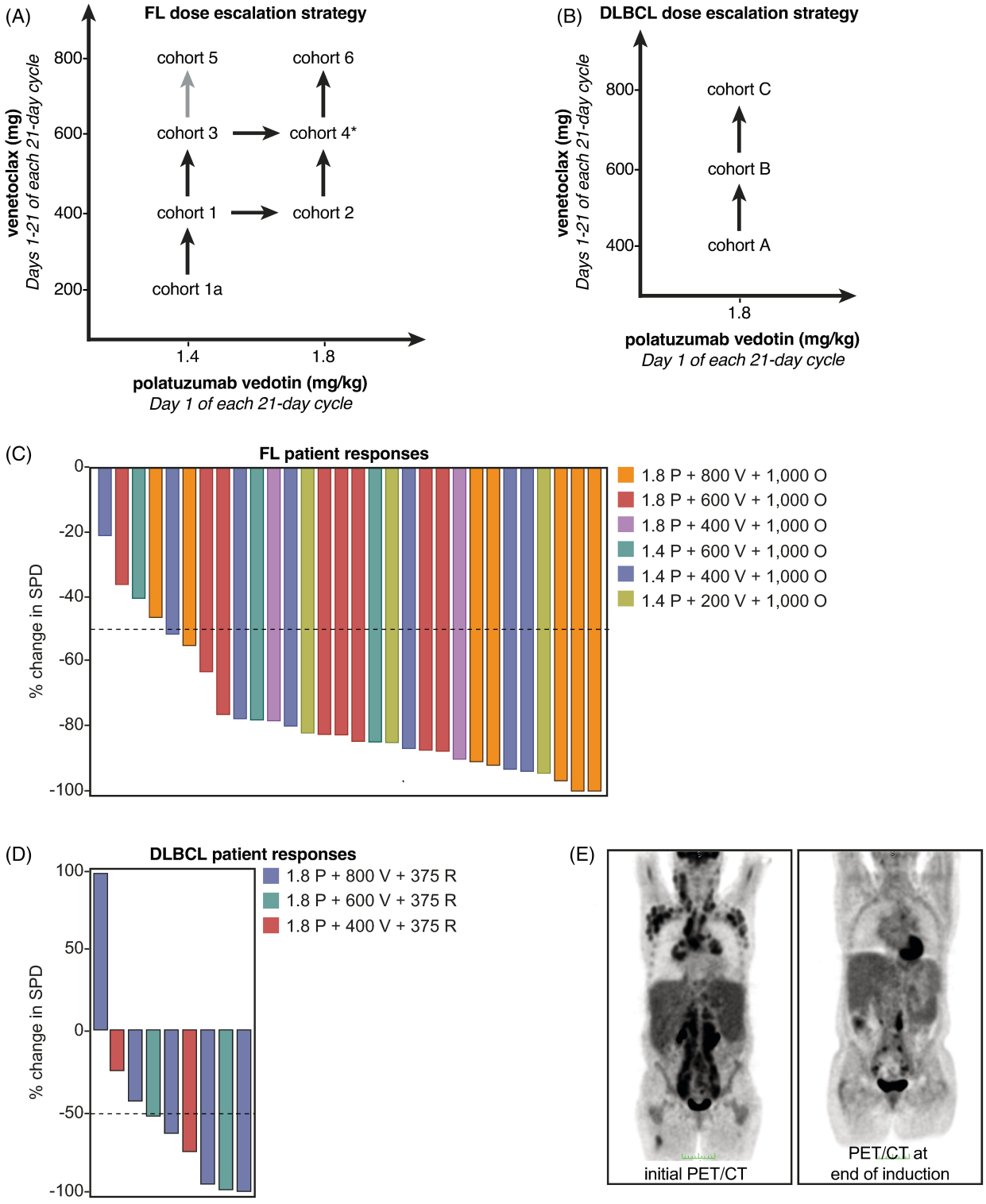

FIGURE 3 Clinical study design, patient responses to therapy, and patient PET/CT images. (A) Dose escalation FL cohorts (3 + 3 design). When doses in cohort 1a were deemed safe and tolerable, escalation continued with cohort 1. Cohorts 2 and 3 enrolled simultaneously once the doses in cohort 1 were determined to also be safe and tolerable. Once both cohort 2 and 3 dose combinations were deemed acceptable, cohorts 4 and subsequently 6 were permitted to open. Cohort 5 would only open if cohort 3, but not 4 cleared. (B) Dose escalation DLBCL cohorts (3 + 3 design). Venetoclax dose escalated with fixed doses of polatuzumab vedotin 1.8 mg/kg and rituximab 375 mg/m^2. (C) Waterfall plot summarizing responses determined by CT scans by percent change in the SPD from baseline at end of induction in patients with FL, separated by cohort. (D) Waterfall plot for DLBCL cohort. (E) PET/CT images of a patient with FL enrolled in cohort 1 at initial screening (left panel) and at the end of induction treatment (right panel), which resulted in a significant signal decrease in cervical, axillary, mediastinal, and abdominal lymph nodes. DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; O, obinutuzumab; P, polatuzumab vedotin; PET/CT, positron emission tomography/computed tomography; R, rituximab; SPD, sum of perpendicular distances; V, venetoclax.

The grade 3/4 infections reported in patients with FL included two cases each of *Clostridium difficile* colitis and pneumonia and one case each of cellulitis, infective exacerbation of chronic obstructive airway disease, lung infection, neutropenic sepsis, *Pseudomonas* infection, rhinovirus infection, sinusitis, and urinary tract infection. In the DLBCL cohorts, reported grade 3/4 infections included pneumonia, urinary tract infection, and vascular device infection. No deaths due to AEs related to study treatment were reported. One patient in DLBCL cohort C discontinued study treatment due to progressive disease and subsequently died due to pneumonia following chimeric antigen receptor T-cell (CAR-T) therapy.

One patient in FL cohort 1 developed laboratory TLS without clinical sequelae on Day 1 of Cycle 1, after the first dose of venetoclax. No additional cases of TLS were reported (Table S11; Table S12). A total of 15 patients (30%) experienced grade 1 or 2 peripheral neuropathy (FL, 11/33 [33%]; DLBCL, 4/17 [24%]) (Table S11; Table S12). No peripheral neuropathy higher than grade 2 in severity was observed in any patient, and the incidence was similar across all cohorts. Two patients (1 FL; 1 DLBCL) required a dose reduction of polatuzumab vedotin from 1.8 to 1.4 mg/kg due to grade 2 peripheral neuropathy.

3.7.5 | Efficacy

Responses were assessed using modified Lugano criteria, based on metabolic response using positron emission tomography/computed tomography (PET/CT) scans at the end of induction (EOI), 6–8 weeks after Cycle 6 Day 1. Response rates are summarized in Table 1, with details by individual cohort in Table S13 and Table S14. The overall response rate (ORR) for patients with FL was 75.8%, with 57.6% of patients achieving a complete response (CR) (Table 1). All patients in FL cohort 6 treated at the identified RP2D dose combination achieved CR at EOI. The ORR observed for patients with DLBCL was 29.4%; 23.5% achieved CR (Table 1). Similar trends were seen in the DLBCL cohorts, with higher response rates in patients treated at the RP2D (37.5% vs. 22.2%). Responses by cohort for patients with FL and DLBCL are shown in Figure 3C,D, confirming that a majority of patients achieved greater than 50% decrease in tumor size from baseline. Representative PET/CT images for a patient in FL cohort 1 who achieved a CR at EOI are shown in Figure 3E, revealing remarkably reduced disease burden after induction treatment in a patient refractory to three prior lines of therapy, including rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone, plus bendamustine. Overall, we provide a comprehensive analysis of combined targeting of BCL-2 and MCL-1 using polatuzumab vedotin and venetoclax in NHL cell lines, mouse models, and primary R/R DLBCL samples, and confirm clinical activity in patients in the context of a Phase Ib clinical trial.

4 | DISCUSSION

R/R NHL continues to be an area of high unmet need, as patients with R/R DLBCL experience a high mortality rate after failing first-line therapy, and those with R/R FL often suffer from multiple episodes of relapse and cumulative toxicities with each line of treatment. Because B-cell NHLs are genetically heterogeneous neoplasms, identifying key disease drivers and designing treatment strategies that simultaneously target them, may provide more efficacious and durable treatment responses.

BCL-2 overexpression or rearrangements are a diagnostic hallmark of FL and are also frequent in DLBCL,[40] with BCL-2 expression levels correlating with in vitro venetoclax sensitivity justifying a targeting strategy in both subtypes. Through in vitro pharmacologic and genetic studies utilizing established NHL cell lines, xenografts, and primary R/R samples, we identified MCL-1, and to a lesser extent BCL-X_L, as key venetoclax resistance factors in NHL. Because BCL-X_L inhibition leads to dose-limiting thrombocytopenia,[41] we focused on treatment regimens that inhibit MCL-1 to overcome venetoclax

TABLE 1 Investigator-assessed clinical response rates at EOI by Modified Lugano criteria for patients with FL and DLBCL

EOI (after 6 cycles)	FL		DLBCL	
n (%)	Cohort 6—RP2D Pola 1.8 mg/kg Ven 800 mg Obi 1000 mg ($N = 8$)	Total patients treated in Phase Ib ($N = 33$)	Cohort C—RP2D Pola 1.8 mg/kg Ven 800 mg Ritux 375 mg/m^2 ($N = 8$)	Total patients treated in Phase Ib ($N = 17$)
ORR	8 (100.0)	25 (75.8)	3 (37.5)	5 (29.4)
CR	8 (100.0)	19 (57.6)	2 (25.0)	4 (23.5)
PR	0	6 (18.2)	1 (12.5)	1 (5.8)
SD	0	5 (15.2)	0	0
PD	0	1 (3.0)	3 (37.5)	7 (41.1)
NE/missing	0	2 (6.1)	2 (25.0)	5 (29.4)

Abbreviations: CR, complete response; DLBCL, diffuse large B-cell lymphoma; EOI, end of induction; FL, follicular lymphoma; NE, not evaluable; Obi, obinutuzumab; ORR, objective response rate; PD, progressive disease; Pola, polatuzumab vedotin; PR, partial response; Ritux, rituximab; RP2D, recommended Phase II dose; SD, stable disease; Ven, venetoclax.

resistance. Mechanistically, the MMAE payload of polatuzumab vedotin promotes ubiquitin-mediated MCL-1 protein degradation both in vitro and in vivo, which enables enhanced cell killing when combined with venetoclax. Further, cell killing induced by this combination required functional BAX/BAK effector proteins, as apoptosis was attenuated in genetically deficient cell lines or R/R primary samples that demonstrate a class B block. In vivo, enhanced anti-tumor activity was demonstrated in both MCL and DLBCL xenograft models for the combination of polatuzumab vedotin and venetoclax compared with monotherapies. The addition of an anti-CD20 antibody enhanced anti-tumor activity relative to polatuzumab vedotin and venetoclax co-treatment, indicating that activation of ADCC and CDC by an anti-CD20 antibody complements apoptosis induced by BCL-2 and MCL-1 inhibition. Importantly, these results were corroborated in primary R/R DLBCL samples ex vivo: 5 of 9 samples were dependent on BCL-2 and MCL-1, predicting sensitivity of these malignancies to the polatuzumab vedotin and venetoclax combination. However, 4 of 9 samples had reduced apoptotic competency, underscoring the need to include therapeutic agents that trigger cell death independent of the mitochondrial pathway, such as anti-CD20 antibodies, in the treatment regimen. Unfortunately, the clinical protocol did not accommodate the collection of primary samples for additional iBH3 profiling. In sum, the preclinical data described herein provide a strong mechanistic rationale for assessing the clinical efficacy of polatuzumab vedotin, venetoclax, and anti-CD20 antibody co-treatment in NHL.

This is the first clinical study to combine polatuzumab vedotin and venetoclax with the anti-CD20 antibodies obinutuzumab or rituximab in patients with R/R NHL. Obinutuzumab was administered to patients with FL based on the favorable efficacy results of previous trials,[42,43] and patients with DLBCL received rituximab, as part of the standard-of-care for patients with DLBCL.[44,45] The Phase Ib dose-finding study identified the RP2D for polatuzumab vedotin as 1.8 mg/kg in combination with venetoclax 800 mg and an anti-CD20 agent. This triplet combination was well tolerated by most patients and had a predictable and acceptable safety profile. Due to the partially overlapping toxicities of the single agents, the most significant safety finding was the trend seen in cytopenias, with higher rates of neutropenia and thrombocytopenia observed as the venetoclax dose increased. However, the myelosuppressive effects of polatuzumab vedotin combined with venetoclax and obinutuzumab were manageable with granulocyte colony-stimulating factor prophylaxis, supportive measures, and dose modifications or delays. The risk mitigation strategies described in the protocol for both TLS and neutropenia provided management guidelines for these known AEs. Further, these safety data suggest that this combination should be explored further in R/R DLBCL and other B-cell NHL subtypes.

Excellent response rates for polatuzumab vedotin and venetoclax combined with an anti-CD20 agent are reported here in heavily pretreated patients with R/R FL and R/R DLBCL, where the majority of patients had disease refractory to their last line of treatment. In the R/R FL population, these results compare favorably with historical response rates seen in studies evaluating doublet combinations such as ROMULUS (polatuzumab vedotin + obinutuzumab/rituximab) or CONTRALTO (venetoclax + rituximab).[34,35,46] In particular, the trend toward higher response rates in patients receiving polatuzumab 1.8 mg/kg and venetoclax 800 mg (FL cohort 6) is notable and distinct from the response kinetics observed at lower dose cohorts. This 100% CR rate for patients with FL in cohort 6 at the EOI is an encouraging finding that is undergoing validation as the RP2D in the ongoing expansion cohort ($n = 40$). The early response rates observed in the R/R DLBCL cohorts confirm the moderate clinical activity of this combination in a difficult to treat aggressive lymphoma and have the potential to offer an alternative treatment option for patients with R/R disease. Although the response rates observed at EOI were lower than those reported with the polatuzumab vedotin + bendamustine + rituximab combination,[24] there are advantages to using the polatuzumab vedotin plus venetoclax combination rather than bendamustine because it is less immunosuppressive and may be a better bridge to a CAR-T therapy, when lymphopenia is undesirable prior to apheresis, as venetoclax does not reduce T cell numbers.[47] When considering options for bridging treatments, toxicity and access pose independent challenges, and for some patients, polatuzumab vedotin + rituximab could also be a suitable option.[34] The impact of post-induction treatment with venetoclax in combination with either obinutuzumab or rituximab is also being evaluated and will be included in future reports.

MCL is an interesting histology for this therapeutic combination, considering the in vitro data reported in this manuscript, showing activity in MCL. Patients with MCL were not included in this clinical study; however, the combination of polatuzumab vedotin, venetoclax, rituximab, and hyaluronidase (human recombinant) is being explored in an ongoing clinical trial (NCT04659044).

In conclusion, this Phase Ib clinical study has demonstrated manageable safety and excellent efficacy for the mechanistically targeted combination of polatuzumab vedotin and venetoclax with obinutuzumab or rituximab in patients with R/R FL or DLBCL. Furthermore, this study established a dosing regimen for the ongoing Phase II study that will provide additional information on the benefit–risk profile of this combination. Because MMAE and other anti-tubulin agents are likely not the only therapeutics that antagonize MCL-1,[49] our study provides scientific rationale and clinical evidence to pursue the broader strategy of identifying additional therapeutics that similarly neutralize MCL-1 function. Notably, single-agent venetoclax has very limited efficacy in acute myeloid lymphoma (AML)[50] and yet combination therapy with azacitidine has proven a highly efficacious breakthrough in AML treatment,[51] whereby azacitidine synergism is thought to be via downregulation/inhibition of MCL-1 and BCL-X_L. Therefore, such agents could also be used in combination with venetoclax to enhance treatment efficacy in other malignancies where MCL-1 is a venetoclax resistance factor. Indeed, the MCL-1 inhibitors under evaluation in clinical studies may provide additional options for therapeutic combinations, pending the establishment of their safety and efficacy. The systematic analysis to identify heterogeneous disease drivers, and translation of our preclinical data in R/R FL and DLBCL patients, support the rational design of mechanism-based treatment regimens that

directly target oncogenic drivers in patients with NHL and may serve as a framework for investigating and treating other malignancies with complex etiologies.

AUTHOR CONTRIBUTIONS

Elisabeth A. Lasater: Data curation, formal analysis, validation, writing—original draft, writing—review and editing, conceptualization, investigation.

Dhara N. Amin: Data curation, formal analysis, validation, writing—original draft, writing—review and editing, conceptualization, investigation.

Rajat Bannerji: Investigation, data curation, formal analysis, validation, writing—review and editing.

Raghuveer Singh Mali: Data curation, formal analysis, validation, writing—original draft, writing—review and editing, conceptualization, investigation.

Kathy Barrett: Data curation, formal analysis, validation, writing—review and editing, conceptualization, investigation.

Ryan N. Rys: Data curation, formal analysis, validation, writing—review and editing, conceptualization, investigation.

Jason Oeh: Data curation, formal analysis, validation, writing—review and editing, conceptualization, investigation.

Eva Lin: Data curation, formal analysis, validation, writing—review and editing, conceptualization, investigation.

Tim Sterne-Weiler: Data curation, formal analysis, validation, writing—review and editing, conceptualization.

Ellen Rei Ingalla: Data curation, formal analysis, validation, writing—review and editing, conceptualization, investigation.

MaryAnn Go: Data curation, formal analysis, validation, writing—review and editing, conceptualization, investigation.

Shang-Fan Yu: Data curation, formal analysis, validation, writing—review and editing, conceptualization, investigation.

Maxwell M. Krem: Investigation, data curation, formal analysis, validation, writing—review and editing.

Chris Arthur: Investigation, data curation, formal analysis, validation, writing—review and editing.

Uwe Hahn: Investigation, data curation, formal analysis, validation, writing—review and editing.

Anna Johnston: Investigation, data curation, formal analysis, validation, writing—review and editing.

Vinit Karur: Investigation, data curation, formal analysis, validation, writing—review and editing.

Nadia Khan: Investigation, data curation, formal analysis, validation, writing—review and editing.

Paula Marlton: Investigation, data curation, formal analysis, validation, writing—review and editing.

Tycel Phillips: Investigation, data curation, formal analysis, validation, writing—review and editing.

Giuseppe Gritti: Investigation, data curation, formal analysis, validation, writing—review and editing.

John F. Seymour: Investigation, data curation, formal analysis, validation, writing—review and editing.

Monica Tani: Investigation, data curation, formal analysis, validation, writing—review and editing.

Sam Yuen: Investigation, data curation, formal analysis, validation, writing—review and editing.

Scott Martin: Data curation, formal analysis, validation, writing—review and editing, conceptualization.

Matthew T. Chang: Data curation, formal analysis, validation, writing—review and editing, conceptualization, investigation.

Christopher M. Rose: Data curation, formal analysis, validation, writing—review and editing, investigation.

Victoria C. Pham: Data curation, formal analysis, validation, writing—review and editing, investigation.

Andrew G. Polson: Data curation, formal analysis, validation, writing—review and editing, conceptualization.

YiMeng Chang: Data curation, formal analysis, validation, writing—review and editing.

Claudia Wever: Data curation, formal analysis, validation, writing—review and editing, conceptualization, investigation.

Nathalie A. Johnson: Data curation, formal analysis, validation, writing—review and editing, conceptualization.

Yanwen Jiang: Data curation, formal analysis, validation, writing—review and editing, conceptualization.

Jamie Hirata: Data curation, formal analysis, validation, writing—review and editing, conceptualization.

Deepak Sampath: Data curation, formal analysis, validation, writing—original draft, writing—review and editing, conceptualization.

Lisa Musick: Data curation, formal analysis, validation, writing—original draft, writing—review and editing.

Christopher R. Flowers: Investigation, data curation, formal analysis, validation, writing—review and editing, conceptualization.

Ingrid E. Wertz: Data curation, formal analysis, validation, writing—original draft, writing—review and editing, conceptualization, investigation.

AFFILIATIONS

[1]Department of Translational Oncology, Genentech, Inc., South San Francisco, California, USA

[2]Department of Discovery Oncology, Genentech, Inc., South San Francisco, California, USA

[3]Department of Early Discovery Biochemistry, Genentech, Inc., South San Francisco, California, USA

[4]Rutgers Cancer Institute of New Jersey, New Brunswick, New Jersey, USA

[5]Department of Biomarker Development, Genentech, Inc., South San Francisco, California, USA

[6]Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, Quebec, Canada

[7]Department of Medicine, McGill University, Montreal, Quebec, Canada

[8]Department of Oncology Bioinformatics, Genentech, Inc., South San Francisco, California, USA

[9]Markey Cancer Center, University of Kentucky College of Medicine, Lexington, Kentucky, USA

[10]Royal North Shore Hospital (RNSH), Sydney, New South Wales, Australia
[11]The Queen Elizabeth Hospital (TQEH), Adelaide, South Australia, Australia
[12]Royal Hobart Hospital (RHH), Hobart, Tasmania, Australia
[13]Baylor Scott & White Healthcare, Temple, Texas, USA
[14]Fox Chase Cancer Center, Philadelphia, Pennsylvania, USA
[15]Princess Alexandra Hospital, and University of Queensland, Brisbane, Queensland, Australia
[16]University of Michigan Comprehensive Cancer Center, Ann Arbor, Michigan, USA
[17]Hematology and Bone Marrow Transplant Unit, Ospedale Papa Giovanni XXIII, Bergamo, Italy
[18]Peter MacCallum Cancer Centre, Royal Melbourne Hospital, and University of Melbourne, Melbourne, Victoria, Australia
[19]Ospedale S. Maria delle Croci, Ravenna, Italy
[20]Calvary Mater Newcastle, Waratah, New South Wales, Australia
[21]Department of Microchemistry, Proteomics and Lipidomics, Genentech, Inc., South San Francisco, California, USA
[22]Hoffmann-La Roche Ltd, Mississauga, Ontario, Canada
[23]Product Development Oncology, Genentech, Inc., South San Francisco, California, USA
[24]Department of Lymphoma and Myeloma, UT MD Anderson Cancer Center, Houston, Texas, USA

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CONFLICT OF INTEREST

Elisabeth A. Lasater, Dhara N. Amin, Raghuveer Singh Mali, Kathy Barrett, Jason Oeh, Eva Lin, Tim Sterne-Weiler, Ellen Rei Ingalla, MaryAnn Go, Shang-Fan Yu, Scott Martin, Matthew T. Chang, Victoria C. Pham, Andrew G. Polson, Yanwen Jiang, Jamie Hirata, Deepak Sampath, Lisa Musick and Ingrid E. Wertz are either currently, or were at the time of study conduct, employees of F. Hoffmann-La Roche Ltd/Genentech, Inc. Nathalie A. Johnson has received consulting fees from F. Hoffmann-La Roche Ltd and AbbVie Inc. John F. Seymour has served on the advisory boards and speakers' bureaus of AbbVie and Roche; has received research funding from AbbVie and Roche; and has provided expert testimony for Roche. YiMeng Chang is an employee of Roche. Maxwell M. Krem and Ryan N. Rys declare no competing interests.

DATA AVAILABILITY STATEMENT

Phase I studies are not in scope of the Roche global policy on data sharing. Given the small study population, the decision to share the patient-level clinical data needs to be handled on a case-by-case basis to determine if the data can be adequately anonymised to give an acceptably low risk of patient re-identification. Qualified researchers may submit an enquiry through the data request platform, Vivli, https://vivli.org/ourmember/roche/, however this does not guarantee that the data can be shared. For up to date details on Roche's Global Policy on the Sharing of Clinical Information and how to request access to related clinical study documents, see here: go.roche.com/data_sharing. Anonymised records for individual patients across more than one data source external to Roche cannot, and should not, be linked due to a potential increase in risk of patient re-identification. Correspondence and request for materials should be addressed to C. R. F. or I. E. W.

ORCID

Tycel Phillips https://orcid.org/0000-0003-2143-9672
Ingrid E. Wertz https://orcid.org/0000-0002-7979-9374

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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