Molecular Determinants of Sensitivity to Polatuzumab Vedotin in Diffuse Large B Cell Lymphoma

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ABSTRACT

Polatuzumab Vedotin (Pola-V) is an antibody-drug conjugate directed to the CD79B subunit of the B cell receptor (BCR). When combined with conventional immunochemotherapy, Pola-V improves outcomes in DLBCL. To identify determinants of sensitivity to Pola-V, we used CRISPR-Cas9 screening for genes that modulated the toxicity of Pola-V for lymphomas or the surface expression of its target, CD79B. Our results reveal the striking impact of CD79B glycosylation on Pola-V epitope availability on the lymphoma cell surface and on Pola-V toxicity. Genetic, pharmacological, and enzymatic approaches that remove sialic acid from N-linked glycans enhanced lymphoma killing by Pola-V. Pola-V toxicity was also modulated by KLHL6, an E3 ubiquitin ligase that is recurrently inactivated in germinal center derived lymphomas. We reveal how KLHL6 targets CD79B for degradation in normal and malignant germinal center B cells, thereby determining expression of the surface BCR complex. Our findings suggest precision medicine strategies to optimize Pola-V as a lymphoma therapeutic.

Statement of Significance:

These findings unravel the molecular basis of response heterogeneity to Pola-V and identify approaches that might be deployed therapeutically to enhance the efficacy of CD79B-specific tumor killing. In addition, they reveal a novel post-translational mechanism used by normal and malignant germinal center B cells to regulate expression of the BCR.

INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is the commonest form of aggressive non-Hodgkin lymphoma, with an estimated incidence of 150,000 cases per year worldwide (1). At the molecular level, it is an exceptionally heterogeneous disease that can be classified by gene expression profiling into subtypes resembling either germinal center B cells (GCB) or activated B cells (ABC) (2) These cell-of-origin (COO) gene expression subtypes can be further divided into genetic subtypes that acquire characteristic constellations of somatic mutations, utilize distinct oncogenic mechanisms, and differ in their response to treatment (3-6). For almost twenty years the first-line therapy for DLBCL has been R-CHOP, an immunochemotherapy regimen comprised of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone that is curative in approximately 60-70% of cases, varying by subtype. Recently, the phase III POLARIX trial reported improved progression free survival when R-CHOP was modified to replace vincristine with the antibody-drug conjugate (ADC) polatuzumab vedotin (Pola-V) (7). While Pola-V has been widely used in the setting of relapsed DLBCL (8-10), the results of the POLARIX trial make Pola-V the first drug to be approved for use in first-line therapy for DLBCL in more than two decades.

Pola-V is synthesized by coupling a monoclonal antibody (polatuzumab) that binds the extracellular domain of CD79B to the cytotoxic microtubule inhibitor monomethyl auristatin E (MMAE) using a protease-cleavable linker (11,12). CD79B is a transmembrane protein with expression restricted to normal B cells and B cell lymphomas, including almost all cases of DLBCL (11). CD79B and CD79A, the signaling subunits of the B cell antigen receptor (BCR), heterodimerize with each other and pair with immunoglobulin heavy chains to form intact BCR on the plasma membrane of normal and malignant B cells (13). Binding of Pola-V to CD79B leads to BCR internalization and trafficking to acidic intracellular vesicles where MMAE is released, thereby killing the targeted malignant lymphoma cells (14).

Not every DLBCL tumor is equally responsive to Pola-V. For example, subgroup analysis from the POLARIX trial suggested that Pola-V may exert a greater effect in ABC DLBCL than in the GCB subtype (7). Since very little is known about the molecular mechanisms that underlie sensitivity or resistance to Pola-V, we sought to identify genes that modulate the effect of Pola-V using unbiased CRISPR-Cas9 functional screens of DLBCL cell lines. We

identified independent mechanisms that converge on the ability of Pola-V to bind to the lymphoma cell surface, suggesting potential therapeutic strategies that could increase the efficacy of CD79B-directed tumor killing by Pola-V.

RESULTS

CRISPR screens identify determinants of CD79B-directed killing by Pola-V.

To identify factors that regulate sensitivity or resistance to Pola-V we performed genome-wide CRISPR-Cas9 drug sensitization screens using a panel of 9 DLBCL cell lines representing both the ABC and GCB subtypes (Fig. 1A; Supplementary Table S1). To allow us to distinguish factors affecting CD79B-directed killing from those affecting sensitivity to MMAE toxin alone, parallel screens were performed under the selective pressure of Pola-V, unconjugated polatuzumab antibody (Pola-Ab), MMAE, and DMSO. Comparing single guide RNA (sgRNA) frequency between Pola-V and Pola-Ab, or between MMAE and DMSO allowed us to calculate a gene-level, Z-normalized CRISPR Screen Score (CSS) (15), where negative scores indicate increased sensitivity to the drug. In parallel, to identify factors that influence expression of the target protein CD79B, we performed CRISPR screens comparing lymphoma cells sorted by flow cytometry for high (top 5%) surface CD79B mean fluorescent intensity (MFI) or low (bottom 5%) CD79B MFI (Fig. 1A; Supplementary Table S1). By comparing sgRNA frequency from the CD79B-high and CD79B-low sorted fractions we generated a gene-level CD79B segregation score, where positive scores indicate increased CD79B surface expression. As expected, sgRNAs targeting CD79B and its binding partner CD79A were associated with strongly reduced CD79B segregation scores (Fig. 1B and 1C). Accordingly, these sgRNAs were associated with reduced sensitivity to Pola-V but did not affect the MMAE CSS. These findings confirm the validity of our screening strategy to identify genes that influence CD79B-directed killing by Pola-V.

We were struck by a cluster of genes, knockout of which increased sensitivity to Pola-V and increased cell surface CD79B levels but did not alter MMAE sensitivity (Fig. 1A and B). Remarkably, almost all these genes belonged to two functional categories related to N-linked glycosylation. The first category included genes encoding subunits of the oligosaccharyltransferase (OST) complex (STT3B, MAGT1, OST4, RPN1, DDOST, RPN2, DAD1,

TMEM258), which attaches a preformed glycan to asparagines of membrane and secreted proteins in the endoplasmic reticulum. The second category included sugar biosynthetic enzymes, sugar transporters, and glycosyltransferases that modify N-linked glycans in the Golgi (ST6GAL1, SLC35A1, SLC35A2, B4GALT1, B3GNT2, GALE, GNE, NANS, CMAS) (Fig. 1D and 1E). Analysis of genes within the Golgi glycosylation pathway suggested involvement of a very specific set of enzymes that assemble an N-linked glycan chain terminating in an α2,6-linked sialic acid residue (Fig. 1E and Supplementary Fig. S1A). This terminal sialic acid moiety is added by the sialyltransferase ST6GAL1 (16). Notably, ST6GAL1, along with multiple genes from the sialic acid synthesis pathway (GNE, NANS, CMAS) and its associated substrate transporter (SLC35A1) were amongst the highest scoring genes in our screens. Also scoring highly were genes involved in the biosynthesis (GALE) and transport (SLC35A2) of galactose, the penultimate sugar moiety in mature glycans that is required for terminal addition of α2,6 sialic acid to N-glycans. Conversely, sgRNAs targeting genes known to oppose development of α2,6-sialylated glycans (MGAT3 (17) and SPPL3 (18)) displayed the opposing phenotype of reduced Pola-V sensitivity and lower CD79B expression (Fig. 1D). Taken together, these findings highlight the importance of terminal sialic acid addition to the N-linked glycan chain in determining sensitivity to CD79B-directed tumor killing by Pola-V.

To investigate how genetic heterogeneity of DLBCL tumors might influence sialylation of N-linked glycans, we interrogated a database of somatic mutations, DNA copy number abnormalities, and digital gene expression from 574 DLBCL tumors (4), focusing on the genes discussed above that influenced sensitivity to Pola-V (Supplementary Fig. S1B). Each of these 9 genes was affected by copy number aberrations in a substantial fraction (>15%) of cases, with 7 of these genes more often altered by DNA copy number gains and amplifications than by copy number losses. In fact, one third of these tumors acquired copy number gains or amplifications in two or more genes that affect N-linked glycosylation. For each gene, there was a roughly linear relationship between the DNA copy number class and mRNA expression levels. These data suggest that the nature and extent of N-linked glycan modifications in DLBCL tumors may vary among patients based on the presence of recurrent genetic alterations targeting key regulators of the N-linked glycosylation pathway, potentially affecting responses to BCR-directed therapies (Supplementary Fig. S1C).

Loss of α2,6 sialylated glycans enhances sensitivity to Pola-V

To confirm the effect of α2,6-sialylation on sensitivity of DLBCL to Pola-V, we generated DLBCL cell lines in which we used CRISPR-Cas9 mutagenesis to inactivate the α2,6-sialyltransferase *ST6GAL1* or the Golgi nucleotide sugar transporters *SLC35A1* and *SLC35A2* that are essential for sialylation. As expected, knockout of any of these genes led to reduced staining with Sambucus Nigra Lectin (SNA), which binds α2,6-linked sialic acid (Supplementary Fig. S2). Compared to wild type control lines, we observed increased sensitivity to Pola-V in all sialylation-incompetent lines, with up to 20-fold reduction in IC_{50} (Fig. 2A). Enhanced sensitivity to Pola-V was further demonstrated in competitive fitness experiments in a panel of six lymphoma lines transduced with Cas9 and sgRNAs targeting the same three genes (*SLC35A1*, *SLC35A2* or *ST6GAL1*) (Fig. 2B). In all cell lines, and for all three deleted genes, we saw strong selection against knockout cells when cultured in the presence of Pola-V. Minimal or no effect was seen in the presence of DMSO or MMAE confirming the importance of α2,6-sialylation in determining sensitivity to CD79B-directed killing by Pola-V. Increased sensitivity was evident in both GCB and ABC DLBCL lines and was independent of immunoglobulin heavy chain isotype.

In addition to the genetic deletion, we observed increased sensitivity to Pola-V in cell lines exposed to the sialyltransferase inhibitor 3Fax-Peracetyl Neu5Ac (Supplementary Fig. S3). To further assess the druggability of this pathway, we engineered the RIVA and TMD8 ABC DLBCL lines to express SLC35A2 fused to $FKBP12^{F36V}$, allowing inducible degradation of the fusion protein by the small molecule dTag-13, as described (19). Addition of dTag-13 reduced SNA staining and increased detection of cell surface CD79B by flow cytometry (Supplementary Fig. S4A). As predicted, dTag-13-induced degradation of SLC35A2 enhanced Pola-V cytotoxicity (Supplementary Fig. S4B). Thus, genetic and pharmacological inhibition of sialylation enhanced Pola-V sensitivity, independent of DLBCL gene expression subtype or immunoglobulin heavy chain isotype.

Finally, we performed xenograft experiments using two cell lines (RIVA and FL-318) in which we had inactivated either *SLC35A1*, *SLC35A2* or *ST6GAL1*. Whilst knockout did not decrease

tumor growth in the absence of Pola-V, inactivation of any of these genes enhanced the sensitivity of the lymphoma tumors to Pola-V (Fig. 2B). Overall, these findings confirm that loss of α2,6-linked, terminal sialic acid residues from N-linked glycans enhances sensitivity to Pola-V both *in vitro* and *in vivo*.

Pola-V sensitivity predicted by surface CD79B flow cytometry

Our CRISPR screening data revealed a strong correlation between increased sensitivity to Pola-V and increased ability to detect CD79B on the cell surface. These data appear at odds with previously published clinical data that found no relationship between Pola-V sensitivity and total CD79B expression, as quantified by immunohistochemistry (IHC) (8,20). To investigate this apparent contradiction, we assessed sensitivity to Pola-V and MMAE in an extended panel of 54 cancer cell lines and compared surface CD79B detection by flow cytometry to drug sensitivity, as quantified by area under the dose-response curve. Surface CD79B detection was strongly correlated (R^2 = 0.64) with sensitivity to Pola-V but not to MMAE (R^2 = 0.09) (Fig. 3A and 3B). We observed no relationship between sensitivity to Pola-V and a previously quantified CD79B expression score (20) as determined by IHC (Supplementary Fig. S5). These methodologies to detect CD79B differ in important ways, including the fact that IHC quantifies both intracellular and surface CD79B expression and uses an antibody (AT107-2) specific for an epitope in the intracellular domain of CD79B. By contrast, the antibody used for flow cytometric assays of surface CD79B recognizes the same epitope as Pola-V, which resides in the extracellular domain (21). In fact, all commercially available antibodies against the extracellular domain of CD79B recognize the same epitope as polatuzumab (21). These findings led us to speculate that α2,6-sialylation might determine sensitivity to Pola-V by influencing either the surface expression of CD79B or, alternatively, the accessibility of the polatuzumab-binding epitope.

α2,6 sialylated N-linked glycans impede binding of polatuzumab to CD79B

To determine whether sialic acid inhibits polatuzumab binding to CD79B, we treated a panel of 12 DLBCL lines with the sialyltransferase inhibitor 3Fax-Peracetyl Neu5Ac and performed flow cytometric analysis of surface sialic acid (using SNA), antibody binding to surface CD79B, and surface immunoglobulin levels at intervals by flow cytometry (Fig. 4A). As expected, sialyltransferase inhibition caused the progressive loss of surface sialic acid

staining. This was accompanied by increased staining for CD79B in 10 of 12 lines tested, reaching almost 600% of baseline by day 4 in the ABC lines RIVA and TMD8. In contrast, levels of surface immunoglobulin were not affected by inhibition of sialyltransferase. Similar findings were made using cell lines in which the sialic acid and galactose transporters SLC35A1 and SLC35A2, respectively, were inactivated by CRISPR-Cas9 mutagenesis in a panel of DLBCL lines (Figs. 4B and 4C). Genetic inactivation of either transporter increased antibody detection of cell surface CD79B but did not alter antibody detection of cell surface immunoglobulin. Since the cell surface BCR is comprised of immunoglobulin heavy chain, CD79B and CD79A with a 2:1:1 stoichiometry (22,23), these findings demonstrate that de-sialylation of the BCR does not alter its transit from the Golgi to the cell surface.

To confirm that loss of α2,6 sialylation did not affect total CD79B protein levels, we performed immunoblot analysis of lysates from cells in which either SLC35A1 or SLC35A2 had been inactivated by CRISPR (Fig. 4D). As an additional control we removed all N-linked glycans by treating samples with the amidase enzyme PNGase-F. While subtle shifts were observed in the migration of CD79B in SLC35A1 and SLC35A2 knockout cells due to loss of terminal glycosylation moieties, a much larger shift was seen with PNGase-F, as expected. In contrast to the considerable increase in binding of antibody to cell surface CD79B seen in flow cytometric assays, total CD79B protein levels did not increase in either SLC35A1 or SLC35A2 knockout cells by immunoblot analysis. Taken together, these data demonstrate that the observed increase in antibody binding to cell surface CD79B upon sialic acid loss was not due to overall increase in CD79B protein or to an increase in transit of the BCR to the cell surface.

We therefore hypothesized that the presence of sialic acid on the cell surface BCR was the determining factor in polatuzumab binding to CD79B. To test this, we exposed a panel of DLBCL cell lines to a purified sialidase enzyme in order to selectively strip sialic acid from cell membrane proteins (Fig. 4E; Supplementary Fig. S6A and S6B). Remarkably, the enzymatic removal of cell surface sialic acid increased the flow cytometric signal for CD79B but did not affect detection of cell surface immunoglobulin. Sialidase treatment had no effect on the cell surface binding of antibodies to CD20 (Rituximab) or CD19 (FMC63) (Supplementary Fig. S7A-7C).

To establish the relevance of these findings to primary human B cells, we repeated the sialidase exposure experiments using primary DLBCL B cells purified from patient biopsies (n=8) as well as germinal center B cells purified from pediatric tonsils (n=4) (Fig. 4F). In both malignant and non-malignant B cells, depletion of sialic acid, as revealed by decreased SNA staining, was associated with increased binding of CD79B antibody to the cell surface, whereas there was no change in anti-CD19 binding.

Finally, to confirm an effect of α2,6-sialylation on binding of antibody to the CD79B epitope, we generated binding curves for polatuzumab, using DLBCL lines transduced with sgRNAs targeting SLC35A1 or SLC35A2, or with a control sgRNA (Fig. 4G). Analysis of the binding curves generated using escalating concentrations of polatuzumab revealed an average decrease of 62% in the K_D of polatuzumab binding to cell surface CD79B in sialylation-incompetent cells compared to control cells (Fig. 4G).

Taken together, these findings in DLBCL cell lines and patient-derived DLBCL B cells confirm that sialylation of N-linked glycans does not affect total CD79B protein abundance or routing of CD79B to the cell surface but instead alters the binding affinity of polatuzumab to its target epitope on CD79B.

Glycosylation of specific residues of CD79A and CD79B impedes polatuzumab binding

Examination of the recently published cryo-electron microscopy structures of the human BCR complex (22,23) revealed four asparagine residues in CD79A and CD79B that are positioned in proximity to the polatuzumab binding site and that reside within an N-linked glycosylation motif (Asn–X–Ser/Thr) (Fig. 5A). We performed glycoproteomic analysis of ABC (TMD8, RIVA) and GCB (FL-318, WSU-FSCCL) cell lines and were able to confirm the presence of glycans on CD79B N101 and N127/N128 as well as on CD79A N112 (Supplementary Table 2).

To pinpoint which of the glycans on CD79A and CD79B might influence polatuzumab binding, we generated CRISPR-Cas9 edited ABC (RIVA) and GCB (SUDHL4) lines in which the asparagine residues of interest were mutated to glutamine, thus precluding glycosylation.

Whereas two glycosylation mutants (CD79A N73Q, CD79B N101Q) had no effect on binding to cell surface CD79B, the CD79A N112Q mutant and the CD79B N127Q/N128Q double mutant significantly increased binding (Fig. 5B, Supplementary Fig. 8A). None of these mutations had any effect on detection of cell surface immunoglobulin, consistent with previous experiments involving genetic and pharmacological inhibition of sialylation. Immunoblot analysis of CD79A and CD79B showed gel mobility shifts consistent with loss of glycosylation, making comparison of total protein abundance challenging (Fig. 5C). To overcome this, we treated lysates with PNGase F to remove all glycosylation, resulting in a single lower molecular weight band in immunoblots for both CD79A and CD79B. This analysis revealed that each glycosylation mutant was expressed at equivalent levels (Fig. 5D), suggesting that the increase in polatuzumab binding to the N-linked glycosylation mutants was due to improved accessibility and/or affinity of polatuzumab for its epitope on CD79B.

The increased polatuzumab binding to the CD79A N112Q and CD79B N127Q/N128Q mutants was mirrored by an increased sensitivity to Pola-V *in vitro* (Fig. 5E). As predicted, the CD79A N73Q and CD79B N101Q mutants, which did not affect polatuzumab binding, did not affect sensitivity to Pola-V, and sensitivity to free MMAE was not altered by any mutant (Supplementary Fig. S9A,B). Finally, we tested the effect of the CD79B N127Q/N128Q mutation on the ability of Pola-V to control tumor growth in a xenograft model of the ABC model RIVA. While the CD79B N127Q/N128Q mutation did not alter tumor growth in vehicle-treated control mice, the CD79B mutant tumors were more sensitive to Pola-V than CD79B wild type tumors (Fig. 5F). These experiments confirm that glycosylation of specific asparagine residues flanking the polatuzumab binding epitope determine sensitivity to Pola-V in vivo.

To model how N-linked glycosylation might be targeted clinically to enhance the efficacy of Pola-V, we examined the effect of NGI-1, a small molecule inhibitor of N-linked glycosylation(24). NGI-1 inhibits N-linked glycosylation by preferentially targeting the enzymatic subunit of the OST-B complex, STT3B, which scored highly in our CRISPR-Cas9 screens as promoting resistance to Pola-V when inactivated (Fig. 1D,E). Treatment of DLBCL cell lines with NGI-1 led to a progressive reduction in surface α2,6 sialic acid staining. This

was associated with a progressive increase in CD79B staining (Fig. 5G) of magnitude similar to that observed in our previous genetic or sialidase experiments. Combinatorial drug studies showed that relatively low concentrations of NGI-1 were able to enhance the toxicity of Pola-V in vitro (Fig. 5H). Moreover, NGI-1 sensitized lymphoma cells to Pola-V when tested in vivo using both RIVA and FL-318 xenograft models (Fig. 5I).

Taken together, these findings confirm that sialylation of N-linked glycans is a major determinant of sensitivity to CD79B-directed killing by Pola-V both *in vitro* and *in vivo.* This is dictated primarily by specific asparagine residues in CD79A and CD79B situated in proximity to the polatuzumab binding epitope on CD79B. These experiments further demonstrate that small molecule inhibitors of N-linked glycosylation can increase Pola-V killing of lymphoma cells both in vitro and in vivo.

KLHL6 regulates sensitivity to Pola-V in GCB DLBCL

While the effect of N-linked glycosylation on Pola-V efficacy was evident in both the ABC and GCB subtypes of DLBCL, we hypothesized that other determinants of sensitivity to Pola-V might be masked by phenotypic differences between these two DLBCL subtypes. We therefore reanalyzed our CRISPR screen data from ABC and GCB models separately. In the analysis of GCB lines, we were struck by the fact that knockout of KLHL6, a subunit of a Cullin3-dependent E3 ubiquitin ligase complex (25), increased cell surface CD79B expression and sensitivity to Pola-V (Fig. 6A) but did not affect sensitivity to MMAE (Fig. 6B). These phenotypes were not evident in the CRISPR screens of ABC lines (Fig. 6C, Supplementary Fig. S10A,B). To confirm the effect of KLHL6 loss on sensitivity to Pola-V, we performed competitive growth assays of cells transduced with a vector expressing GFP along with a KLHL6 sgRNA or a control sgRNA. In the absence of Pola-V, knockout of *KLHL6* had no effect on the proportion of GFP^+/$sgKLHL6^+$ cells over time. However, the presence of Pola-V led to strong selection against *KLHL6*-inactivated cells in GCB but not ABC cell lines (Fig. 6D). KLHL6 inactivation did not affect sensitivity to MMAE in pooled competition assays (Supplementary Fig. S10C), confirming that loss of KLHL6 sensitizes cells to CD79B-directed killing by Pola-V. Finally, in dose response experiments, KLHL6 knockout in 3 GCB lines rendered them sensitive to lower concentrations of Pola-V (Fig. 6E).

KLHL6 is a germinal center-specific tumor suppressor in DLBCL

The GCB-specific effect of KLHL6 knockout on Pola-V sensitivity prompted us to investigate KLHL6 expression in lymphoma subtypes and during B cell differentiation, using previously published RNA-Seq data (4). KLHL6 mRNA expression was greater in GCB DLBCL than in ABC DLBCL, both in tumor biopsies and cell lines, in line with previous immunohistochemical observations (26), while Unclassified cases had an intermediate expression (Fig. 6F). Furthermore, separation of genetically subtyped tumors by cell of origin classification showed that independent of genetic subtype, GCB tumors expressed higher levels of KLHL6 mRNA than ABC or unclassified tumors (Fig. 6G). KLHL6 mRNA levels were also strongly upregulated in germinal center dark zone and light zone B cells isolated from human tonsils compared with naïve mature B cells (Fig. 6H). Of note, CD79B protein expression showed an opposite distribution, with lower protein levels in the germinal center compared to the surrounding mantle zone (Fig. 6I). The reduced abundance of CD79B protein in germinal center B cells was not due to changes in CD79B or CD79A mRNA levels (Fig. 6H), suggesting a post-translational mechanism, as has been reported by others, albeit without previous mechanistic explanation(27,28).

Given the restricted expression of KLHL6 in normal and malignant germinal center B cells, we investigated which DLBCL subtypes might preferentially acquire KLHL6 mutations and abrogate its tumor suppressor activity. We pooled mutation data from 51 sequencing studies of DLBCL tumors (see Methods) and observed 535 KLHL6 mutations in 6% (431/7146) of cases (Fig. 6J). Analysis of individual mutations revealed hotspots within both the amino-terminal BTB domain and the carboxy-terminal Kelch domain (Fig. 6K,L). *KLHL6* mutations were significantly enriched in GCB DLBCL (8.8%) relative to the other gene expression subtypes and were especially prevalent in the ST2 genetic subtype (15% of cases) (Fig. 6J).

KLHL6 negatively regulates CD79B protein expression and BCR cell surface levels

While KLHL6 was previously shown to regulate NF-κB activity in the context of ABC DLBCL (25), *KLHL6* inactivation did not affect surface CD79B levels in CRISPR-Cas9 screens of four ABC lines, suggesting that KLHL6 may play a greater role in determining levels of surface CD79B in GCB DLBCL (Fig. 6C, Supplementary Fig. 10A,B). To extend these findings, we

transduced a panel of ABC (n=2) and GCB (n=5) DLBCL lines with an sgRNA targeting KLHL6 and induced Cas9 expression. Over time, we observed a progressive increase in cell surface CD79B expression in the GCB but not ABC cell lines (Fig. 7A). Immunoblotting revealed an increase in total cellular CD79B abundance in KLHL6-deficient GCB lines, which was likely responsible for the increased detection of CD79B on the cell surface of these cells (Fig. 7B).

We next investigated whether KLHL6 regulates CD79B protein expression in normal germinal center B cells. For this, we transduced Cas9 and a KLHL6 sgRNA into human tonsillar germinal center B cells that were expanded *ex vivo* using a coculture system that mimics the GC microenvironment (29). Inactivation of *KLHL6* resulted in a significant increase in CD79B surface expression by flow cytometry (Fig. 7C; Supplementary Fig. S11A). Moreover, immunoblot analysis demonstrated that KLHL6 inactivation caused a pronounced increase in total cellular CD79B (Fig. 7D), confirming that KLHL6 negatively regulates CD79B expression in non-malignant germinal center B cells. Increased CD79B expression was also associated with increased CD79A and increased phospho-CD79A, suggesting that KLHL6 loss enhanced proximal BCR signaling (Fig. 7D).

To confirm these findings in a physiological germinal center response, we retrovirally transduced control or *klhl6*-targeting sgRNAs into bone marrow cells from mice expressing Cas9 under the control of the germinal center-specific AID-Cre. Transduced cells were injected into wild type mice and germinal centers from Peyer's patches and mesenteric lymph nodes were analyzed 7 weeks later. Germinal center B cells expressing the *Klhl6*-targeting sgRNA had significantly higher surface immunoglobulin levels than non-transduced germinal center B cells, irrespective of immunoglobulin heavy chain isotype, as well as elevated levels of surface CD79B, whereas the control sgRNA had no effect (Fig. 7E; Supplementary Fig. S12C).

Taken together these findings demonstrate that KLHL6 negatively regulates the abundance of CD79B protein in both GCB DLBCL and in normal germinal center B cells. In so doing, KLHL6 loss increases the surface expression of the BCR complex and the sensitivity of cells to Pola-V.

KLHL6 directly regulates CD79B protein turnover

KLHL6 has not previously been implicated in the regulation of CD79B or other BCR proteins. To test whether the activity of KLHL6 as a ubiquitin E3 ligase is necessary for CD79B regulation, we took advantage of KLHL6 BTB domain mutations that were previously shown to disrupt interaction with Cullin3, thereby preventing the formation of a functional Cullin-RING ubiquitin ligase complex (25,30). We used CRISPR-Cas9 mutagenesis to create knock-in clones bearing one such loss-of-function mutation, KLHL6^{L90F}. From two GCB lines, SUDHL5 and WSU-DLCL2, we established 3 independent KLHL6^{L90F} clones, each of which had significantly higher expression of CD79B on the cell surface than in the parental lines (Fig. 7F). Immunoblot analysis revealed a marked increase in total cellular CD79B levels in the L90F knock-in mutants relative to the parental lines (Fig. 7G). Moreover, the increased CD79B expression seen in KLHL6 L90F knock-in mutant clones translated into increased sensitivity to Pola-V in survival assays when compared to WT KLHL6 (Fig. 7H).

To establish the mechanism for this observed effect, we performed quantitative whole proteome mass spectrometry to identify proteins with altered expression after knockout of *KLHL6.* In parallel, we identified KLHL6-interacting proteins by ectopically expressing a KLHL6-BioID2 fusion construct (31) in SUDHL5 GCB DLBCL cells and identified biotinylated proteins by streptavidin pulldown and mass spectrometry (Fig. 7I, Supplementary Table 3). Remarkably, CD79B was identified amongst proteins with the greatest increase in expression after *KLHL6* deletion (Fig 7I, Supplementary Table 3). Moreover, components of the BCR complex (including CD79A, CD79B, IgM) were identified as KLHL6-proximal proteins, suggesting that CD79B protein levels might be regulated through direct interaction with KLHL6.

To further confirm CD79B as a direct target of KLHL6, we sought to identify the lysine residues in CD79B that might be ubiquitinated by KLHL6. To this end, we transduced SUDHL5 GCB cells with a KLHL6 sgRNA or a control sgRNA and performed quantitative whole ubiquitinome profiling by mass spectrometry to identify KLHL6-dependent ubiquitinated lysines. This revealed lysine ubiquitination of CD79B at K152 and K219 (Fig. 7J, Supplementary Table 3). K152 resides in the extracellular portion of CD79B whereas K219 is in the intracellular tail, C-terminal to the ITAM motif that mediates signaling (Supplementary

Fig. S13). Interestingly, CD79B K219 is recurrently mutated in DLBCL, albeit rarely, with this lysine changed to arginine or threonine in 4 reported DLBCL cases (Supplementary Fig. S13) (4,32-34). We generated knock-in mutant clones in the GCB line WSU-DLCL2 in which these ubiquitinated lysines were changed to arginine (K152R, K219R). For completeness, we also created clones in which the other two intracellular lysines in CD79B were changed to arginine (K183R, K187R). For each mutation, we isolated at least 2 independent clones to ensure the reproducibility of their associated phenotypes. Cell surface CD79B and surface immunoglobulin levels were increased in the K219R mutant clones relative to control clones with wild type CD79B, whereas the other lysine mutants had no effect (Fig. 7K, Supplementary Fig. S11). Immunoblot analysis confirmed an increase in CD79B protein abundance exclusively in the CD79B K219R mutant clones (Fig. 7L).

KLHL6 partners with Cullin3 through its BTB domain to form a functional E3 ligase that directs ubiquitin to target proteins, ultimately leading to their degradation. Cullin3 stability and activation requires neddylation, a process that can be inhibited by the small molecule MLN4924. By immunoblot analysis, MLN4924 treatment increased CD79B protein and, to a lesser degree, CD79A (Fig. 7M, Supplementary Fig. S14). In cells in which KLHL6 was inactivated by CRISPR-Cas9 mutagenesis, CD79B levels were elevated constitutively but MLN4924 treatment did not further increase expression, supporting the hypothesis that an E3 ubiquitin ligase complex containing Cullin3 and KLHL6 mediates CD79B ubiquitination and degradation. In contrast, treatment with the proteasome inhibitor MG132 did not alter CD79B protein levels, suggesting ubiquitinated CD79B was not degraded via the proteasome (Fig. 7N; Supplementary Fig. S15A). Ubiquitination of cell surface proteins most often directs proteins for degradation through the lysosome rather than the proteasome (35). Abrogation of lysosome function using the V-ATPase inhibitor Bafilomycin A1 increased CD79A and CD79B protein abundance in control cells but not cells in which KLHL6 was inactivated (Fig. 7O; Supplementary Fig. S15B). These findings reveal a previously unappreciated role for KLHL6 in the regulation of CD79B by ubiquitination of lysine 219, thereby routing the CD79B protein for lysosomal degradation, providing a mechanistic basis for the enhanced sensitivity of KLHL6-deficient and KLHL6-mutant cells to Pola-V.

DISCUSSION

Pola-V is the first new drug approved for use in the initial therapy of DLBCL in almost two decades. By coupling the microtubule inhibitor MMAE to a CD79B-directed antibody, Pola-V widens the therapeutic window of MMAE by enhancing its delivery to malignant B cells while limiting its effect on other cell types, resulting in a very favorable safety profile. Our CRISPR screening strategy uncovered molecular determinants of sensitivity and resistance to Pola-V that were distinct from those that influence sensitivity to the microtubule inhibitor. Specifically, our study revealed that sialylation of N-linked glycans determines accessibility of Pola-V to its epitope on CD79B, providing a road map for new therapeutics that could increase the efficacy of Pola-V by removing sialylated glycans from CD79A and CD79B. We additionally discovered that a lymphoma tumor suppressor, KLHL6, is a CD79B ubiquitin ligase that regulates CD79B levels on the cell surface such that inactivating KLHL6 mutations in DLBCL enhance Pola-V sensitivity. Finally, we observed a strong correlation between sensitivity to Pola-V and surface CD79B staining when quantified by flow cytometry, in contrast to the lack of correlation reported between clinical response to Pola-V and CD79B expression assessed by IHC (11,20). This suggests that flow cytometry might serve as an important clinical biomarker for the efficacy of Pola-V. By providing mechanistic insights into the determinants of Pola-V sensitivity, our study has precision medicine implications for the deployment of this important new lymphoma drug and suggests a developmental therapeutics strategy to improve its efficacy.

Our CRISPR screens for modifiers of Pola-V sensitivity implicated a specific set of enzymes and small molecule transporters that converge on the addition of a terminal α2,6-linked sialic acid residue on N-linked glycans. Knockout of these enzymes and transporters increased sensitivity of DLBCL models to Pola-V, as did treatment of cells with a sialyltransferase inhibitor or OST-B inhibitor. Importantly, extracellular treatment of lymphoma lines with sialidase exposed the Pola-V epitope on CD79B while not altering the binding of anti-immunoglobulin heavy chain antibodies to cell surface BCR. Importantly, our *ex vivo* experiments with primary tumor biopsy material confirm the broad relevance of these cell line findings to human patients with DLBCL. It is fascinating that the removal of a single terminal sugar moiety from the glycans attached to CD79B facilitates Pola-V binding to such an extent. Sialic acid is negatively charged, so electrostatic repulsion could limit Pola-V accessibility to its epitope. Although the three-dimensional disposition of the glycan

chains on CD79B is not known, it is also possible that the absence of terminal sialic acid moieties alters the glycocalyx surrounding CD79B in a more complex fashion.

Our analysis specifically implicated glycans attached to asparagines in CD79B (N127/N128) and CD79A (N112) as modulators of Pola-V binding to the BCR. In the three-dimensional model of the BCR, these asparagines are in close proximity to the CD79B epitope recognized by Pola-V, and mutation of these residues decreased the apparent molecular weight of CD79B, consistent with a loss of glycosylation. Critically, mutation of these asparagines did not affect total cellular protein levels of CD79B or the trafficking of the BCR to cell surface but rather altered the accessibility of the Pola-V epitope on CD79B. Accordingly, loss of glycosylation at these residues enhanced sensitivity to Pola-V, comparable to the effect of a full knockout of the sialylation machinery, with a lowering of the *in vitro* IC_{50} of Pola-V by up to 20-fold and a significantly greater effect of Pola-V on tumor growth *in vivo*. Sialylation has been implicated previously in the resistance of a Burkitt lymphoma line to an anti-CD22 ADC, and the resistance of breast and gastric lines to trastuzumab (36). This resistance was due to increased lysosomal delivery of the ADCs and release of their payloads, with no apparent contribution of ADC binding. In contrast, we showed that the presence of sialylated glycans on specific CD79A and CD79B asparagine residues impedes the binding of Pola-V to its target epitope, with no impact on CD79B protein abundance.

The binding of Pola-V to the surface of malignant lymphoma cells could be influenced by genetic or epigenetic alterations that affect sialylation of glycoproteins. Indeed, our genetic analysis of DLBCL tumors uncovered multiple mutations and copy number alterations that target the sialylation machinery. *ST6GAL1*, *B4GALT1*, *B3GNT2*, and *SLC35A2* were frequently amplified and overexpressed in DLBCL, often in the same tumors, whereas *SLC35A1* was recurrently deleted and decreased in expression (4,5). Additionally, these genes acquire coding region mutations in DLBCL, including multiple truncating mutations targeting SLC35A2, as well as non-coding mutations targeting the *ST6GAL1* superenhancer that could alter its expression (37). As has been suggested in other cancer types (38-40), these genetic alterations may have been selected in DLBCL to alter the glycocalyx as a means to evade the host immune response. Genetic or epigenetic alterations that influence the sialylation of CD79A and CD79B could have an impact on the Pola-V susceptibility of individual DLBCL

tumors. Indeed, we observed considerable variability in the binding of anti-CD79B antibody to the surface of DLBCL cell lines, which correlated with Pola-V sensitivity. From a practical standpoint, we showed that flow cytometry for CD79B epitope binding rather than CD79B immunohistochemistry could serve as a useful Pola-V response biomarker, a possibility that should be addressed in prospective clinical trials.

Our findings also suggest exciting potential approaches to enhance the tumor-specific activity of Pola-V. One strategy would be to inhibit N-linked glycosylation by targeting the OST complex or more discretely inhibiting the addition of α2,6-linked sialic acid to glycoproteins by developing inhibitors of ST6GAL1. While systemic inhibition of protein glycosylation could be complicated by toxicity, it is notable that an OST inhibitor could control growth of DLBCL tumors in mouse xenografts without overt side effects, suggesting a therapeutic index might be achievable (41). Indeed, using mouse xenograft models, we demonstrated that the small molecule OST inhibitor NGI-1 can be successfully co-administered with Pola-V to deplete surface glycans and enhance lymphoma killing *in vivo* without excess toxicity to the animal.

An alternative strategy to enhance Pola-V sensitivity would be to remove sialic acid from cell surface glycoproteins by targeting recombinant sialidase enzyme to the tumor surface. Such an approach has been developed to increase immune destruction of breast cancer cells using a trastuzumab-sialidase conjugate (42). Other antibody sialidase conjugates are currently under clinical development and have shown a positive safety profile (ClinicalTrials.gov identifier: NCT05259696). A similar strategy might be employed in DLBCL by conjugating sialidase to one of several currently used monoclonal antibodies directed to B cell-specific antigens, such as CD19, CD20, or CD22. By removing sialic acid from B cell tumors, such sialidase conjugated antibodies theoretically might have additional antitumor activity by fostering an effective host cellular immune response.

In contrast to the effects of sialylation on CD79B epitope availability, we show that the effect of KLHL6 on sensitivity to Pola-V results from its regulation of CD79B protein abundance. In our experiments, this effect was only observed in GCB DLBCL lines, perhaps related to their derivation from normal germinal center B cells, which express higher levels

of KLHL6 (43). Inactivating mutations in *KLHL6* are frequent in DLBCL, most commonly within GCB DLBCL and especially the GCB cases belonging to the ST2 genetic subtype, which acquires *KLHL6* mutations in 15% of tumors. Previous studies examined *KLHL6* mutation in ABC DLBCL, revealing an impact upon NF-κB signaling via the ubiquitination of the mRNA decay factor Roquin2 (25). Our study reveals that KLHL6 also directly reduces CD79B protein abundance by ubiquitinating lysine-219 of CD79B, thereby triggering its lysosomal degradation. Because of the stoichiometric contribution of CD79B to BCR formation, KLHL6 dictates the expression of the BCR on the cell surface. Consequently, inactivation of KLHL6 raised surface BCR expression in DLBCL lines and increased CD79A phosphorylation, a marker of proximal BCR signaling. Taken together, we hypothesize that increased constitutive BCR signaling may at least partly explain the recurrent acquisition of KLHL6-inactivating mutations in GCB DLBCL, although we do not exclude other, as yet unknown targets of KLHL6. Further, our findings explain the recurrent mutation of CD79B lysine-219 in DLBCL biopsies since these mutations prevent KLHL6 from ubiquitinating CD79B and promoting its lysosomal degradation.

Our finding that *KLHL6* deletion leads to increased surface BCR expression in non-malignant, human and mouse germinal center B cells suggests a previously unappreciated physiological function for KLHL6 in the normal germinal center reaction. KLHL6 mRNA expression increases almost 10-fold as B cells enter the germinal center reaction. This germinal center-specific expression of KLHL6 may explain the known suppression of BCR expression observed in germinal center B cells, which we confirmed by immunochemical analysis of CD79B in human tonsillar germinal centers. Indeed, by limiting surface BCR expression in germinal center B cells, KLHL6 may play a pivotal role in positive selection for B cells with higher affinity immunoglobulin variable regions. A recent intriguing study identified inactivating *KLHL6* mutations in expanded B cell clones producing a rheumatoid factor autoantibody from a patient with mixed cryoglobulinemia (30). These KLHL6 mutant cells had abnormally high levels of somatic hypermutation as did a mouse model with a heterozygous mutant *Klhl6* allele. Survival signals emanating from the BCR are essential for persistence of light zone B cells and their re-entry into the dark zone where somatic hypermutation occurs (44). We therefore speculate that increased BCR expression in *KLHL6* mutant cells may enhance BCR-derived survival signals and persistence within the germinal

center, leading to additional cycles of dark zone re-entry and hypermutation of clones that might otherwise be deleted at an earlier stage of the germinal center reaction.

The POLARIX clinical trial was a randomized Phase III trial that examined the benefit of adding Pola-V to chemoimmunotherapy in previously untreated patients with DLBCL(7). A subset analysis of this trial revealed a benefit of Pola-V addition to chemotherapy in ABC but not GCB DLBCL(45). An interesting possibility is that the lower expression of KLHL6 in ABC DLBCL may have contributed to its susceptibility to Pola-V. Ultimately, the efficacy of Pola-V is likely to be multifactorial and influenced by genetic and epigenetic factors that regulate BCR expression on the cell surface, such as KLHL6, as well as the accessibility of the polatuzumab binding epitope on CD79B, which is modulated by N-linked glycosylation. From this perspective, a flow cytometric assay for binding of polatuzumab to the surface of malignant cells in DLBCL biopsies might serve as an integrative biomarker of Pola-V efficacy, a hypothesis that requires confirmation in future clinical trials of Pola-V.

In summary, our findings reveal molecular determinants of sensitivity to Pola-V that have precision medicine implications for the optimal deployment of this important new anti-lymphoma drug. More generally, our study demonstrates that an unbiased functional genomic assessment of sensitivity and resistance to antibody-drug conjugates can reveal mechanisms of sensitivity and resistance as well as suggest new therapeutic strategies to prevent and overcome resistance. Given the diversity of cell surface receptors that are being used to deliver toxins to cancer cells, it is likely that functional genomic studies of each antibody-drug conjugate will yield different and fascinating new mechanisms that could be exploited to optimize efficacy.

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Data Availability

The whole proteome and ubiquitylation mass spectrometry data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD043670.

FIGURE LEGENDS

Figure 1. Whole genome CRISPR screens reveal the impact of N-glycosylation upon CD79B-directed killing by Pola-V.

(A) Scheme and workflow of surface CD79B-sorted CRISPR screens and drug-modified CRISPR screens in nine DLBCL cell lines. **(B)** Comparison of mean Pola-V CRISPR Screen Score (CSS) to mean CD79B Segregation Score (CD79B High CSS – CD79B Low CSS). Pola-V CRISPR Screen Score is the average of 4 ABC (HBL1, TMD8, RIVA, OCI-Ly10) and 5 GCB (OCI-Ly1, OCI-Ly8, WSU-FSCCL, WSU-DLCL2, SUDHL5) cell lines. CD79B Segregation score is the average of 3 ABC (HBL1, TMD8, RIVA) and 5 GCB (OCI-Ly1, OCI-Ly8, WSU-FSCCL, WSU-DLCL2, SUDHL5) cell lines. Genes are colored by function according to the color scheme shown. **(C)** Comparison of Pola-V CSS to MMAE CSS in 4 ABC (HBL1, TMD8, RIVA, OCI-Ly10) and 5 GCB (OCI-Ly1, OCI-Ly8, WSU-FSCCL, WSU-DLCL2, SUDHL5) cell lines. Genes are colored by function according to the color scheme shown. **(D)** Ranked list of genes by CD79B segregation score. Color pattern is same as in (B). **(E)** Ranked list of genes by Pola-V CSS. Genes are colored by function according to the color scheme shown. **(F)** Schematic of the Golgi glycosylation pathway, showing the enzymes and transporters that mediate the stepwise assembly of an α2,6 sialylated N-linked glycan chain. Genes are colored by function as shown.

Figure 2. Loss of terminal sialic acid residues enhances sensitivity to Pola-V.

(A) Drug sensitivity assays in TMD8, RIVA, FL-318, or SUDHL4 cells, either unmodified or after knockout of genes required for α2,6-linked sialic acid linkage. Cells were treated with the indicated doses of Pola-V. ABC mutant lines are denoted in blue and GCB mutant lines are denoted in orange. Data show mean and SEM of 3 independent experiments, each with 3 technical replicates. **(B)** Tumor volumes from NOD *scid* gamma (NSG) mice xenografted with the DLBCL line RIVA or FL-318, either wild type or CRISPR modified to delete the indicated gene (*SLC35A1*, SLC35A2 or *ST6GAL1*) to preclude α2,6-linked sialic acid linkage. Mice were treated once on Day 2 with either 0.5mg/kg Pola-V or vehicle. Data shows the mean and SEM of at least 4 mice per treatment group. **(C)** Competitive growth experiment in a panel of 6 DLBCL lines transduced with the indicated sgRNAs (*SLC35A1*, SLC35A2 or *ST6GAL1*) marked with GFP. Cells were cultured for 7 days after the induction of Cas9 before addition of DMSO, MMAE, or Pola-V as indicated. The proportion of gRNA transduced (GFP^+) cells was then

tracked using flow cytometry and presented as a ratio normalized to that of day 7. Data shows mean and SEM of at least 3 independent experiments.

Figure 3. Surface CD79B determined by flow cytometry correlates with sensitivity to Pola-V
Area under the curve (AUC) sensitivity analysis of MTS drug sensitivity assays for 54 cancer cell lines treated with Pola-V **(A)** or MMAE **(B)** compared to surface CD79B staining determined by flow cytometry. AUC data represent the mean AUC determined using Prism (GraphPad) of at least 3 independent experiments, each with 3 technical replicates. CD79B staining data represent the log2 of the mean fluorescent intensity (MFI) determined by flow cytometry from 3 separate experiments. Pola-V dosing ranged from 1ug/ml to 0.1ng/ml in decreasing half log doses. MMAE proliferation assay dosing ranged from 10nM to 1pM in decreasing half log doses.

Figure 4. Loss of cell surface sialic acid increases the binding of Pola-V to CD79B
(A) Mean Fluorescence Intensity (MFI) of CD79B, immunoglobulin (Ig), CD19, and α2,6-linked sialic acid (SNA) examined by flow cytometry after treatment with 50uM of the competitive sialic acid inhibitor (SA-I) 3-Fax-Peracetyl Neu5Ac relative to control (DMSO) samples. Data shown are Mean and SEM of at least 3 independent experiments. ABC DLBCL cell lines are denoted in blue, GCB DLBCL cell lines in orange. **(B, C)** Time course after Cas9 induction of MFI of CD79B and immunoglobulin (Ig) of cell lines transduced with the indicated sgRNAs marked with GFP. MFI of transduced cells is presented relative to the non-transduced population normalized to day 0. Mean and SEM of at least 3 independent experiments. **(D)** Immunoblot using the indicated antibodies in ABC (HBL1, TMD8, RIVA) and GCB (FL-318, SUDHL4, WSU-FSCCL) DLBCL cell lines with knockout of *SLC35A1* or *SLC35A2*, or removal of glycosylation via PNGase F treatment. **(E)** MFI of CD79B and Immunoglobulin (Ig) after 30-minute treatment with recombinant sialidase shown relative to untreated cells. Mean and SEM of at least 3 independent experiments. **(F)** MFI of SNA, CD19(FMC63), and CD79B of primary human germinal center B cells and viably frozen DLBCL patient tumors treated with or without recombinant sialidase. (*P=0.0348, **P=0.0072, ***P=0.0001, ****P=<0.0001 ratio paired T-test). **(G)** Binding curve of escalating concentrations of polatuzumab antibody detected with anti-IgG-PE. K_D determined by nonlinear fit analysis of one-site binding in Prism (GraphPad). Mean and SEM of 3 independent experiments.

Figure 5. Sialylation of specific residues of CD79A and CD79B impedes Pola-V binding.

(A) Three-dimensional model of the human IgM B-Cell receptor (23) annotated with potential N-linked glycosylation sites and the Pola-V binding site. **(B)** Mean fluorescence Intensity (MFI) of CD79B and immunoglobulin measured by flow cytometry in N-glycosite-mutant knock-in clones with the indicated asparagine mutations of *CD79A* and *CD79B*. Data show mean and SEM of at least 3 replicates relative to WT line in the same experiment. **(C)** Immunoblot of BCR components from N-glycosite-mutant knock-in clones with the indicated asparagine mutations of *CD79A* and *CD79B*. **(D)** Immunoblot of BCR components from N-glycosite-mutant knock-in clones with the indicated asparagine mutations of *CD79A* and *CD79B* compared to removal of all glycosylation via PNGase F treatment. **(E)** MTS proliferation assays of parental WT or *CD79A/B* knock-in mutated cell lines exposed to escalating concentrations of Pola-V. Normalized growth represents the mean compared to control of 3 independent experiments with 3 technical replicates of each drug concentration. Error bars represent SEM. **(F)** Tumor volumes from NOD *scid* gamma (NSG) mice xenografted with WT or the indicated mutation knock-in RIVA cells treated once on Day 2 with either 0.5mg/kg Pola-V or vehicle. Data show mean and SEM of at least 4 separate mice per treatment group. **(G)** Mean Fluorescence Intensity (MFI) of CD79B and α2,6-linked sialic acid (SNA) examined by flow cytometry after treatment with 5uM of the OST-B inhibitor NGI-1 relative to control (DMSO) samples. Data shown are Mean and SEM of at least 3 independent experiments. ABC DLBCL cell lines are denoted in blue, GCB DLBCL cell lines in orange. **(H)** MTS proliferation assays for the indicated cell lines co-treated with escalating doses of Pola-V (x-axis) and the OST-B inhibitor NGI-1**.** Curves are normalized to absorbance in NGI-1 treatment alone before normalizing to 0ng/ml Pola-V to view synergy between dual treatment. Mean and error represent SEM of at least 3 independent replicates. **(I)** Tumor volumes from NOD *scid* gamma (NSG) mice xenografted with the DLBCL line RIVA or FL-318. For Pola-V, mice were treated once on Day 2 with either 0.5mg/kg Pola-V (RIVA) or 0.25mg/kg Pola-V (FL-318) or vehicle. For NGI-1, mice were treated every other day with 15mg/kg NGI-1 or vehicle starting on day 2. Data shows the mean and SEM of at least 4 mice per treatment group.

Figure 6. KLHL6 regulates sensitivity of GCB DLBCL to Polatuzumab-Vedotin

(A) Comparison of mean Pola-V CRISPR Screen Score (CSS) to mean CD79B Segregation Score (CD79B High CSS – CD79B Low CSS) in 5 GCB DLBCL cell lines (OCI-Ly1, OCI-Ly8, WSU-FSCCL, WSU-DLCL2, SUDHL5). Genes encoding BCR subunits, N-linked glycosylation pathway components, and KLHL6 are highlighted. **(B)** Comparison of Pola-V CSS to MMAE CSS in 5 GCB DLBCL cell lines. **(C)** Additive CSS for ABC (blue) and GCB (orange) DLBCL cell lines for the gene *KLHL6*. **(D)** Competitive growth experiments for the indicated cell lines transduced with *KLHL6*-targeting sgRNA marked with GFP. Pola-V or vehicle control was added to cultures at day 7 of Cas9 induction with doxycycline. Pola-V concentration was chosen as the approximate IC_{50} for each line as follows: SUDHL5 (30 ng/ml), DOHH2 (50 ng/ml), WSU-DLCL2 (70 ng/ml), HBL1 (30 ng/ml), TMD8 (30 ng/ml), OCI-Ly10 (90 ng/ml). Data show the proportion of transduced (GFP^+) cells at each timepoint relative to that of day 7. GCB and ABC DLBCL cell lines are denoted with orange and blue lines respectively. Mean and SEM of at least 3 independent experiments are shown **(E)** Drug sensitivity assays in SUDHL5, WSU-DLCL2, and DOHH2 cells, either unmodified or after knockout of KLHL6. Cells were treated with the indicated doses of Pola-V. Data show mean and SEM of at least 2 independent experiments, each with 3 technical replicates. **(F)** KLHL6 mRNA expression from RNA-seq of DLBCL tumors (left panel) and cell lines (middle panel) subtyped by gene expression profiling, as well as the indicated sorted tonsillar B cell subpopulations (right panel). **** $P < 0.0001$, two-tailed unpaired T-test. **(G)** KLHL6 mRNA expression from RNA-seq of DLBCL tumors subtyped by gene expression profiling and grouped by genetic subtype determined by LymphGen **(H)** KLHL6 and CD79B mRNA expression determined via RNA-seq from the indicated B-cell populations **(I)** Immunohistochemistry of CD79B in a reactive tonsil. Scale bar represents 100μM **(J)** Frequency of KLHL6 mutations found in DLBCL tumors by gene expression subtype (left) and genetic subtype (right) **(K)** Distribution of mutations targeting KLHL6 in DLBCL tumors. **(L)** Representation of location of common KLHL6 mutations in KLHL6 crystal structure predicted by AlphaFold.

Figure 7. CD79B protein is regulated by KLHL6-dependent ubiquitination.

(A) Time course after Cas9 induction of CD79B Mean Fluorescence Intensity (MFI) of cell lines transduced with *KLHL6* sgRNA marked with GFP. MFI of transduced cells is presented relative to the non-transduced population, normalized to day 0. Mean and SEM of at least 3 independent experiments. **(B)** Immunoblot for BCR components of GCB DLBCL cell lines

transduced with either control or *KLHL6* sgRNA. **(C)** CD79B expression (MFI) by flow cytometry of human germinal center B cells expanded *ex vivo* and transduced with Cas9 and control or *KLHL6*-targeting sgRNAs. Data show mean and SEM of 3 different human donors. **P=0.0066 via paired T-test. **(D)** Immunoblot of cells from (C) probed with the indicated antibodies. **(E)** Average MFI of denoted surface immunoglobulin heavy chain isotypes or CD79B in immunoglobulin-positive cells from mouse Peyer's Patch germinal center B cells expressing an AID-driven Cas9 and the indicated sgRNAs. IgA ***P=0.0001, IgG1 **P=0.0018, IgG2b *P=0.0169, IgM ***P=0.0010 paired T-test. **(F)** Surface levels of CD79B detected by flow cytometry comparing the indicated parental cell lines to the KLHL6 L90F knock-in mutant clones. **(G)** Immunoblot for CD79B comparing the parental SUDHL5 GCB lines to KLHL6 L90F knock-in mutants. **(H)** Drug sensitivity assays in SUDHL5 or WSU-DLCL2 cells, either unmodified or after knock-in of specified KLHL6 mutations. Cells were treated with the indicated doses of Pola-V. Data show mean and SEM of 3 independent experiments, each with 3 technical replicates. **(I)** KLHL6-BioID2 interactome in SUDHL5 cells compared to global proteome comparison in SUDHL5 cells comparing cells expressing a KLHL6 sgRNA to a control sgRNA. L2FC = $\log_2$ fold change. **(J)** Ubiquitin site profiling in SUDHL5 cells comparing cells expressing *KLHL6*-targeting sgRNA to control sgRNA compared to whole proteome data from SUDHL5 cells expressing KLHL6 sgRNA relative to control sgRNA. Mean of 4 biological replicates. L2FC = Log_2 Fold Change. **(K)** Relative mean fluorescence intensity (MFI) compared to WT of surface CD79B and surface immunoglobulin in WSU-DLCL2 cells with denoted endogenous CD79B mutations. Mean of at least 2 clones and at least 3 biological replicates per clone. Error bars represent SEM. *** P = 0.0003, ** P = 0.0034 Two-tailed T test. **(L)** Immunoblot of WSU-DLCL2 GCB cells edited to express the indicated CD79B lysine mutations. Quantification represents average of 2 clones per mutation normalized to beta-actin and compared to WT. **(M)** Immunoblot of SUDHL5 GCB cells expressing either control or *KLHL6*-tareging sgRNA treated with the neddylation inhibitor MLN4924 (1μM) or control for 14 hours. **(N)** Immunoblot of SUDHL5 GCB cells expressing either control or *KLHL6*-targeting sgRNA treated with MG132 (10μM) for 6 hours. Mean of at least 2 independent experiments **(O)** Immunoblot of SUDHL5 GCB cells expressing either control or *KLHL6*-targeting sgRNA treated with the lysosome inhibitor Bafilomycin A1 (100nM) for 16 hours. Immunoblots are representative of at lead two independent experiments.

Supplemental Figure 1. CD79B Segregation Score and Pola-V CSS for Golgi glycosylation genes shows specific sugar regulators of surface CD79B and Pola-V sensitization.

(A) Additive score by cell line of CD79B Segregation Score and Pola-V CSS for all Golgi sialyltransferases, Golgi galactosyltransferases, and Golgi nucleotide sugar transporters, as indicated, expressed in DLBCL cell lines. Scores from ABC cell lines in blue, GCB cell lines in orange. General function of genes noted below gene name. **(B)** Oncoprint matrix of the indicated genetic aberrations in each of 574 DLBCL biopsy specimens. **(C)** Relationship between mRNA digital gene expression (DGE) in each indicated gene within each indicated DNA copy number category.

Supplemental Figure 2 – Knockout of *SLC35A1*, *SLC35A2*, and *ST6GAL1* decreases surface α2,6-linked sialic acid.

(A) Representative flow plots of the ABC DLBCL cell line RIVA WT and knockout cells stained for surface α2,6-linked sialic acid (SNA). **(B)** Same as in (A) but for the GCB DLBCL line FL-318

Supplemental Figure 3. Small-molecule sialyltransferase inhibition synergizes with Pola-V in ABC and GCB DLBCL.

(A) MTS proliferation assays for the indicated cell lines co-treated with escalating doses of Pola-V (x-axis) and the sialylation inhibitor 3Fax-Peracetyl Neu5Ac**.** Curves are normalized to absorbance in sialylation inhibitor treatment alone before normalizing to 0ng/ml Pola-V to view synergy between dual treatment. ABC DLBCL cell lines denoted in blue; GCB DLBCL cell lines denoted in orange. Mean and error represent SEM of at least 3 independent replicates.

Supplemental Figure 4. SLC35A2 targeted degradation by dTag modification synergizes with Pola-V.

(A) RIVA SLC35A2 knockout cells engineered to re-express SLC35A2 with an N-terminus FKBP12 degradation domain were treated with either DMSO or 100nM dTag13. Surface CD79B and α2,6-linked sialic acid (SNA) levels were measured over 6 days comparing MFI in dTag13 treated samples to DMSO. **(B)** MTS proliferation assays for RIVA cells in (A) TMD8 cells engineered using the same method. Cell lines were co-treated with escalating doses of Pola-V (x-axis) and dTag13**.** Curves are normalized to absorbance in dTag13 treatment alone before

normalizing to 0ng/ml Pola-V to view synergy between dual treatment. Mean and error represent SEM of at least 3 independent replicates.

Supplemental Figure 5 – CD79B immunohistochemistry is not predictive of Pola-V sensitivity.

(A) CD79B H-Score adapted from Pfeifer *et al.* 2015 graphed against area under the curve (AUC) sensitivity analysis of MTS proliferation assays with Pola-V of DLBCL cell lines. AUC data represent the average AUC determined by Prism (GraphPad) of at least 3 independent experiments with 3 technical replicates each. ABC DLBCL cell lines denoted in blue; GCB DLBCL cell lines denoted in orange.

Supplemental Figure 6 - Sialidase treatment reduces surface α2,6-linked sialic acid.

(A) Ratio of surface α2,6-linked sialic acid (SNA) mean MFI measured by flow cytometry between cells either treated or untreated with exogenous sialidase from Figure 4E for CD79B co-stained samples. Mean and error represent SEM of at least 3 independent replicates. ABC DLBCL cell lines denoted in blue; GCB DLBCL cell lines denoted in orange. **(B)** Same as in (A) but for Immunoglobulin co-stained samples.

Supplemental Figure 7 - Sialidase treatment does not increase binding of other therapeutic antibodies.

(A) Ratio of surface CD19-FMC63 mean MFI measured by flow cytometry between cells either treated or untreated with exogenous sialidase. Mean and error represent SEM of at least 3 independent replicates. ABC DLBCL cell lines denoted in blue; GCB DLBCL cell lines denoted in orange. **(B)** Same as in (A) but for Rituximab. **(C)** Same as in (A) but for α2,6-linked sialic acid (SNA) for cells in (A) and (B).

Supplemental Figure 8 – Knock-In mutations to specific N-linked glycosylation sites results in higher CD79B but not immunoglobulin MFI by flow cytometry.

(A) Representative flow plots of surface CD79B expression after knock-in mutation of the indicated N-linked glycosylation sites in the ABC DLBCL cell line RIVA and GCB DLBCL cell line SUDHL4.

Supplemental Figure 9 - Mutation of *CD79A* and *CD79B* glycosylated residues does not alter sensitivity to MMAE.

(A) MTS proliferation assays for RIVA and SUDHL4 cells with either WT CD79A and CD79B or the indicated knock-in mutations treated with escalating doses of MMAE. Mean and error represent SEM of at least 3 independent replicates.

Supplemental Figure 10 – KLHL6 loss does not score as a negative regulator of surface CD79B or Pola-V sensitivity in CRISPR screens of ABC DLBCL cell lines and KLHL6 loss does not sensitize to MMAE.

(A) Comparison of mean Pola-V CRISPR Screen Score (CSS) to mean CD79B Segregation Score (CD79B High CSS – CD79B Low CSS) in 4 ABC DLBCL cell lines (OCI-Ly10, RIVA, TMD8, HBL1). Genes encoding BCR subunits, N-linked glycosylation pathway components, and KLHL6 are highlighted. **(B)** Comparison of Pola-V CSS to MMAE CSS in 4 ABC DLBCL cell lines. **(C)** Competitive growth experiment co-culturing the indicated cell lines transduced with *KLHL6*-targeting sgRNAs marked with GFP. Vehicle or drug was added after 7 days of Cas9 induction by doxycycline addition. Lines display relative GFP+ cells normalized to that of day 7. Graphs with orange lines represent GCB DLBCL cell lines and graphs with blue lines represent ABC DLBCL cell lines. Mean and error represent SEM of at least 2 independent replicates.

Supplemental Figure 11 – Knockout of the E3 ligase KLHL6 results in increased cell surface CD79B in a human model of GCB DLBCL and ubiquitination of CD79B by KLHL6 on CD79B K219 recapitulates this phenotype in GCB DLBCL cell lines.

(A) Representative flow plots of surface CD79B expression of human germinal center B cells from separate donors expanded *ex vivo* and transduced with Cas9 and control or *KLHL6*-targeting sgRNAs. **(B)** Representative flow plots of surface CD79B or surface immunoglobulin expression after knock-in mutation of the indicated lysines in the GCB DLBCL cell line WSUDLCL2.

Supplemental Figure 12 – Knockout of *KLHL6* in mouse germinal center B cells increases surface BCR expression.

(A) Gating strategy for germinal center B-cells for surface immunoglobulin analysis (upper panel) and surface CD79B analysis (lower panel) **(B)** Flow cytometry plots for Peyer's Patch surface immunoglobulin from Figure 6N. **(C)** Comparison of surface immunoglobulin-positive GC B cells from mesenteric lymph node in control or KLHL6 sgRNA treated mice. IgG1**** P<0.0001, IgG2b ** P=0.0069, IgM * P=0.0203 paired T-test.

Supplemental Figure 13 – DLBCL mutations targeting the CD79B intracellular domain.
(A) Reported mutations in DLBCL tumors are shown, highlighting mutations targeting the 4 lysine residues as well as highly recurrent mutations targeting the ITAM motif. K219, the only intracellular lysine identified as ubiquitinated in DLBCL cell lines (Fig. 6B), affected by recurrent missense mutations changing this residue to threonine or arginine.

Supplemental Figure 14 – Inhibition of neddylation increases expression of BCR subunits.
(A) Immunoblot of DOHH2 cells expressing either control or KLHL6 sgRNA treated +/- 1μM neddylation inhibitor MLN4924 for 14 hours.

Supplemental Figure 15 – CD79B is degraded in a lysosomal, not proteasomal manner.
(A) Immunoblot of WSU-DLCL2 cells expressing either control or *KLHL6* sgRNA treated +/- 10uM proteasome inhibitor MG132 for 6 hours. Representative blot of at least 2 independent experiments **(B)** Immunoblot of WSU-DLCL2 cells expressing either control or KLHL6 sgRNA treated with 100nM of the lysosome inhibitor Bafilomycin A1 for 16 hours. Representative blot of at least 2 independent experiments.

REFERENCES

1. Sehn LH, Salles G. Diffuse Large B-Cell Lymphoma. N Engl J Med **2021**;384(9):842-58 doi 10.1056/NEJMra2027612.
2. Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A*, et al.* Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature **2000**;403:503-11.
3. Chapuy B, Stewart C, Dunford AJ, Kim J, Kamburov A, Redd RA*, et al.* Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes. Nat Med **2018**;24(5):679-90 doi 10.1038/s41591-018-0016-8.
4. Schmitz R, Wright GW, Huang DW, Johnson CA, Phelan JD, Wang JQ*, et al.* Genetics and Pathogenesis of Diffuse Large B-Cell Lymphoma. N Engl J Med **2018**;378(15):1396-407 doi 10.1056/NEJMoa1801445.
5. Wright GW, Huang DW, Phelan JD, Coulibaly ZA, Roulland S, Young RM*, et al.* A Probabilistic Classification Tool for Genetic Subtypes of Diffuse Large B Cell Lymphoma with Therapeutic Implications. Cancer Cell **2020**;37(4):551-68 e14 doi 10.1016/j.ccell.2020.03.015.
6. Lacy SE, Barrans SL, Beer PA, Painter D, Smith AG, Roman E*, et al.* Targeted sequencing in DLBCL, molecular subtypes, and outcomes: a Haematological Malignancy Research Network report. Blood **2020**;135(20):1759-71 doi 10.1182/blood.2019003535.
7. Tilly H, Morschhauser F, Sehn LH, Friedberg JW, Trneny M, Sharman JP*, et al.* Polatuzumab Vedotin in Previously Untreated Diffuse Large B-Cell Lymphoma. N Engl J Med **2022**;386(4):351-63 doi 10.1056/NEJMoa2115304.
8. Sehn LH, Herrera AF, Flowers CR, Kamdar MK, McMillan A, Hertzberg M*, et al.* Polatuzumab Vedotin in Relapsed or Refractory Diffuse Large B-Cell Lymphoma. J Clin Oncol **2020**;38(2):155-65 doi 10.1200/JCO.19.00172.
9. Palanca-Wessels MC, Czuczman M, Salles G, Assouline S, Sehn LH, Flinn I*, et al.* Safety and activity of the anti-CD79B antibody-drug conjugate polatuzumab vedotin in relapsed or refractory B-cell non-Hodgkin lymphoma and chronic lymphocytic leukaemia: a phase 1 study. Lancet Oncol **2015**;16(6):704-15 doi 10.1016/s1470-2045(15)70128-2.
10. Morschhauser F, Flinn IW, Advani R, Sehn LH, Diefenbach C, Kolibaba K*, et al.* Polatuzumab vedotin or pinatuzumab vedotin plus rituximab in patients with relapsed or refractory non-Hodgkin lymphoma: final results from a phase 2 randomised study (ROMULUS). Lancet Haematol **2019**;6(5):e254-e65 doi 10.1016/s2352-3026(19)30026-2.
11. Dornan D, Bennett F, Chen Y, Dennis M, Eaton D, Elkins K*, et al.* Therapeutic potential of an anti-CD79b antibody-drug conjugate, anti-CD79b-vc-MMAE, for the treatment of non-Hodgkin lymphoma. Blood **2009**;114(13):2721-9 doi 10.1182/blood-2009-02-205500.
12. Okazaki M, Luo Y, Han T, Yoshida M, Seon BK. Three new monoclonal antibodies that define a unique antigen associated with prolymphocytic leukemia/non-Hodgkin's lymphoma and are effectively internalized after binding to the cell surface antigen. Blood **1993**;81(1):84-94.

13. Young RM, Phelan JD, Wilson WH, Staudt LM. Pathogenic B-cell receptor signaling in lymphoid malignancies: New insights to improve treatment. Immunol Rev **2019**;291(1):190-213 doi 10.1111/imr.12792.
14. Polson AG, Yu SF, Elkins K, Zheng B, Clark S, Ingle GS*, et al.* Antibody-drug conjugates targeted to CD79 for the treatment of non-Hodgkin lymphoma. Blood **2007**;110(2):616-23 doi 10.1182/blood-2007-01-066704.
15. Phelan JD, Young RM, Webster DE, Roulland S, Wright GW, Kasbekar M*, et al.* A multiprotein supercomplex controlling oncogenic signalling in lymphoma. Nature **2018**;560(7718):387-91 doi 10.1038/s41586-018-0290-0.
16. Schjoldager KT, Narimatsu Y, Joshi HJ, Clausen H. Global view of human protein glycosylation pathways and functions. Nature Reviews Molecular Cell Biology **2020**;21(12):729-49 doi 10.1038/s41580-020-00294-x.
17. Nakano M, Mishra SK, Tokoro Y, Sato K, Nakajima K, Yamaguchi Y*, et al.* Bisecting GlcNAc Is a General Suppressor of Terminal Modification of N-glycan. Mol Cell Proteomics **2019**;18(10):2044-57 doi 10.1074/mcp.RA119.001534.
18. Voss M, Kunzel U, Higel F, Kuhn PH, Colombo A, Fukumori A*, et al.* Shedding of glycan-modifying enzymes by signal peptide peptidase-like 3 (SPPL3) regulates cellular N-glycosylation. EMBO J **2014**;33(24):2890-905 doi 10.15252/embj.201488375.
19. Nabet B, Roberts JM, Buckley DL, Paulk J, Dastjerdi S, Yang A*, et al.* The dTAG system for immediate and target-specific protein degradation. Nat Chem Biol **2018**;14(5):431-41 doi 10.1038/s41589-018-0021-8.
20. Pfeifer M, Zheng B, Erdmann T, Koeppen H, McCord R, Grau M*, et al.* Anti-CD22 and anti-CD79B antibody drug conjugates are active in different molecular diffuse large B-cell lymphoma subtypes. Leukemia **2015**;29(7):1578-86 doi 10.1038/leu.2015.48.
21. Zheng B, Fuji RN, Elkins K, Yu SF, Fuh FK, Chuh J*, et al.* In vivo effects of targeting CD79b with antibodies and antibody-drug conjugates. Mol Cancer Ther **2009**;8(10):2937-46 doi 10.1158/1535-7163.MCT-09-0369.
22. Ma X, Zhu Y, Dong D, Chen Y, Wang S, Yang D*, et al.* Cryo-EM structures of two human B cell receptor isotypes. Science **2022**;377(6608):880-5 doi 10.1126/science.abo3828.
23. Su Q, Chen M, Shi Y, Zhang X, Huang G, Huang B*, et al.* Cryo-EM structure of the human IgM B cell receptor. Science **2022**;377(6608):875-80 doi 10.1126/science.abo3923.
24. Lopez-Sambrooks C, Shrimal S, Khodier C, Flaherty DP, Rinis N, Charest JC*, et al.* Oligosaccharyltransferase inhibition induces senescence in RTK-driven tumor cells. Nat Chem Biol **2016**;12(12):1023-30 doi 10.1038/nchembio.2194.
25. Choi J, Lee K, Ingvarsdottir K, Bonasio R, Saraf A, Florens L*, et al.* Loss of KLHL6 promotes diffuse large B-cell lymphoma growth and survival by stabilizing the mRNA decay factor roquin2. Nat Cell Biol **2018**;20(5):586-96 doi 10.1038/s41556-018-0084-5.
26. Kunder CA, Roncador G, Advani RH, Gualco G, Bacchi CE, Sabile JM*, et al.* KLHL6 Is Preferentially Expressed in Germinal Center-Derived B-Cell Lymphomas. Am J Clin Pathol **2017**;148(6):465-76 doi 10.1093/ajcp/aqx099.
27. Todo K, Koga O, Nishikawa M, Hikida M. Modulation of Igβ is essential for the B cell selection in germinal center. Sci Rep **2015**;5:10303 doi 10.1038/srep10303.
28. Stewart I, Radtke D, Phillips B, McGowan SJ, Bannard O. Germinal Center B Cells Replace Their Antigen Receptors in Dark Zones and Fail Light Zone Entry when Immunoglobulin Gene Mutations are Damaging. Immunity **2018**;49(3):477-89.e7 doi 10.1016/j.immuni.2018.08.025.

29. Caeser R, Di Re M, Krupka JA, Gao J, Lara-Chica M, Dias JML*, et al.* Genetic modification of primary human B cells to model high-grade lymphoma. Nature Communications **2019**;10(1):4543 doi 10.1038/s41467-019-12494-x.
30. Singh M, Jackson KJL, Wang JJ, Schofield P, Field MA, Koppstein D*, et al.* Lymphoma Driver Mutations in the Pathogenic Evolution of an Iconic Human Autoantibody. Cell **2020** doi 10.1016/j.cell.2020.01.029.
31. Kim DI, Jensen SC, Noble KA, Kc B, Roux KH, Motamedchaboki K*, et al.* An improved smaller biotin ligase for BioID proximity labeling. Molecular biology of the cell **2016**;27(8):1188-96 doi 10.1091/mbc.E15-12-0844.
32. Sha C, Barrans S, Cucco F, Bentley MA, Care MA, Cummin T*, et al.* Molecular High-Grade B-Cell Lymphoma: Defining a Poor-Risk Group That Requires Different Approaches to Therapy. J Clin Oncol **2019**;37(3):202-12 doi 10.1200/jco.18.01314.
33. Dubois S, Viailly PJ, Bohers E, Bertrand P, Ruminy P, Marchand V*, et al.* Biological and Clinical Relevance of Associated Genomic Alterations in MYD88 L265P and non-L265P-Mutated Diffuse Large B-Cell Lymphoma: Analysis of 361 Cases. Clin Cancer Res **2017**;23(9):2232-44 doi 10.1158/1078-0432.CCR-16-1922.
34. Radke J, Ishaque N, Koll R, Gu Z, Schumann E, Sieverling L*, et al.* The genomic and transcriptional landscape of primary central nervous system lymphoma. Nat Commun **2022**;13(1):2558 doi 10.1038/s41467-022-30050-y.
35. Foot N, Henshall T, Kumar S. Ubiquitination and the Regulation of Membrane Proteins. Physiol Rev **2017**;97(1):253-81 doi 10.1152/physrev.00012.2016.
36. Tsui CK, Barfield RM, Fischer CR, Morgens DW, Li A, Smith BAH*, et al.* CRISPR-Cas9 screens identify regulators of antibody-drug conjugate toxicity. Nat Chem Biol **2019**;15(10):949-58 doi 10.1038/s41589-019-0342-2.
37. Bal E, Kumar R, Hadigol M, Holmes AB, Hilton LK, Loh JW*, et al.* Super-enhancer hypermutation alters oncogene expression in B cell lymphoma. Nature **2022**;607(7920):808-15 doi 10.1038/s41586-022-04906-8.
38. Pearce OM, Läubli H. Sialic acids in cancer biology and immunity. Glycobiology **2016**;26(2):111-28 doi 10.1093/glycob/cwv097.
39. Stanczak MA, Rodrigues Mantuano N, Kirchhammer N, Sanin DE, Jacob F, Coelho R*, et al.* Targeting cancer glycosylation repolarizes tumor-associated macrophages allowing effective immune checkpoint blockade. Sci Transl Med **2022**;14(669):eabj1270 doi 10.1126/scitranslmed.abj1270.
40. Gray MA, Stanczak MA, Mantuano NR, Xiao H, Pijnenborg JFA, Malaker SA*, et al.* Targeted glycan degradation potentiates the anticancer immune response in vivo. Nat Chem Biol **2020**;16(12):1376-84 doi 10.1038/s41589-020-0622-x.
41. Scheich S, Chen J, Liu J, Schnutgen F, Enssle JC, Ceribelli M*, et al.* Targeting N-linked Glycosylation for the Therapy of Aggressive Lymphomas. Cancer Discov **2023** doi 10.1158/2159-8290.Cd-22-1401.
42. Xiao H, Woods EC, Vukojicic P, Bertozzi CR. Precision glycocalyx editing as a strategy for cancer immunotherapy. Proc Natl Acad Sci U S A **2016**;113(37):10304-9 doi 10.1073/pnas.1608069113.
43. Gupta-Rossi N, Storck S, Griebel PJ, Reynaud CA, Weill JC, Dahan A. Specific over-expression of deltex and a new Kelch-like protein in human germinal center B cells. Mol Immunol **2003**;39(13):791-9 doi 10.1016/s0161-5890(03)00002-6.

44. Chen ST, Oliveira TY, Gazumyan A, Cipolla M, Nussenzweig MC. B cell receptor signaling in germinal centers prolongs survival and primes B cells for selection. Immunity **2023**;56(3):547-61 e7 doi 10.1016/j.immuni.2023.02.003.
45. Palmer AC, Kurtz DM, Alizadeh AA. Cell-of-Origin Subtypes and Therapeutic Benefit from Polatuzumab Vedotin. N Engl J Med **2023**;389(8):764-6 doi 10.1056/NEJMc2306105.