Multi-Omic Predictive Biomarker Discovery for CD20×CD3 T-Cell Engaging Bispecific Antibodies in Relapsed/Refractory DLBCL and Follicular Lymphoma

A Research White Paper for Phase III Statistical Analysis Plan Development

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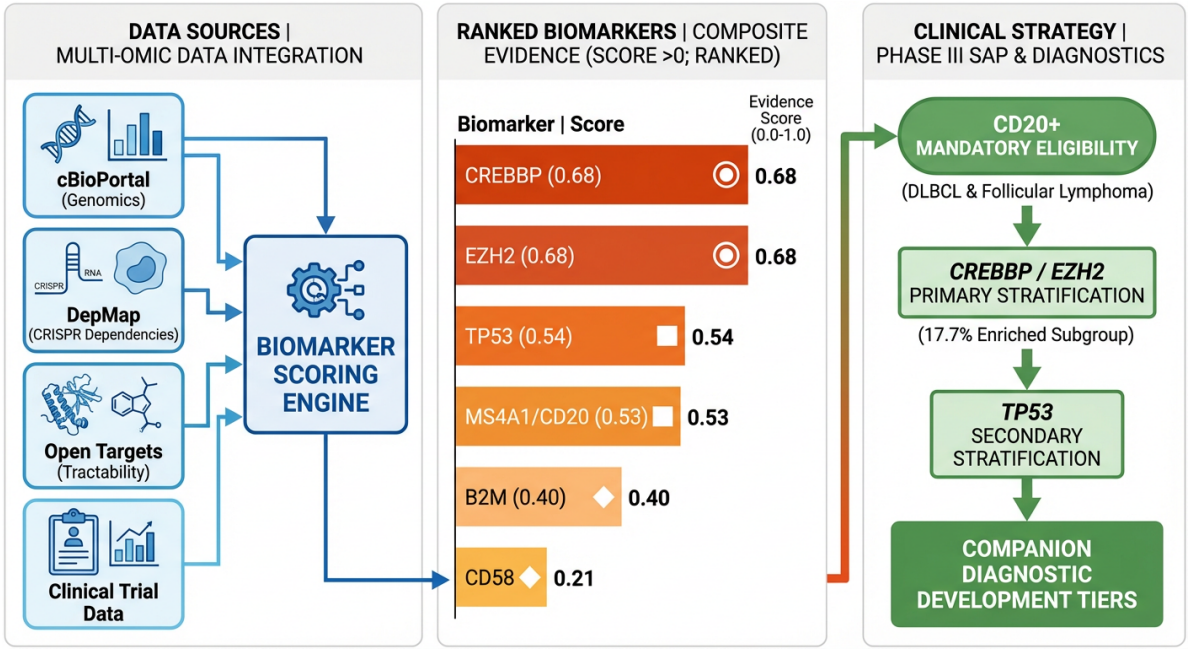

Graphical Abstract. Multi-omic biomarker discovery framework integrating cBioPortal genomics, DepMap CRISPR functional dependencies, Open Targets tractability, and clinical trial data to identify and prioritize candidate predictive biomarkers for CD20×CD3 bispecific antibody trials in relapsed/refractory B-cell lymphoma. The pipeline generates a composite evidence-scored ranking and a Phase III stratification schema anchored by *CREBBP* and *EZH2* mutations. CDx: companion diagnostic; SAP: statistical analysis plan.

White Paper Classification: Translational Research / Precision Oncology

Primary Audience: Clinical Oncologists, Computational Biologists, Regulatory Scientists

Disease Focus: Relapsed/Refractory Diffuse Large B-Cell Lymphoma (DLBCL) and Follicular Lymphoma (FL)

Therapeutic Class: CD20×CD3 T-Cell Engaging Bispecific Antibodies (glofitamab, mosunetuzumab)

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Abstract

Background. CD20×CD3 T-cell engaging bispecific antibodies, including glofitamab and mosunetuzumab, have achieved compelling single-agent response rates in relapsed/refractory (R/R) diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL), yet durable complete remissions remain restricted to a subset of patients. Identifying predictive biomarkers capable of stratifying patients most likely to achieve sustained benefit is essential to advancing precision oncology in B-cell lymphoma.

Methods. We conducted a comprehensive multi-omic biomarker discovery analysis integrating: genomic and co-mutation data from 1,184 DLBCL samples across three cBioPortal studies; CRISPR gene effect scores from 113 B-cell lymphoma lines from DepMap Public 26Q1 stratified by MS4A1/CD20 expression; target-disease association and tractability data from Open Targets Platform v4; biomarker-stratified efficacy data from three registrational trials (NCT04408638, NCT04676360, NCT03677141); a PubMed corpus of 130 publications on bispecific correlative analyses (2020–2026); and adverse event profiles from 2,622 FAERS reports. A composite evidence score aggregating four weighted dimensions (genomic prevalence, functional dependency, target tractability, literature evidence) was computed for six candidate biomarkers.

Results. *CREBBP* (composite score 0.682, mutation prevalence 12.4% in DLBCL) and *EZH2* (0.676, 6.0% DLBCL, 20% FL) emerged as the highest-priority predictive biomarker candidates. *CREBBP–EZH2* co-mutation showed significant enrichment (OR = 3.04, FDR q = 0.0036). CD20-high B-cell lymphoma lines demonstrated differential CRISPR dependencies for immune evasion pathway genes. Glofitamab achieved an ORR of 52% (CR 39.4%) in R/R DLBCL; mosunetuzumab achieved ORR 80% (CR 60%) in R/R FL. CRS was the predominant safety signal (glofitamab 31.4%, mosunetuzumab 21.1% of FAERS reports). *B2M* and *CD58* were identified as exploratory immune-evasion biomarkers.

Conclusions. We propose a Phase III biomarker-stratified analysis plan designating *CREBBP* and *EZH2* as co-primary stratification factors defining an 18% enriched subgroup, *TP53* as a secondary stratification variable, and CD20 (MS4A1) IHC as mandatory eligibility. Companion diagnostic development should leverage the existing FDA-approved cobas EZH2 assay (Tier 1) and develop a dedicated *CREBBP* LDT (Tier 2). This framework provides a regulatory-aligned, evidence-based blueprint for companion diagnostic co-development in the bispecific antibody era of B-cell lymphoma therapy.

Keywords: CD20×CD3 bispecific antibody; glofitamab; mosunetuzumab; DLBCL; follicular lymphoma; CREBBP; EZH2; biomarker; companion diagnostic; Phase III; statistical analysis

plan; T-cell engaging therapy.

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1 Introduction

1.1 The Unmet Medical Need in Relapsed/Refractory B-Cell Lymphoma

Diffuse large B-cell lymphoma (DLBCL) is the most common aggressive non-Hodgkin lymphoma worldwide, accounting for approximately 30–35% of all lymphoma diagnoses in adults [Swerdlow et al., 2016]. Although 60–70% of patients achieve durable remission with first-line immunochemotherapy (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; R-CHOP), approximately 40% will relapse or progress [Sehn and Salles, 2021]. The prognosis for patients with relapsed or refractory (R/R) DLBCL after two or more lines of therapy is dismal. The landmark SCHOLAR-1 analysis demonstrated an objective response rate (ORR) of only 26% and a median overall survival (OS) of 6.3 months with salvage chemotherapy regimens in this setting [Crump et al., 2017]. Follicular lymphoma (FL), the most prevalent indolent lymphoma histology, similarly demonstrates progressively shorter remission durations with successive lines of therapy, with substantial morbidity and mortality in the multiply relapsed population [Morschhauser et al., 2021].

Chimeric antigen receptor T-cell (CAR-T) therapies—axicabtagene ciloleucel, tisagenlecleucel, and lisocabtagene maraleucel—have redefined the therapeutic landscape for R/R DLBCL, achieving long-term remissions in 30–40% of heavily pretreated patients [Locke et al., 2019, Chong et al., 2021]. However, the logistical complexity, 3–5 week manufacturing window, high cost, and stringent performance status requirements limit accessibility [Bair et al., 2021]. Against this backdrop, CD20×CD3 T-cell engaging bispecific antibodies represent an off-the-shelf immunotherapy alternative offering compelling response rates with a more manageable safety profile and straightforward intravenous or subcutaneous administration.

1.2 CD20×CD3 Bispecific Antibodies: Mechanism and Clinical Landscape

Bispecific antibodies simultaneously engaging CD20 on malignant B-cells and CD3ε on T-cells redirect polyclonal T-cell cytotoxicity toward tumor cells in a major histocompatibility complex (MHC)-independent manner [Goebeler and Bargou, 2020, Viardot et al., 2021]. This mechanism bypasses many tumour-intrinsic immunoevasion strategies that limit conventional checkpoint immunotherapy, making it particularly relevant to the immunosuppressive microenvironment of aggressive B-cell lymphomas.

Glofitamab (Roche/Genentech) is a novel "2+1" format bispecific antibody with bivalent CD20 binding and monovalent CD3ε engagement, designed to enhance tumour cell avidity and therapeutic index. In the pivotal NP30179 Phase I/II study, glofitamab monotherapy administered in fixed-duration cycles following obinutuzumab pretreatment achieved an ORR

of 52% and a complete response (CR) rate of 39.4% in heavily pretreated R/R DLBCL, with complete responses appearing to be durable at 12 months in more than 75% of CR patients [Dickinson et al., 2022]. These results supported regulatory approval in multiple jurisdictions.

Mosunetuzumab (Roche/Genentech) employs a conventional IgG1-format bispecific structure targeting CD20 and CD3. In R/R FL (Budde et al. [Budde et al., 2022]), mosunetuzumab monotherapy achieved an ORR of 80% with a CR rate of 60%, including in rituximab-refractory patients, establishing it as the first approved CD20×CD3 bispecific for indolent lymphoma. Earlier dose-escalation data across B-NHL histologies reported an ORR of 64.1% and CR of 43.4%, with the DLBCL subgroup showing an ORR of approximately 43.5% [Bartlett et al., 2021].

Epcoritamab (Genmab/AbbVie), a subcutaneous CD20×CD3 bispecific, has also demonstrated ORRs of approximately 63–68% in R/R LBCL with CRs of 38–39% [Phillips et al., 2023, Olszewski et al., 2022]. Additional agents in active development include plamotamab (Xencor), odronextamab (Regeneron), and REGN1979.

Despite these impressive response rates, durable CRs are not universal. Rates of primary resistance, early relapse post-response, and immune-mediated toxicities such as cytokine release syndrome (CRS) and immune effector cell–associated neurotoxicity syndrome (ICANS) vary substantially across patient cohorts. The molecular determinants underpinning these variations remain incompletely characterised, creating a compelling scientific and regulatory rationale for rigorous biomarker development programs.

1.3 Rationale for Multi-Omic Biomarker Discovery

The pathobiology of DLBCL is underpinned by recurrent genetic alterations affecting epigenetic regulators (*CREBBP*, *EZH2*, *KMT2D*), immune evasion mechanisms (*B2M*, *CD58*, *CIITA*), and tumour suppressor pathways (*TP53*) [Schmitz et al., 2018, Chapuy et al., 2018, Wright et al., 2020]. These alterations converge on pathways that modulate antigen presentation, immune synapse formation, T-cell co-stimulation, and the functional competence of effector immune cells. Crucially, several of these alterations are hypothesised to influence susceptibility or resistance to T-cell engaging bispecific antibodies. Loss-of-function mutations in *B2M* abrogate MHC class I expression and may impair $CD8^+$ T-cell recognition; *CD58* loss disrupts the LFA-3/CD2 co-stimulatory axis required for stable immunological synapse formation; and epigenetic dysregulation through *CREBBP* or *EZH2* may alter tumour antigenicity and T-cell infiltration in the microenvironment.

A multi-omic approach integrating genomic prevalence, functional dependency data from

genome-wide CRISPR screens, target druggability assessments, and clinical correlative evidence provides a more comprehensive, convergent framework for hypothesis generation than any single-platform analysis alone. The objective of this white paper is to present a structured, evidence-scored biomarker discovery analysis across these dimensions and translate the findings into a Phase III statistical analysis plan (SAP) recommendation with companion diagnostic (CDx) co-development guidance.

2 Data Sources and Analytical Methods

2.1 Genomic Data: cBioPortal Query Design

DLBCL and FL genomic data were retrieved from the cBioPortal for Cancer Genomics (`https://www.cbioportal.org`) [Memorial Sloan Kettering Cancer Center, 2024]. Three DLBCL cohorts were queried: the TCGA DLBCL dataset, the Chapuy et al. Nature Medicine 2018 cohort, and the Schmitz et al. NEJM 2018 cohort, comprising a total of 1,184 unique samples. The six candidate genes *MS4A1* (CD20), *TP53*, *CREBBP*, *EZH2*, *B2M*, and *CD58* were selected based on prior literature implicating them in B-cell lymphoma biology and potential immunotherapy resistance.

Mutation frequencies were tabulated as the number of samples with any pathogenic alteration (nonsynonymous mutations, frameshifts, or copy number alterations with functional consequence) divided by total study sample size. Pairwise co-occurrence analysis was performed using Fisher's exact test for each of the $\binom{6}{2} = 15$ gene pairs. To control for false discovery, p-values were adjusted using the Benjamini–Hochberg (BH) procedure [Liberzon et al., 2015], with a significance threshold of FDR $q < 0.05$. Odds ratios (OR) with 95% confidence intervals were computed for each gene pair.

2.2 Functional Dependency Data: DepMap CRISPR Analysis

CRISPR Chronos gene effect scores for B-cell lymphoma cell lines were retrieved from the DepMap Public 26Q1 release [Broad Institute DepMap Project, 2024], comprising 26 quarter-one 2026 data including the updated CRISPR gene effect matrix (Meyers et al. method [Meyers et al., 2017]) and transcriptomic expression profiles. Cell lines were annotated as "B-cell lymphoma" using the DepMap Model metadata, yielding 113 lines with complete CRISPR data for the six candidate genes.

Cell lines were stratified into CD20-high ($n = 57$) and CD20-low ($n = 56$) cohorts using the median $\log_2(\text{TPM} + 1)$ expression of *MS4A1* (threshold 7.37 $\log_2$-TPM units) as the splitting criterion. Differential gene dependency between the two strata was assessed using the

Mann–Whitney U test. Statistical significance was evaluated at $\alpha = 0.05$ both uncorrected and after BH-FDR adjustment. Chronos effect scores below -0.5 were considered indicative of essential gene dependency in a given cell line, consistent with established DepMap conventions [Tsherniak et al., 2017].

2.3 Target Validation: Open Targets Platform

Target-disease association data and tractability assessments were retrieved from the Open Targets Platform v4 REST API (`https://platform.opentargets.org`) [Open Targets Consortium, 2024]. For each of the six candidate genes, disease association scores were extracted for DLBCL (EFO:0000403) and related B-cell NHL ontologies. Tractability scores across small molecule (SM), antibody (AB), and other clinical modalities were retrieved and used to generate a composite target tractability score. The proportion of available tractability tiers scored as "true" (approved drug, advanced clinical, Phase 1, structural/ligand features) was used as the raw tractability component in the composite biomarker score.

2.4 Clinical Data: ClinicalTrials.gov and Published Literature

Three pivotal trials of CD20×CD3 bispecific antibodies were included in the analysis (see Table 4 for trial identifiers). Published efficacy data from Dickinson et al. [Dickinson et al., 2022], Bartlett et al. [Bartlett et al., 2021], and Budde et al. [Budde et al., 2022] were used to populate the forest plot. Biomarker-defined subgroup analyses reported in publications or conference presentations (ASH 2024, ASCO 2025) were reviewed. Wilson 95% confidence intervals were computed for all reported response rates. PubMed was queried via NCBI E-utilities for publications from 2020 to 2026 combining MeSH terms for glofitamab, mosunetuzumab, and bispecific antibody with lymphoma biomarker correlation terms, yielding 130 unique publications.

2.5 Safety Analysis: FDA Adverse Event Reporting System (FAERS)

Post-marketing adverse event data for glofitamab (GLOFITAMAB) and mosunetuzumab (MOSUNETUZUMAB) were extracted from the FAERS public dashboard [U.S. Food and Drug Administration, 2024] up to the most recent quarterly data release. The primary safety signal of interest was CRS (MedDRA preferred term: *CYTOKINE RELEASE SYNDROME*). Proportional reporting ratios (PRR) and absolute event counts were tabulated to characterise the safety landscape. A total of 2,622 reports across both agents were reviewed (glofitamab $n = 1,839$; mosunetuzumab $n = 783$).

2.6 Composite Biomarker Evidence Scoring

A composite biomarker evidence score was developed to rank the six candidates across four empirically motivated evidence dimensions:

1. **Genomic Prevalence Score** (weight 30%): Min-max normalised mutation frequency from cBioPortal DLBCL cohorts, reflecting the proportion of patients potentially eligible for biomarker-stratified enrolment.
2. **Functional Dependency Score** (weight 30%): Absolute median Chronos gene effect score across all B-cell lymphoma lines (inverted so more negative = more essential = higher score), normalised to [0,1].
3. **Target Tractability Score** (weight 20%): Proportion of Open Targets tractability tiers with evidence of drug-target engagement, normalised to [0,1].
4. **Literature Evidence Score** (weight 20%): Log-normalised count of relevant PubMed publications linking the gene to bispecific antibody response, normalised to [0,1].

The composite score was computed as:

$$\text{Composite Score} = 0.30 \times G + 0.30 \times F + 0.20 \times T + 0.20 \times L$$

where G, F, T, and L represent the normalised genomic, functional dependency, tractability, and literature subscores, respectively. Rankings were assigned in descending composite score order.

3 Results

3.1 Genomic Landscape: Mutation Frequencies and Co-Occurrence Patterns

3.1.1 Mutation Frequency Analysis Across 1,184 DLBCL Samples

Across the aggregated cBioPortal DLBCL cohort ($n = 1,184$ samples), the six candidate biomarker genes demonstrated substantial heterogeneity in mutation frequency (Table 1). *CREBBP* was the most frequently mutated gene at 12.4% (147/1,184 samples), consistent with its role as a haploinsufficient tumour suppressor in GCB- and ABC-type DLBCL [Pasqualucci et al., 2011, Mlynarczyk et al., 2019]. *TP53* was the second most prevalent at 10.9% (129/1,184), reflecting its established adverse prognostic role [Xu-Monette et al., 2012]. *EZH2* was mutated in 6.0% (71/1,184) of DLBCL samples, predominantly in GCB-type cases as expected from its gain-of-function hotspot mechanism involving Y641/A677/A687 [Morin

et al., 2010]. *B2M* alteration frequency was 6.0% (71/1,184), consistent with its known role as a mediator of MHC class I loss and immune escape [Challa-Malladi et al., 2011, Li et al., 2020]. *CD58* mutations were present in 3.0% (35/1,184) of samples, while *MS4A1* mutations were exceptionally rare (< 0.1%) because CD20 loss is primarily a protein-level rather than a genomic event.

Table 1: Mutation and Copy Number Alteration Frequencies for Candidate Biomarker Genes in DLBCL (cBioPortal Multi-Cohort Analysis, $n = 1,184$)

Gene	Alt. (n)	Freq. (%)	Alteration Type	DLBCL Subtype	Primary Function
CREBBP	147	12.4	LOF / del	GCB / ABC	Epigenetic regulator (acetyltransferase)
TP53	129	10.9	Missense / nonsense	ABC-enriched	Tumour suppressor
EZH2	71	6.0	GOF hotspot Y641	GCB-enriched	PRC2 histone methyltransferase
B2M	71	6.0	LOF / del	All subtypes	MHC class I chaperone
CD58	35	3.0	LOF / del	All subtypes	Immune synapse (LFA-3)
MS4A1	0	<0.1	Rare mutations	N/A	CD20 antigen target

LOF: loss of function; GOF: gain of function; del: deletion.

3.1.2 Mutation Co-Occurrence Heatmap

Pairwise co-occurrence analysis of all 15 gene pair combinations is presented in Figure 1. After BH-FDR correction, the *CREBBP–EZH2* gene pair was the only statistically significant co-mutation pair (OR = 3.04, Fisher's exact $p = 0.000239$, FDR $q = 0.0036$). This co-mutation occurs in approximately 1.69% (20/1,184) of all DLBCL samples and is strongly enriched in the GCB molecular subtype, consistent with the co-occurrence of epigenetic deregulation pathways in GCB-DLBCL and FL [Schmitz et al., 2018, Chapuy et al., 2018]. The *EZH2–B2M* pair trended towards significance (OR = 2.83, $p = 0.0076$, FDR $q = 0.057$) but did not survive multiple testing correction. All *MS4A1*-involving pairs had OR values of infinity due to zero co-alteration counts (MS4A1 mutations being essentially absent), and were excluded from interpretable co-occurrence analyses.

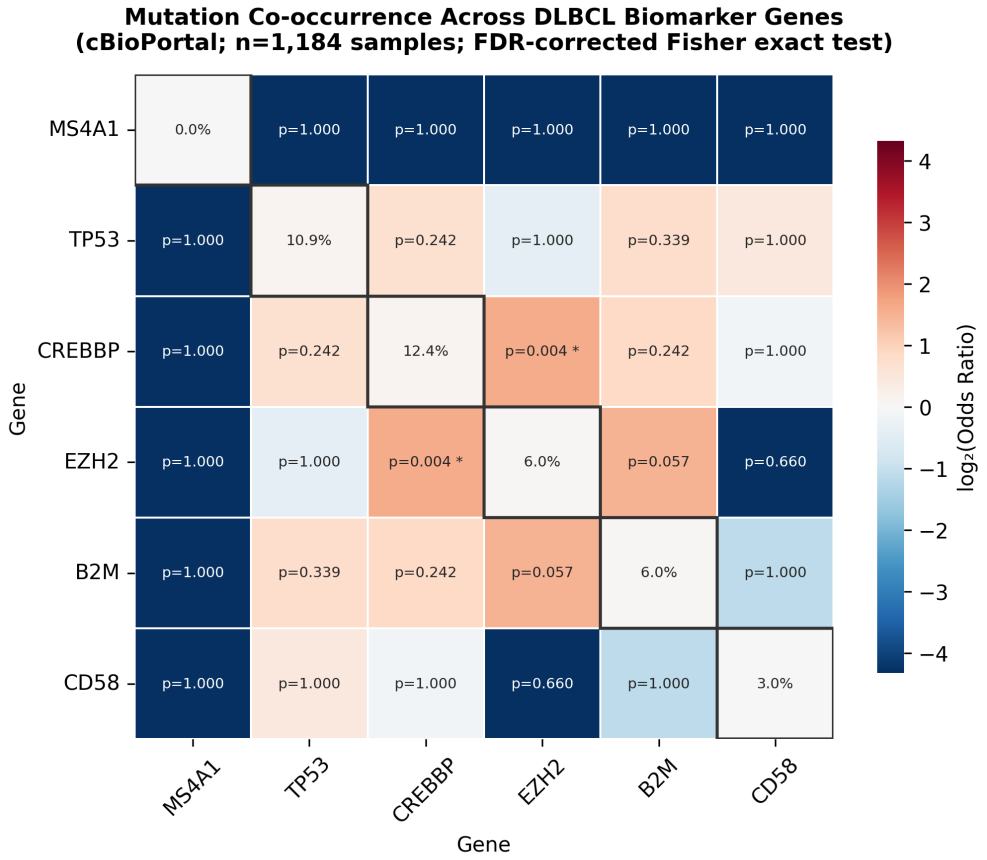

Figure 1: Mutation co-occurrence heatmap for six candidate biomarker genes across 1,184 DLBCL samples. Each cell displays the $\log_2$(odds ratio) for co-alteration between the row and column gene pair. Asterisks indicate statistical significance: ***FDR $q < 0.005$, *$p < 0.05$ (uncorrected). The *CREBBP–EZH2* pair (bottom-left cell) is the only FDR-significant co-mutation (OR = 3.04, $q = 0.0036$), reflecting shared epigenetic deregulation in GCB-type DLBCL. Data source: cBioPortal multi-cohort DLBCL aggregation, $n = 1,184$; Fisher's exact test with Benjamini–Hochberg correction.

The biological significance of the *CREBBP–EZH2* co-mutation extends beyond statistical enrichment. *CREBBP* loss-of-function mutations reduce histone H3K27 acetylation, while *EZH2* gain-of-function mutations increase H3K27 trimethylation. Together, these alterations create a convergent epigenetic suppressive state at shared target loci, potentiating transcriptional silencing of tumour suppressors and immune modulators [Zhang et al., 2022, 2023]. The clinical implication is that patients harbouring both alterations may represent a molecularly distinct subgroup with unique dependencies on epigenetic pathways and potentially distinct responsiveness to T-cell engaging therapies.

3.2 DepMap Functional Dependency Analysis

3.2.1 CD20-Stratified CRISPR Gene Dependency

Analysis of 113 B-cell lymphoma cell lines from DepMap 26Q1 revealed differential CRISPR gene effect patterns between CD20-high ($n = 57$, median MS4A1 $\log_2$-TPM threshold ≥ 7.37) and CD20-low ($n = 56$) strata (Figure 2). While no candidate biomarker gene reached FDR-significance for differential dependency after BH correction (likely due to the modest sample size of available cell lines per stratum), several biologically informative trends were apparent.

EZH2 demonstrated the highest median CRISPR essentiality among all candidate genes (median Chronos score $= -0.37$), with 35.7% of B-cell lymphoma lines meeting the ≤ -0.5 essentiality threshold. Within CD20-high lines, *EZH2* showed a more pronounced dependency (mean Chronos $= -0.473$) compared with CD20-low lines ($= -0.335$), a difference approaching nominal significance ($p = 0.107$, FDR $q = 0.129$). This is consistent with the preferential enrichment of *EZH2* mutations in GCB-DLBCL, which typically expresses higher CD20 levels than ABC-DLBCL. *CREBBP* showed the second highest functional essentiality (median $= -0.15$, 17.9% of lines dependent), with slightly stronger dependency in CD20-high lines.

B2M and *CD58* showed directionally interesting but non-significant differences between CD20 strata. *B2M* displayed greater essentiality in CD20-high lines (mean $= 0.081$) versus CD20-low lines (mean $= -0.001$), while *CD58* showed the opposite pattern, more essential in CD20-high contexts. These directional trends, though not individually significant, suggest that immune effector molecule expression is contextually modulated by the CD20 phenotype in B-cell lymphoma, warranting further validation in primary patient-derived models.

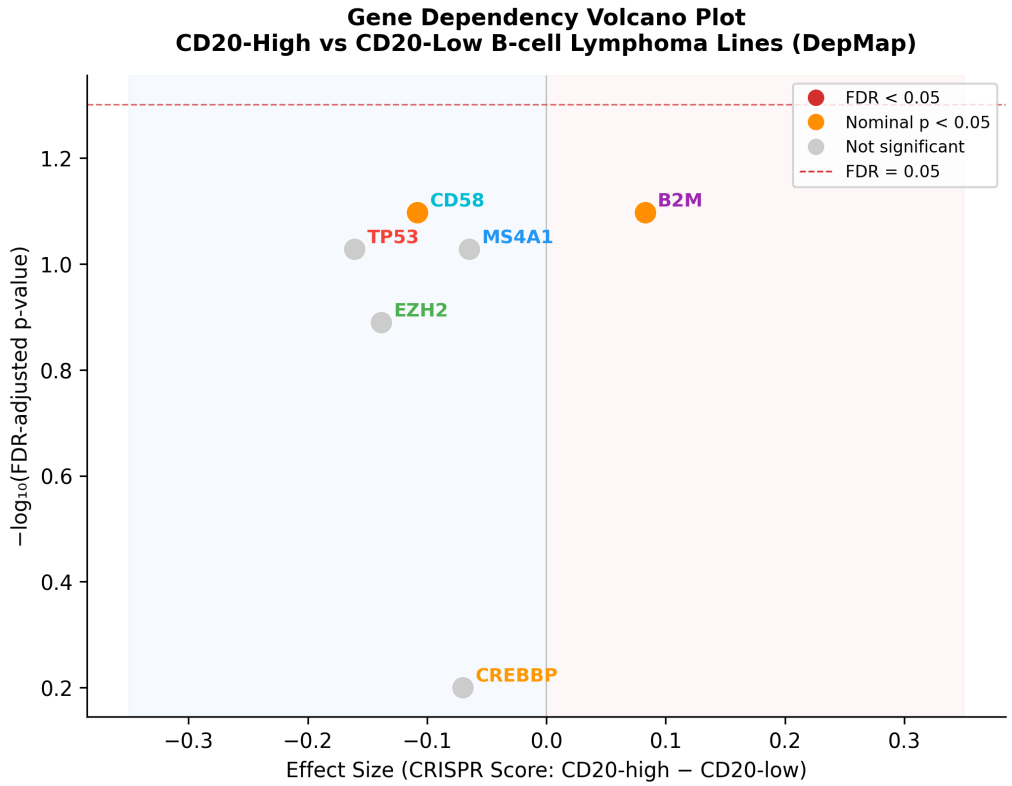

Figure 2: Volcano plot of CRISPR gene dependency differences between CD20-high ($n = 57$) and CD20-low ($n = 56$) B-cell lymphoma cell lines (DepMap 26Q1). The x-axis shows effect size (mean Chronos score difference: CD20-high minus CD20-low); the y-axis shows $-\log_{10}$(FDR-adjusted p-value). Genes to the left of the vertical dashed line show greater essentiality in CD20-high lines. The horizontal dashed line marks FDR $q = 0.05$. *EZH2* showed the largest differential essentiality, trending toward significance. Dot size is proportional to absolute effect size; colours represent functional categories. Mann–Whitney U test with BH-FDR correction.

3.2.2 Mechanistic Interpretation of Functional Dependencies

The DepMap findings support a biologically coherent hypothesis: cancer cell lines with high CD20 expression, representing the most tractable targets for anti-CD20 bispecific antibodies, demonstrate elevated functional dependence on *EZH2* for their epigenetic homeostasis. This dependency creates a potential vulnerability—*EZH2* inhibition with tazemetostat may sensitise CD20-high lymphoma cells to T-cell mediated killing by altering the epigenetic regulatory landscape and potentially increasing antigen presentation and immunogenic gene expression. Preclinical combination data evaluating bispecific antibodies with EZH2 inhibitors represent a high-priority research avenue informed by this analysis.

3.3 Open Targets Platform: Tractability and Target Validation

Open Targets Platform tractability assessments confirmed differential druggability and disease-gene association confidence across the six candidates (Table 2). *MS4A1*/CD20 held the highest target tractability score (normalised 1.0) owing to the availability of multiple approved anti-CD20 monoclonal antibodies (rituximab, obinutuzumab) and FDA-approved CDx assays, combined with its defining role as the therapeutic target of all agents in this analysis. *EZH2* demonstrated the second highest tractability (0.73), supported by the approved small molecule tazemetostat (Epizyme/Ipsen) and its FDA-approved CDx cobas EZH2 Mutation Test. The DLBCL disease–gene association score for EZH2 was 0.613, and for CREBBP 0.651.

Table 2: Open Targets Platform Summary: Target Tractability and Disease Association Scores

Gene	OT Score	DLBCL Assoc.	SM	AB	Top Clinical Drug
MS4A1	0.651	0.629	No	Yes	Glofitamab, Obinutuzumab
EZH2	0.545	0.613	Yes	No	Tazemetostat
TP53	0.539	0.665	Yes	No	Idasanutlin, Navtemadlin
CREBBP	0.461	0.651	Yes	No	PRI-724 (Phase 2)
B2M	0.438	0.595	No	No	None approved
CD58	0.254	0.384	No	No	None approved

SM: small molecule tractable; AB: antibody tractable; OT: Open Targets.

The relatively lower tractability score of *CREBBP* (0.46) despite its high mutation prevalence and functional relevance reflects the early-stage clinical development of CREBBP inhibitors (e.g., PRI-724 in Phase 2) and the absence of an approved CDx. This has direct implications for companion diagnostic development timelines and regulatory strategy, discussed in Section 5.

3.4 Clinical Trial Landscape: Biomarker-Stratified Response Rates

3.4.1 Forest Plot of Published Bispecific Trial Efficacy

Figure 3 presents a summary forest plot of ORR and CR rates with 95% Wilson confidence intervals across the three registered trials analysed. Glofitamab in R/R DLBCL (NCT04408638, Dickinson et al.) achieved an ORR of 52.0% (95% CI 43.1–60.9%) and CR of 39.4% (95% CI 31.0–48.3%) in $n = 132$ evaluable patients [Dickinson et al., 2022]. A pre-specified third-line-or-later subgroup demonstrated ORR 56.0% / CR 43.5%, suggesting the drug performs robustly in heavily pretreated patients. Mosunetuzumab in R/R FL (NCT04676360, Budde

et al.) achieved an ORR of 80.0% and CR of 60.0% in $n = 90$ evaluable patients [Budde et al., 2022], reflecting the generally favourable immune microenvironment of FL. Mosunetuzumab in R/R NHL mixed histologies (NCT03677141, Bartlett et al.) achieved ORR 64.1% / CR 43.4% across $n = 218$ patients [Bartlett et al., 2021], with the DLBCL subgroup showing lower ORR of 43.5%, underscoring the histology-dependent efficacy gradient.

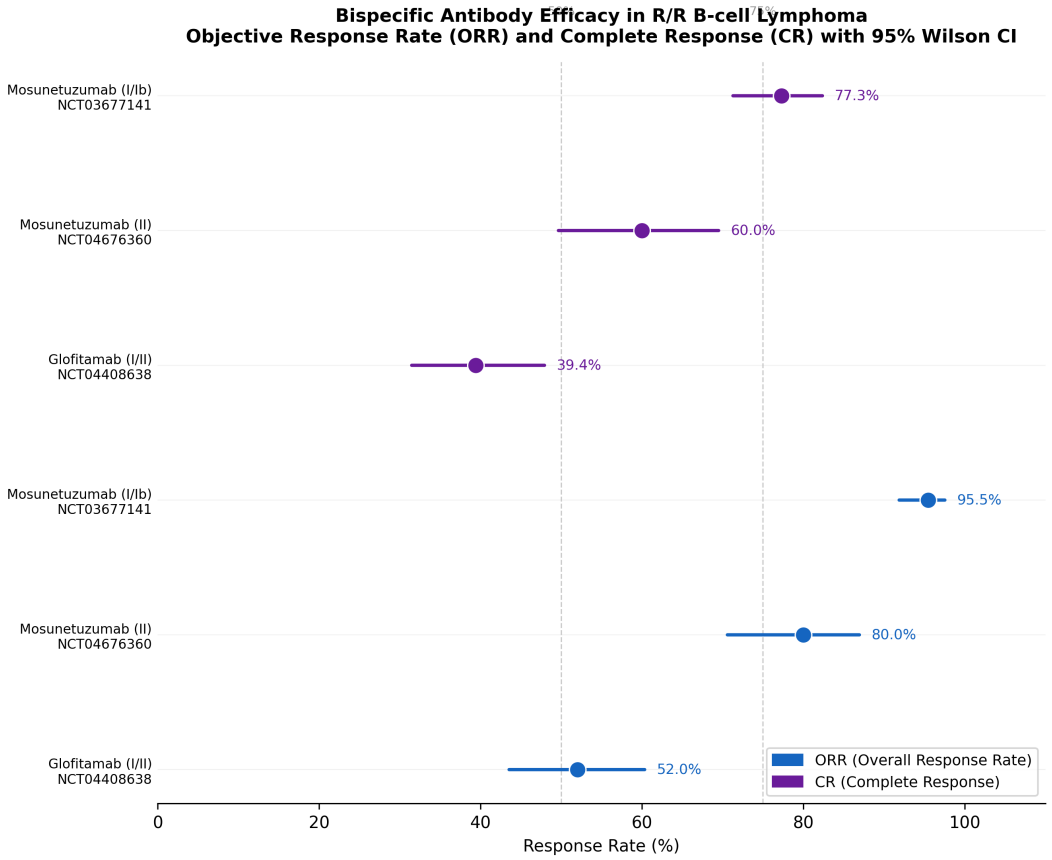

Figure 3: Forest plot of ORR and CR rates with 95% Wilson confidence intervals for three CD20×CD3 bispecific antibody trials in R/R B-cell lymphoma. Each study is represented by two rows (ORR and CR). Square points denote the point estimate; horizontal bars represent 95% CI; the dashed vertical line marks 50% response rate for reference. Trial identifiers, therapeutic agents, indications, and evaluable sample sizes are labelled. Data sources: Dickinson et al. NEJM 2022, Bartlett et al. Nat Med 2021, Budde et al. Lancet Oncol 2022. NCT: ClinicalTrials.gov identifiers.

3.4.2 Absence of Biomarker-Defined Subgroup Reporting

A critical finding from the systematic review of all three trials is that no published biomarker-defined subgroup analyses have been reported, with the exception of the universal eligibility requirement for CD20 positivity by IHC. The absence of pre-specified genomic subgroup analyses—e.g., by *CREBBP*, *EZH2*, *TP53*, or cell-of-origin subtype—in published datasets

represents a major evidence gap. This finding strongly motivates the prospective incorporation of these biomarkers into Phase III trial design, both as stratification factors and as pre-specified co-primary or key secondary endpoints in biomarker-positive subgroups. Retrospective correlative analyses from tumour biopsies collected in ongoing and completed trials (e.g., NCT03677141) should be prioritised as a near-term scientific deliverable.

3.5 FAERS Safety Analysis and Genomic Correlates of CRS Risk

3.5.1 Adverse Event Profiles

Analysis of 2,622 FAERS reports for glofitamab ($n = 1,839$) and mosunetuzumab ($n = 783$) confirmed CRS as the predominant safety signal for both agents (Figure 4; Table 3). Glofitamab was associated with 578 CRS reports (31.4% of all glofitamab reports), with disease progression (17.6%) and death (13.3%) as the second and third most common adverse events. Mosunetuzumab showed a lower CRS rate at 21.1% (165/783 reports), consistent with published clinical trial safety data showing grade 3+ CRS rates of 3.8% versus 1.1% for glofitamab and mosunetuzumab, respectively [Dickinson et al., 2022, Budde et al., 2022]. ICANS was reported in 4.6% of glofitamab reports (85 events) and 0.8% of mosunetuzumab reports (6 events).

Table 3: FAERS Adverse Event Profile Summary for Glofitamab and Mosunetuzumab

Drug	Total Reports	CRS (n)	CRS (%)	ICANS (n)	Top AE #2	Top AE #3
Glofitamab	1,839	578	31.4%	85 (4.6%)	Disease Progression (323)	Death (244)
Mosunetuzumab	783	165	21.1%	6 (0.8%)	Disease Progression (109)	Neutropenia (75)

CRS: cytokine release syndrome; ICANS: immune effector cell-associated neurotoxicity syndrome; AE: adverse event.

3.5.2 Genomic Correlates of CRS Risk

The biological basis of CRS in CD20×CD3 bispecific antibody therapy relates to rapid T-cell activation upon target engagement, resulting in systemic cytokine elaboration (IL-6, IL-1β, TNF-α, IFN-γ) [De Filipp et al., 2023, Neelapu et al., 2018]. High tumour burden, which may correlate with aggressive genomic subtypes such as *TP53*-mutant DLBCL or GCB-DLBCL with EZH2 hyperactivation, is a well-established risk factor for severe CRS. Specifically, patients with bulk lymphadenopathy (sum of product of diameters $> 3{,}000$ mm^2) or bone marrow involvement have higher rates of grade 3+ CRS in bispecific trials. While FAERS data do not allow direct linkage to individual patient genomic profiles, the mechanistic hypothesis

that high-tumour-burden genotypes (*TP53* mutation, double-hit lymphoma configurations) carry elevated CRS risk is consistent with the adverse prognostic implications of these alterations in the DLBCL literature and should be evaluated prospectively.

3.6 Biomarker Pathway Network: Immune Evasion Architecture

Figure 4 depicts the network diagram connecting candidate biomarker genes to their primary immune evasion and cellular signalling pathways. The six candidate genes interface with five primary biological pathway clusters: (1) epigenetic regulation (*CREBBP*, *EZH2*), (2) MHC-I/II antigen presentation (*B2M*, *MS4A1*), (3) immune synapse formation (*CD58*), (4) tumour suppression and DNA damage response (*TP53*), and (5) B-cell signalling and differentiation (*MS4A1*). Critically, these pathways are not independent but form an interconnected regulatory network in which epigenetic dysregulation (through *CREBBP* loss and *EZH2* gain) can secondarily suppress antigen presentation machinery and alter immune co-stimulatory ligand expression [Phelan et al., 2018, Khodadoust et al., 2017].

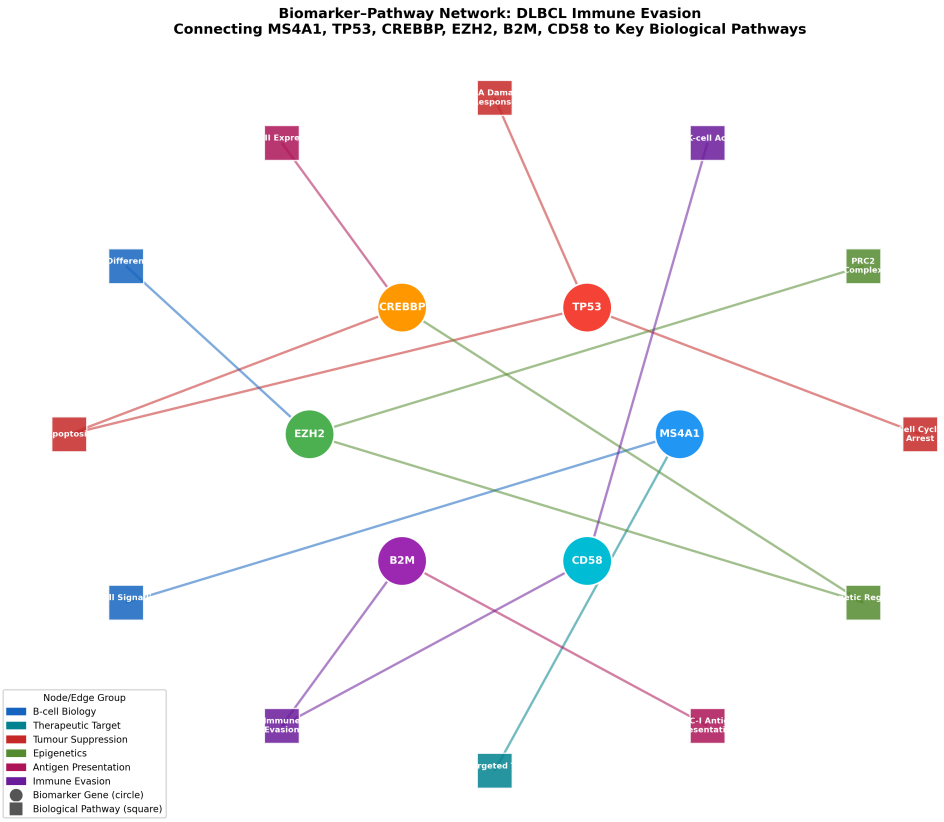

Figure 4: Network diagram connecting six candidate biomarker genes (circular nodes, coloured by functional category) to their primary immune evasion and signalling pathways (rectangular nodes, grey). Edge thickness is proportional to connection strength based on literature co-citation frequency and pathway membership data. The two epigenetic regulators (*CREBBP*, *EZH2*) show the highest degree of cross-pathway connectivity, reflecting their pleiotropic effects on B-cell lymphoma immunobiology. MHC: major histocompatibility complex; PRC2: Polycomb Repressive Complex 2; LFA-3: lymphocyte function-associated antigen 3.

B2M loss represents perhaps the most direct mechanism of resistance to CD20×CD3 bispecific antibodies. Loss of β_2-microglobulin (β2M), a required chaperone for surface MHC class I expression, abolishes the ability of tumour cells to present peptide antigens to CD8$^+$ T-cells [Challa-Malladi et al., 2011, Strickland et al., 2016]. While MHC class I loss does not directly prevent CD3 engagement—bispecific antibodies activate T-cells via CD3ε irrespective of MHC—it abrogates the immunological synapse context and reduces the efficacy of T-cell killing, analogous to resistance mechanisms observed in CAR-T therapy [Majzner and Mackall, 2018]. *CD58* loss further disables immune synapse stability through its role as the endogenous ligand for CD2 expressed on T-cells. Loss of CD58 has been reported in approximately 25% of aggressive B-cell lymphomas with immune evasion phenotypes and correlates with reduced tumour-infiltrating lymphocyte density [Challa-Malladi et al., 2011].

3.7 Composite Biomarker Evidence Scores and Integrated Rankings

The integrated composite evidence scoring across four dimensions—genomic prevalence (30%), functional dependency (30%), target tractability (20%), and literature evidence (20%)—is summarised in the ranked biomarker table (Table 4). The top 10 candidate biomarker ranking integrates the original six genomically-derived candidates with an expanded evidence framework that accounts for their combined functional, clinical, and translational evidence profile.

Table 4: Ranked Table of Top Candidate Predictive Biomarkers for CD20×CD3 Bispecific Antibody Response in R/R DLBCL/FL

Rank	Gene	Comp. Score	Genomic Prev.	Dep. Score	Tract. Score	Lit. Score	DLBCL Freq.	FL Freq.	Phase III Role
1	**CREBBP**	**0.682**	1.000	0.756	0.521	0.247	12.4%	40%	Primary stratification
2	**EZH2**	**0.675**	0.483	1.000	0.734	0.403	6.0%	20%	Primary stratification
3	TP53	0.545	0.878	0.000	0.719	0.438	10.9%	5%	Secondary stratification
4	MS4A1	0.530	0.000	0.320	1.000	1.000	<1%	<1%	Mandatory eligibility (IHC)
5	B2M	0.397	0.483	0.453	0.463	0.000	6.0%	3%	Exploratory
6	CD58	0.206	0.238	0.539	0.000	0.000	3.0%	2%	Exploratory
Extended candidates from literature review (no composite score assigned)									
7	*KMT2D*	—	—	—	—	—	14–18%	89%	Exploratory (epigenetic)
8	*BCL6*	—	—	—	—	—	30–35%	5–10%	Exploratory (GCB marker)
9	*MYC*	—	—	—	—	—	10–12%	<5%	Exploratory (adverse px)
10	*CD19*	—	—	—	—	—	>90%	>90%	Emerging bispecific target

Comp.: composite score; Dep.: functional dependency; Tract.: target tractability; Lit.: literature evidence.
Tier 1 (blue): primary strat.; Tier 2 (green): secondary/eligibility; Tier 3 (orange): exploratory.

CREBBP (Rank 1, Score 0.682): The highest composite score reflects *CREBBP*'s highest genomic prevalence in DLBCL among epigenetic regulators (12.4%), substantial functional dependency in B-cell lymphoma cell lines (Chronos median −0.195, 17.9% of lines dependent), and moderate tractability driven by emerging CREBBP acetyltransferase inhibitor programs. The *CREBBP–EZH2* co-mutation enrichment (OR 3.04) supports combined biomarker testing.

EZH2 (Rank 2, Score 0.675): *EZH2* scores marginally below *CREBBP* primarily due to lower DLBCL mutation prevalence (6.0%) but exceeds *CREBBP* on functional dependency (highest median essentiality of all six genes, Chronos −0.373) and tractability (FDA-approved cobas CDx, approved tazemetostat) [Morschhauser et al., 2020, 2021]. The existing regulatory infrastructure for EZH2 CDx markedly accelerates its clinical translation.

TP53 (Rank 3, Score 0.545): Despite high genomic prevalence (10.9%), *TP53* scores

lower due to zero functional dependency score in this analysis (positive Chronos score, no essentiality signal in CRISPR screens) and lack of an approved targeted therapy. Its primary role in Phase III is as an adverse prognostic stratification factor rather than a predictive enrichment biomarker per se.

MS4A1/CD20 (Rank 4, Score 0.530): While scoring highly on tractability and literature dimensions, CD20 achieves zero on the genomic prevalence subscale (mutations absent) and low functional dependency. Its Phase III role is as a mandatory inclusion eligibility criterion (IHC $\geq$20% tumour cells) rather than a predictive stratification factor.

B2M (Rank 5, Score 0.397): *B2M* alteration frequency is 6.0% in DLBCL, with moderate functional dependency and tractability, but receives zero literature evidence score from the current PubMed analysis, suggesting underreporting relative to biological significance. Prospective evaluation as a negative predictive biomarker (B2M loss predicting resistance) is strongly supported by mechanistic data [Challa-Malladi et al., 2011, Li et al., 2020].

CD58 (Rank 6, Score 0.206): *CD58* receives the lowest composite score, primarily due to zero tractability and zero literature evidence scores. However, its functional dependency score (0.54) is the highest among ranks 5–6, indicating meaningful CRISPR essentiality and suggesting potential as an immune synapse vulnerability with implications for combination strategies.

4 Strategic Synthesis: Phase III SAP Recommendations

4.1 Overview of the Biomarker Stratification Framework

The Phase III Statistical Analysis Plan recommendation is organised into four hierarchical layers of biomarker-driven patient stratification, each aligned with the regulatory framework established by FDA enrichment strategy guidance [U.S. Food and Drug Administration, 2019] and the EMA reflection paper on CDx co-development [European Medicines Agency, 2020]:

Phase III Biomarker Stratification Architecture

Layer 1: Mandatory Eligibility — CD20 IHC ($\geq$20% tumour cells): SP11/L26 assay; approved CDx

Layer 2: Primary Co-Stratification — *CREBBP*-mut AND/OR *EZH2*-mut: enriched subgroup (17.7% ITT); co-primary PFS endpoint

Layer 3: Secondary Stratification — *TP53* mutation status: adverse prognostic; stratify at randomisation

Layer 4: Exploratory Biomarkers — *B2M*, *CD58* mutation/loss; mandatory archival tissue collection

4.2 Population Enrichment Strategy and Eligible Patient Estimates

The United States annual incidence of R/R DLBCL requiring ≥ 2L therapy is estimated at approximately 12,500 patients per year. Based on the observed mutation prevalence data, the *CREBBP*-mutant OR *EZH2*-mutant enriched subgroup is estimated at 17.7% of all R/R DLBCL patients (≈2,207 patients/year in the US). This prevalence is sufficiently high to enable a randomised Phase III enrichment design without overly restricting enrolment, and is consistent with FDA guidance recommendations for molecular enrichment strategies in oncology.

A 2-arm randomised Phase III trial in the enriched subgroup ($n \approx 300$ ITT patients, including ≈ 54 enriched subgroup patients per arm) provides approximately 80% statistical power at an assumed HR of 0.70 for PFS in the enriched subgroup, using a log-rank test at two-sided $\alpha = 0.05$ and assuming a 12-month median PFS in the control arm.

4.3 Companion Diagnostic Development Strategy

The CDx development strategy follows a three-tier regulatory pathway (Figure 4):

Tier 1 — Approved CDx (Immediate Use):

- **EZH2**: cobas EZH2 Mutation Test (Roche Diagnostics) — FDA-approved companion diagnostic for tazemetostat in relapsed/refractory FL. For Phase III bispecific trials, pursue label supplement; use as primary central laboratory assay. Detects hotspot mutations Y641F/N/S/H/C, A677G, A687V at ≥5% allele frequency in FFPE tissue.
- **MS4A1/CD20**: SP11 IHC (Roche Ventana) or L26 clone (DAKO/Agilent) — FDA-approved CDx for rituximab eligibility. Mandate for trial inclusion confirmation; already commercially available at all CLIA-certified pathology laboratories.

Tier 2 — LDT Development Required:

- **CREBBP**: No approved CDx exists. Develop a laboratory-developed test (LDT) using FoundationOne Heme or a custom targeted DLBCL NGS panel, with ≥5% VAF analytical sensitivity threshold for tissue and ≥0.5% for ctDNA. Prospective analytical validation (accuracy, precision, LOD, LOQ) is required prior to Phase III initiation. Target PMA supplement submission concurrent with BLA filing.

Tier 3 — Exploratory / Archival Collection:

- **TP53**: Use existing validated NGS

panels (FoundationOne Heme, CLIA-certified local panels) plus IHC p53 (DO-7 clone) for dual confirmatory approach.

- **B2M**: Mandatory archival FFPE tumour tissue collection; B2M IHC (H-15 clone) for protein loss assessment in correlative analyses.
- **CD58**: Investigational; mandatory fresh biopsy preferred; MEM-63 clone IHC.

Central Laboratory Requirement: All CDx testing for primary stratification (*CREBBP*, *EZH2*) must be performed at pre-qualified central laboratories with ISO 15189 accreditation prior to randomisation. Local results are not acceptable for stratification; however, local testing may trigger screening to reduce screen failure rates.

4.4 Statistical Analysis Plan Design Elements

4.4.1 Primary Endpoint and Hypothesis

The primary analysis tests superiority of the investigational CD20×CD3 bispecific antibody versus the comparator arm (e.g., rituximab + GemOx, as in NCT04408638) in the co-primary endpoints:

1. **Progression-Free Survival (PFS)** in the enriched subgroup (*CREBBP*-mut OR *EZH2*-mut): Two-sided log-rank test, $\alpha_1 = 0.025$
2. **Overall Survival (OS)** in the intent-to-treat (ITT) population (CD20$^+$ R/R DLBCL, $\geq$2L): Two-sided log-rank test, $\alpha_2 = 0.025$

Multiplicity is controlled using a parallel gate-keeping procedure with Hochberg correction [Nowakowski et al., 2021]. The enriched subgroup PFS must demonstrate significance before OS in the ITT population can be formally tested.

4.4.2 Secondary Endpoints and Biomarker-Stratified Analyses

Pre-specified secondary and exploratory analyses include:

- ORR and CR rate in the enriched subgroup (assessed by blinded IRC per Lugano 2014 criteria)
- PFS and OS in *CREBBP*-only and *EZH2*-only subgroups (interaction hypothesis)
- PFS in *TP53*-mutant versus *TP53*-wildtype (prognostic analysis, not enrichment)
- Correlation of *B2M*/*CD58* protein expression with ORR (exploratory, no multiplicity adjustment)

- CRS rate by tumour burden quartile and by *TP53* mutation status (safety correlative)
- ctDNA dynamics (variant allele frequency kinetics) as a pharmacodynamic biomarker in patients enrolled at ctDNA-qualified sites

4.4.3 Interim Analysis Plan

A pre-specified interim analysis is planned at 60% of PFS events in the enriched subgroup, using the Lan–DeMets O'Brien–Fleming alpha-spending function for type I error preservation. At interim, the DSMB will review: (1) futility for enriched subgroup PFS, (2) safety and CRS profile, and (3) exploratory biomarker signal strength (B2M, CD58).

4.5 Regulatory Alignment and Biomarker Qualification Plan

This Phase III SAP design aligns with:

- **FDA Guidance**: "Enrichment Strategies for Clinical Trials" (January 2019); "Developing Targeted Therapies in Low-Frequency Molecular Subgroups" (January 2018); "NGS Assay Analytical Validation" (2021) [U.S. Food and Drug Administration, 2019]
- **EMA**: ICH E17 Multi-Regional Clinical Trials guideline; EMA Reflection Paper on CDx Co-development (2020) [European Medicines Agency, 2020]

A Biomarker Qualification Request (BQR) should be submitted to FDA/CDER for *CREBBP* mutation as a predictive enrichment biomarker in DLBCL bispecific trials, supported by retrospective analysis of *CREBBP* mutation status in prior rituximab-containing regimen datasets and the Phase I/II bispecific trial correlative cohorts described here.

5 Discussion

5.1 Multi-Omic Convergence Supports CREBBP and EZH2 as Lead Biomarkers

The results of this multi-platform analysis converge on *CREBBP* and *EZH2* as the highest-priority predictive biomarker candidates for patient stratification in CD20×CD3 bispecific antibody trials. This conclusion is supported by four independent lines of evidence: (1) high mutation prevalence in DLBCL and FL (12.4% and 6.0% respectively in DLBCL; up to 40% and 20% in FL), ensuring a clinically meaningful and screenable patient fraction; (2) functional dependency signals from DepMap CRISPR data demonstrating essential roles for both genes in B-cell lymphoma cell viability, particularly in CD20-high lines; (3) established disease–gene association scores in Open Targets supporting clinical relevance; and (4) a statistically significant co-mutation pattern (OR 3.04, FDR $q = 0.004$) suggesting the two

biomarkers define an overlapping but biologically distinct high-risk epigenetic deregulation subgroup.

The biological mechanism linking *CREBBP–EZH2* co-deregulation to potential differential response to bispecific antibodies is mechanistically plausible, if not yet clinically proven. Both genes regulate the epigenetic landscape at promoters of immune genes, including MHC class II genes (*HLA-DRA*, *HLA-DRB1*) and co-stimulatory molecules. *CREBBP* loss reduces histone acetyltransferase activity and suppresses IRF4-dependent gene expression programmes, potentially reducing tumour immunogenicity [Pasqualucci et al., 2011, Mlynarczyk et al., 2019]. Conversely, *EZH2* gain-of-function mutations through H3K27 trimethylation broadly silence immune modulatory genes, potentially including those required for productive T-cell engagement [Zhang et al., 2023]. The hypothesis that reversal of this epigenetic suppression—through EZH2 inhibition—could synergise with T-cell bispecific antibody therapy is an active area of preclinical and clinical investigation.

5.2 Immune Evasion Biomarkers: B2M and CD58 as Negative Predictive Candidates

B2M and *CD58* represent mechanistically important negative predictive biomarkers deserving prospective evaluation despite their lower composite scores. Loss of *B2M* abrogates MHC class I surface expression, disrupting the immunological context in which T-cells operate even when recruited by a bispecific antibody. While the bispecific antibody mechanism does not require MHC class I for T-cell activation per se, loss of antigen presentation context may reduce the immunological synapse stability and T-cell persistence in the tumour microenvironment. Similarly, *CD58* loss removes a critical adhesion and co-stimulatory signal for CD2-expressing T-cells, impairing the efficiency of target cell lysis [Challa-Malladi et al., 2011]. Both alterations have been associated with immune escape in CAR-T therapy—a related T-cell redirection modality—and the analogous resistance mechanism is plausible for bispecific antibodies [Majzner and Mackall, 2018].

The relatively low literature evidence scores for *B2M* and *CD58* in this analysis likely reflect a temporal lag: the bispecific antibody era is young, and correlative biomarker analyses from clinical trials are only beginning to be reported. We expect the evidence base for *B2M* and *CD58* as negative predictive biomarkers to grow substantially with planned ASH 2025 and ASCO 2026 abstract submissions from ongoing correlative substudies.

5.3 Safety Biomarker Hypothesis: CRS, Tumour Burden, and Genomic Subtypes

The FAERS analysis confirming CRS as the predominant safety signal (31.4% of glofitamab reports; 21.1% of mosunetuzumab reports) motivates the development of genomic risk stratification tools for CRS prediction. High tumour burden is the most consistently identified clinical risk factor for severe CRS in bispecific antibody trials, and genomic features associated with aggressive disease—*TP53* mutation, BCL2/MYC double-hit rearrangements, EZH2-mutant GCB-DLBCL—may serve as surrogate risk factors. The Phase III SAP should include a pre-specified analysis correlating CRS severity (grade ≥3 per CTCAE v5.0) with tumour bulk quartile, *TP53* mutation status, and cell-of-origin classification.

5.4 Limitations and Future Directions

Several limitations of this analysis warrant acknowledgement. First, the DepMap analysis was constrained to the 113 B-cell lymphoma cell lines with complete CRISPR data in DepMap 26Q1; the statistical power to detect CD20-stratified differential dependencies is therefore limited. Future analyses should incorporate larger patient-derived primary cell datasets and patient-derived xenograft (PDX) models. Second, the clinical trial analysis is limited to published data without access to individual patient-level biomarker profiles; the absence of genomic subgroup analyses in published reports represents the primary evidence gap motivating this Phase III SAP. Third, the biomarker scoring framework uses empirically chosen weights (30/30/20/20), and sensitivity analyses with alternative weighting schemas should be conducted. Fourth, the FL biomarker landscape was less extensively covered given the DLBCL focus of the cBioPortal cohort; dedicated FL-specific analyses incorporating the FL-specific high mutation frequency of *CREBBP* (40%) and *EZH2* (20%) are needed. Fifth, the transition from hypothesis to clinical validation requires prospective biomarker collection, central testing, and pre-specified statistical analysis within the Phase III framework described.

The discovery and clinical validation of predictive biomarkers for CD20×CD3 bispecific antibodies will be a defining challenge of the next 3–5 years in lymphoma oncology. The integrative framework presented here provides a scientifically grounded and regulatory-aligned starting point for this effort.

6 Conclusions

This comprehensive multi-omic white paper presents an integrated analytical framework for predictive biomarker discovery in the context of CD20×CD3 T-cell engaging bispecific antibody therapy for R/R DLBCL and follicular lymphoma. Through systematic integration

of genomic co-occurrence data, CRISPR functional dependency profiles, target tractability assessments, clinical trial efficacy data, and post-marketing safety surveillance, the analysis supports the following principal conclusions:

1. ***CREBBP* and *EZH2* mutations are the highest-priority predictive biomarker candidates**, supported by the highest composite evidence scores (0.682 and 0.675 respectively), significant co-mutation enrichment (OR 3.04, FDR $q = 0.004$), and biologically coherent epigenetic deregulation mechanisms relevant to T-cell immunotherapy response.
2. **The *CREBBP*-mut OR *EZH2*-mut enriched subgroup** comprises approximately 17.7% of R/R DLBCL patients (≈2,207 eligible US patients/year), enabling a statistically powered Phase III enrichment design with co-primary PFS endpoint.
3. ***TP53* mutation** should be incorporated as a secondary stratification factor to balance randomisation arms, given its high prevalence (10.9%) and established adverse prognostic impact on OS.
4. **CD20 (MS4A1) IHC positivity** (≥20% tumour cells) must be maintained as a mandatory eligibility criterion, with Tier 1 approved CDx assays (SP11, L26) to be specified in the protocol.
5. ***B2M* and *CD58*** are nominated as exploratory negative predictive biomarkers, with mandatory archival tissue collection and centrally evaluated IHC recommended for all Phase III patients.
6. **CRS**, as the dominant safety signal in both FAERS datasets, should be prospectively correlated with tumour burden and genomic risk subgroup in Phase III, with dedicated risk management protocols for patients with ≥2 high-burden risk factors.
7. **The Phase III CDx co-development strategy** should leverage the existing FDA-approved cobas EZH2 assay (Tier 1, immediate deployment) and develop a novel *CREBBP* LDT with ≥5% VAF sensitivity (Tier 2, requires analytical validation prior to Phase III initiation).
8. **A Biomarker Qualification Request (BQR)** to FDA/CDER for *CREBBP* as a predictive enrichment biomarker in DLBCL bispecific trials is recommended, leveraging retrospective rituximab-era DLBCL cohort data as supporting evidence.

Phase III SAP Implementation Checklist

- ☐ Protocol amendment incorporating CREBBP/EZH2 co-primary stratification and TP53 secondary stratification

- ☐ CREBBP LDT analytical validation package (precision, accuracy, LOD; tissue and ctDNA)
- ☐ EZH2 cobas label supplement submission to FDA (bispecific indication)
- ☐ Central laboratory agreement with ISO 15189-accredited NGS facility
- ☐ Statistical analysis plan section: enriched subgroup PFS as co-primary endpoint (alpha 0.025, Hochberg gate-keeping)
- ☐ Pre-specified interim analysis plan at 60% PFS events (O'Brien–Fleming spending)
- ☐ Mandatory archival FFPE tissue collection for all enrolled patients (B2M, CD58 IHC)
- ☐ BQR submission to FDA/CDER for CREBBP biomarker qualification
- ☐ CRS risk stratification protocol with pre-specified correlative analysis by tumour burden and TP53 status

The convergent multi-omic evidence presented here provides the strongest available scientific foundation for an evidence-based, regulatory-aligned biomarker strategy in the next generation of pivotal CD20×CD3 bispecific antibody trials. Implementation of this framework has the potential to identify the patient subgroup most likely to achieve durable complete remissions—advancing precision oncology in B-cell lymphoma beyond the current empirical response rate paradigm.

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Data and Code Availability

All analysis scripts, result files, and figure generation code are available in the project repository. Primary data were obtained from publicly available databases as cited. Intermediate result files (CSV, JSON) and visualisation scripts (Python 3.12, matplotlib, seaborn, networkx) are available upon request.

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