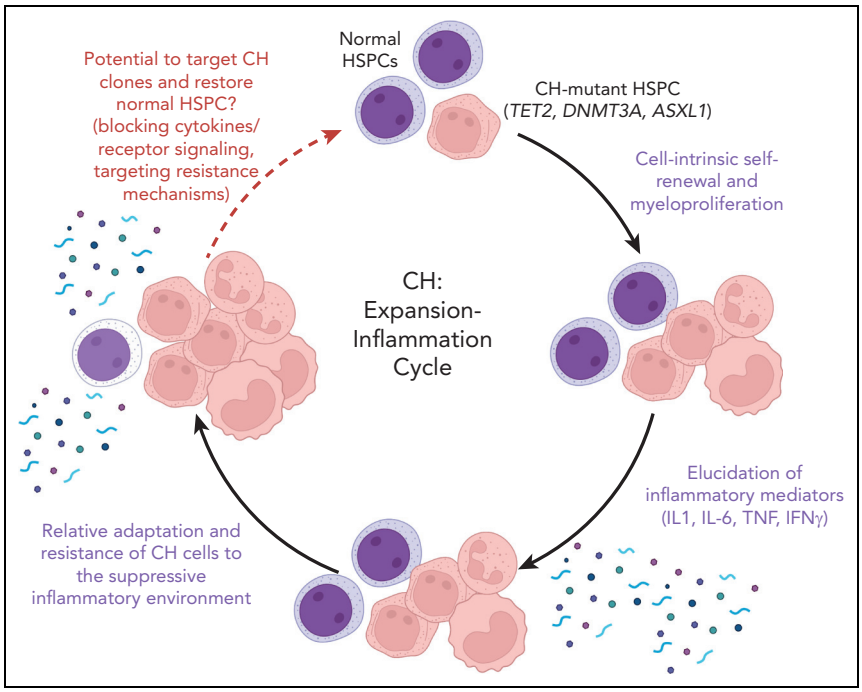

The CH expansion-inflammation cycle. Age-associated CH is most commonly driven by somatic mutations in HSPCs, involving the epigenetic regulator genes *TET2*, *DNMT3A*, and *ASXL1* (top). Enhanced fitness imparted by increased self-renewal leads to CH-mutant HSPC expansion and myeloproliferation (right). In turn, and in the context of aging, this leads to increased and chronic expression of proinflammatory cytokines and other mediators that alter the hematopoietic milieu (bottom). While this environment suppresses normal HSPC, gene expression and other adaptations in CH-mutant HPSC lead to a vicious cycle of enhanced CH cell fitness and inflammation (left). This environment may increase the risk of cancer and exacerbate diseases associated with inflammation. The findings of Caiado et al in this issue of *Blood* (and other recent publications) suggest that targeting inflammatory cytokines, associated receptors, signaling, or other adaptive mechanisms may break this vicious CH cycle and decrease the risk of cancer and comorbid inflammatory diseases. Figure created with BioRender. IFN-γ, interferon gamma; TNF, tumor necrosis factor.

resistance to inflammation and enhances the fitness of *asxl1*-mutant zebrafish HSPC.[8]

It remains to be determined whether breaking the cycle of CH and inflammation (see figure) will prove to be effective in controlling CH clones and clinical outcomes in humans. Patients with previous myocardial infarction and *TET2*-mutant CHIP may experience less adverse cardiac events when treated with an anti–IL-1β antibody.[9] Another study involving 3 macaque nonhuman primates with established *TET2*, *DNMT3A*, and *ASXL1* CH clones treated with a monoclonal antibody that blocks IL-6 signaling found a specific slowing of *TET2*-mutant clones, although the growth rate rebounded following treatment discontinuation.[10]

The next important steps are to examine the effect of anti-inflammatory therapy on CH clonal dynamics in humans. Longitudinal cohorts offered anti-inflammatory therapy as part of the standard of care for inflammatory diseases, with serial blood sampling, will permit the analysis of changes in CH clones. It is also unclear whether anti-inflammatory or other therapies will be effective in controlling established clones, better suited to prevent clone emergence, or if caution is necessary to prevent the outgrowth of other or resistant mutants. These are important considerations for future prospective CH studies and clinical trials, but the findings of Caiado et al and others offer promising targets for breaking the vicious inflammation-expansion cycle and decreasing the risk of cancer and CH-associated comorbidities.

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LYMPHOID NEOPLASIA

Comment on Thomas et al, page 904

Meet the Burkitts: a dark zone family

Adam J. Olszewski | Alpert Medical School of Brown University and Rhode Island Hospital

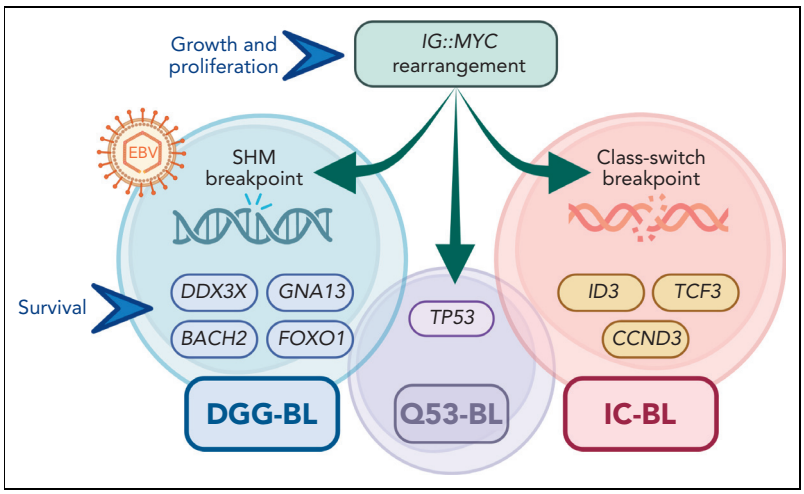
In this issue of *Blood*, Thomas et al[1] present a whole-genome sequencing analysis of 230 pediatric and adult Burkitt lymphomas (BLs). The researchers

wished to discover genomic subgroups within this rare disease by feeding data on somatic mutations, copy number alterations, and structural variants into a consensus clustering algorithm. Previously, this approach established 5 subtypes of diffuse large B-cell lymphoma (DLBCL) with compelling biological and clinical differences.[2] In BLs, the result is less clear-cut, and our imperfect diagnostic criteria may be largely to blame. Although some clusters emerged from the BL cases, the researchers obtained a more robust classification after adding and comparing 295 DLBCL genomes to the BL genomes.

Consistent with prior experience from gene expression profiling (GEP) studies, quite a few histologically confirmed BL tumors turned out to group with DLBCL and vice versa. The 3 main identified subtypes (termed DGG-BL [defined by mutations in *DDX3X*, *GNA13*, *GNAI2*, *BACH2*, and *FOXO1*], IC-BL [defined by mutations in *ID3*, *TCF3*, and *CCND3*], and Q53-BL [defined by mutations in *TP53*]; see figure) illustrate various genomic pathways that allow BL cells to overcome vulnerability to cell death under the common *MYC*-driven cell growth and proliferation program. We can also glimpse hints of prognostic relevance for specific DNA alterations.

BL seems deceptively homogeneous: it has the nearly universal *IG::MYC* translocation, a monotonous morphology, and a simple karyotype, and it is consistently responsive to intensive, unsophisticated blasts of chemotherapy.[3] Historical classifications of BL used patient age or epidemiologic attributes, distinguishing the endemic, immunosuppression-associated, and sporadic variants. Apart from the striking association between the endemic variant and an EBV infection, these approaches provided little insight to influence treatments or patient outcomes. Prognosis and therapeutic decisions rely on gross indicators of disease burden and have not taken advantage of any molecular factors.[4] Uncovering driver alterations that could help replace cytotoxic agents with more targeted therapeutic approaches is a high research priority in BL. In this context, the utility of genomic subclassification in BL will depend on whether it can help us understand the clinical and epidemiologic associations, clarify the diagnostic conundrums, and inform prognostication or treatment.

Previous studies have treated BL as a fixed entity separable from DLBCL, with frequent mutations in genes involved in the germinal center dark zone program (*TCF3*, *ID3*, *CCND3*, and *FOXO1*). The EBV status proved more relevant for genomic profiling than age or epidemiologic context.[5,6] As identified in this study, 72% of DGG-BL tumors were EBV positive. Consequently, they showed a relatively high mutation load related to overexpression of activation-induced cytidine deaminase and aberrant SHM. SHM-related *IG::MYC* breakpoints were also more common in EBV-positive cases, even though class-switch recombination had been considered the predominant mechanism for such breakpoints in BL.[7] The most perplexing finding is that tumors classified as IC-BL unexpectedly overexpressed *IRF4* and *TNFRSF13B* as well as gene sets associated with post–germinal center phenotypes. The clinicopathologic significance of this discovery awaits exploration. The differential therapeutic vulnerability of the DGG-BL and IC-BL subtypes to PI3K signaling inhibition or immunomodulation is an intriguing possibility.



Main discerning features of the BL genomic subgroups: rearrangements between *MYC* and *IG* genes are universal, but the SHM breakpoints are more common in DGG-BL, which is strongly associated with EBV infections; DGG-BL and IC-BL acquire separate sets of additional mutations that act in concert with the *MYC*-induced cell growth and proliferation program; *TP53* mutations occur frequently in all subtypes, but a small subset (termed Q53-BL) lacks any additional driver mutations. IG, immunoglobulin; SHM, somatic hypermutation; EBV, Epstein-Barr virus.

The genomic subgroups of BL are perhaps best thought of as segregated pathogenic mechanisms that give rise to a common phenotype, rather than different diseases. The double-hit lymphoma story illustrates that high-grade B-cell lymphoma (HGBL) biology requires 2 concurrent genomic hits: one to facilitate uncontrolled proliferation (eg, *IG::MYC*) and another to provide protection from cell death (eg, *BCL2::IGH*). We can now discern that in BL, this second survival-promoting hit can be acquired through several pathways: the *TCF3/ID3* axis supporting constitutive PI3K signaling (IC-BL), *DDX3X*-mediated attenuation of proteotoxic stress (DGG-BL), or even simple inactivation of p53 (Q53-BL). These mechanisms are not mutually exclusive, as evidenced by substantial overlap in the relevant gene mutations among the subgroups. *TP53* mutations are common in all 3 BL subtypes and are overrepresented in relapsed disease, potentially contributing to treatment refractoriness regardless of the mutational profile. Nevertheless, the quiet Q53-BL subgroup intriguingly lacks any driver mutations beyond those in *MYC* and *TP53*, and it does not show the genomic complexity typically associated with p53 aberrations (in contrast to the aneuploid A53 subtype of DLBCL).

Approximately 9% of DLBCL tumors (as well as most BLs whose diagnosis were rejected upon central review) were classified within the BL genomic clusters. This finding replicates prior GEP experience and contributes to our understanding of the entire spectrum of

mature HGBL. HGBLs include BL, double-hit lymphomas, HGBL with 11q aberration, and the rare HGBL, not otherwise specified, as well as perhaps 10% to 15% of tumors currently diagnosed as DLBCL. Despite the confusing range of histologic patterns, these aggressive lymphomas seem to use consistent sets of mutated transcription factors to hijack mechanisms that operate within the germinal center dark zone. The emerging GEP and genomic classifiers need translation into trials that would separate the HGBL-like tumors from DLBCL to identify more efficacious treatments and mitigate their refractoriness to the standard-intensity chemotherapy.

The study by Thomas et al does not paint an easily interpretable picture of prognostic relevance for the discovered BL subgroups. The overall survival of the pediatric and adult patients included in the study exceeded 80%, much higher than observed in clinical practice in either North America or Europe.[4] In aggregate, outcomes did not differ between DGG-BL and IC-BL, and borderline (and contradictory) associations from the pediatric and adult cohorts are hard to interpret with as few as 5 to 8 patients in the subgroups and the lack of stratification in baseline variables or treatment. Nevertheless, the notable prognostic disadvantage for adult BL carrying *TP53* mutations provides a hint that sequencing data might add value to clinical prognosis. The constellation of *MYC* rearrangement with *TP53* mutation, regardless of histology, may define one of the worst categories of HGBL.[8] Future research should include more cases of disseminated, extranodal BL, which is often diagnosed using blood, bone marrow, or cerebrospinal fluid. Such tumors carry significantly worse prognosis in both experimental and observational cohorts[4,9,10] but may be underrepresented in sequencing studies due to unavailability of archival paraffin-embedded nodal tissue. Analysis of prospectively collected samples from patients who are uniformly treated on clinical trials will be the next critical step to examine the prognostic impact of specific genomic alterations in BL and the practical relevance of the subtypes.

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MYELOID NEOPLASIA

Comment on Pecquet et al, page 917

CALR goes rogue

Johanna Melo-Cardenas and John D. Crispino | St. Jude Children's Research Hospital

In this issue of *Blood*, Pecquet et al[1] report that hematopoietic cells with a mutation in calreticulin (*CALR*) secrete a soluble form of the protein that acts in paracrine fashion to enhance the growth of surrounding tumor cells.

The BCR-ABL-negative myeloproliferative neoplasms (MPNs) are caused by mutations in the kinase JAK2, the thrombopoietin receptor MPL (also known as TpoR), and the endoplasmic reticulum (ER) chaperone CALR.[2] Although it was readily apparent that *JAK2* and *MPL* mutations promote cytokine independent growth through activation of the JAK/STAT signaling pathway, how mutations in *CALR* contribute to the MPNs was not obvious. CALR is involved in the quality control of newly synthesized proteins and glycoproteins that prevent misfolded proteins from leaving the ER prematurely. CALR is also involved in the regulation of intracellular calcium levels, integrin signaling, and loading of antigens onto the major histocompatibility complex. CALR can be found on the cell surface where it initiates prophagocytic signals and mediates immunostimulatory effects. Mutations of *CALR* in the MPNs result in an altered C terminus that selectively binds TpoR in the ER and leads to its activation independent of its ligand thrombopoietin.[3] Mutant CALR is then transported to the cell surface along with TpoR, where it leads to JAK/STAT pathway activation and promotes malignant growth. Glycosylation of TpoR is necessary for the CALR binding through its lectin binding domain, and this former modification was recently shown to be a therapeutic vulnerability in CALR mutant MPNs.[4] In addition to binding TpoR, previous reports showed that mutant CALR is also found as a soluble form.[5-7]

Pecquet et al sought to understand the biological function of soluble mutant