● ● ● **LYMPHOID NEOPLASIA**

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Recurrent mutations and targeted therapy in FL

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In this issue of *Blood*, Krysiak et al expand our understanding of the genetic events which drive follicular lymphoma (FL), and provide insights into potential applications of targeted therapy.[1]

FL is a pathologically and clinically heterogeneous disease. For the majority of patients, FL presents at advanced stage with multifocal lymph node involvement, and follows an indolent natural history with a favorable prognosis. In the modern era, most patients diagnosed with FL will have a life expectancy similar to age-matched controls, whereas ~20% will experience a more aggressive course and ultimately die of their disease.[2] Unique FL variants have also been identified with distinct clinical presentations, pathologic findings, and natural histories. These include pediatric-type FL and primary intestinal FL, each of which has been found to be biologically distinct from typical nodal FL cases.[3,4] The t(14;18) is present in the majority of FLs and serves as a defining molecular feature. This translocation, which results in constitutive expression of the antiapoptotic BCL2 protein, appears to be an early genetic event that alone is insufficient to result in lymphomagenesis. The development of lymphoma, therefore, is driven by a diverse collection of additional genetic events, the types of which determine the clinical and biologic heterogeneity within this disease.

Krysiak et al performed whole-exome sequencing on a training set of 24 FL biopsy specimens and, based on mutations identified in this cohort, along with those in the existing literature, proceeded to sequence 1716 genes in 113 FL biopsies drawn from 105 individual patients, most of whom were treatment naive. They identified 39 recurrently mutated genes, some of which have been previously identified, whereas others represent novel findings.

Newly identified mutations which warrant further investigation for their role in follicular lymphomagenesis include *EGR1/2*, *POU2AF1*, *ZNF608*, and *HVCN1*. Among previously identified mutations in FL, the investigators confirmed that histone modifiers are among the most commonly mutated genes, including *MLL2*, *CREBBP*, *EP300*, *EZH2*, and *MEF2B*. Additionally, 44% of cases had at least 1 mutation in 1 of 25 histone genes. These speak to a likely critical role for epigenetic regulators in FL pathogenesis. Frequent mutations of histone-modifying genes also suggest promise for epigenetically targeted therapies in FL. To date, the histone deacetylase (HDAC) inhibitors vorinostat and abexinostat have both shown encouraging activity in relapsed/refractory FL.[5,6] Similarly, tazemetostat, an inhibitor of the recurrently mutated *EZH2*, has shown early evidence of activity in an ongoing phase 1 study.[7]

Another interesting finding is that ~40% of patients had mutations affecting the B-cell receptor (BCR) signaling pathway. These included previously identified recurrent mutations affecting *CD79B*, *CARD11*, and *CXCR4*, as well as *BTK* mutations in the tyrosine kinase domain. Multiple other critical components of the BCR pathway were also found mutated at lower levels, including *CD22*, *BLNK*, *PLCγ2*, *BCL10*, and *NFκB2*. These findings support a role for constitutive BCR signaling as a component of FL pathogenesis in some cases, and also have implications for therapy. The BCR pathway and Bruton tyrosine kinase (BTK) specifically have emerged as critical therapeutic targets in B-cell lymphomas, with US Food and Drug Administration (FDA) approvals for the BTK inhibitor ibrutinib in chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), and Waldenstrom macroglobulinemia (WM). In these diseases, the majority of patients with relapsed or refractory disease respond to ibrutinib, whereas development of resistance has been tied to mutations of *BTK* and *PLCγ2*.[8] Despite evidence of recurrent BCR pathway mutations in a large proportion of patients with FL, the activity of ibrutinib in this disease has been decidedly more modest than in CLL, MCL, or WM, with an overall response rate of only 30%.[9] Krysiak et al provide potential explanations for this. First, fewer than half of FLs have activating mutations within the BCR pathway, and are therefore likely to be driven by other mutations which confer proliferative and survival advantage that would not be targeted with ibrutinib. Among FL cases that are driven by BCR signaling, multiple mutations have now been identified which could confer ibrutinib resistance, including *BTK*, *PLCγ2*, *CARD11*, and *NFκB*. A similar phenomenon has been observed in diffuse large B-cell lymphoma (DLBCL) where the activated B-cell (ABC) type of DLBCL relies on chronic active BCR signaling which may render sensitivity to ibrutinib. The response rate within ABC-DLBCL in a phase 2 study was 37%, with *CARD11* mutations, among others, being identified as potential mechanisms of resistance.[10]

These findings highlight that our application of targeted therapies in FL is still in its infancy. Although ibrutinib clearly has meaningful clinical activity in a subset of FL cases, determination of sensitivity cannot be predicted by histology alone. Rather, to select appropriate candidates for this therapy, we will need to identify cases driven by BCR signaling and that lack resistance mutations in *BTK*, or activating mutations in downstream signaling molecules. Similarly, the abundance of mutations in histone-modifying genes, along with early clinical activity of HDAC and EZH2 inhibitors, warrants further investigation as to which genes are being selectively modified by these agents to confer sensitivity so that optimal candidates for such therapy can be identified a priori. We will also need to identify recurrent genetic events enriched in patients who are at risk of early progression and ultimately dying of their disease, so they can be selected for relevant targeted therapies. As additional novel agents emerge in FL which target epigenetic modifiers, oncogenic signaling pathways, and the immune microenvironment, it will be critically important to understand the genomic substructure of a given patient in order to select among the available options with distinct

mechanisms of action. In so doing, we can look forward to entering the era of personalized medicine in FL.

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● ● ● **PLATELETS AND THROMBOPOIESIS**

Parsing the repertoire of GPIb-IX-V disorders

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In this issue of *Blood*, Sivapalaratnam et al[1] report an association between unique, rare monoallelic variants in *GP1BB*, which encodes the glycoprotein (GP)Ibβ subunit of the platelet GPIb-IX-V complex, and autosomal dominant macrothrombocytopenia.

Platelet GPIb-IX-V is a major player in hemostasis, serving as the receptor for von Willebrand factor (VWF) and mediating the platelet–subendothelium interactions. Deficiencies of this multiprotein complex or the plasma VWF result in a bleeding diathesis. The GPIb-IX-V complex, expressed on megakaryocytes and platelets, contains 4 distinct transmembrane subunits, GPIbα, GPIbβ, GPIX, and GPV (see figure).[2] Each subunit is a distinct gene product.[2]

The best recognized inherited bleeding disorder involving the GPIb-IX-V complex is the Bernard-Soulier syndrome (BSS), an autosomal recessive disorder arising from homozygous or compound heterozygous variants of *GP1BA*, *GP1BB*, and *GP9*.[3] In 1948, 2 French physicians, Jean Bernard and Jean-Pierre Soulier, first reported a severe bleeding disorder associated with thrombocytopenia and giant platelets, later shown to be characterized by decreased ristocetin-induced platelet agglutination.[3,4] To date, 45, 39, and 28 variants in *GP1BA*, *GP1BB*, and *GP9*, respectively, have been identified in the BSS.[4] *GP5* variants have not been implicated in the BSS. In contrast to these loss-of-function variants, some *GP1BA* variants have conferred an increase in affinity of the platelet complex for VWF, resulting in the platelet-type vWD associated with a secondary decrease in plasma high molecular weight VWF multimers.[3]

More recently, an association between monoallelic variants in *GP1BA* and an autosomal dominant macrothrombocytopenia has emerged (see figure).[4-6] The most frequent of these is the *GP1BA* variant (p.Ala172Val), labeled as the "Bolzano" variant, associated with mild thrombocytopenia and increased platelet volume, with or without mild bleeding symptoms.[4-6] Three other monoallelic *GP1BA* variants have also been implicated.[4] Although *GP1BB* variants have been linked to autosomal dominant macrothrombocytopenia in 3 Japanese families,[1,4] this association is not well established.

Sivapalaratnam et al extend the repertoire of GPIb-V-IX complex-related disorders by documenting a statistical association between rare nonsynonymous monoallelic variants in *GP1BB* and autosomal dominant macrothrombocytopenia. Their approach used high-throughput whole-genome DNA sequencing coupled with human phenotype ontology coding of clinical and laboratory phenotypes. The study population consisted of 1 discovery and 2 validation cohorts. The discovery cohort comprised 1542 patients with a suspected inherited bleeding or platelet disorder with unknown molecular etiology, or their relatives, enrolled in the National Institute for Health Research BioResource. One validation cohort contained 75 patients with thrombocytopenia evaluated with the ThromboGenomics gene panel, whereas another contained 301 patients from a Japanese cohort with suspected inherited thrombocytopenia and their relatives. Within the discovery cohort, there was a strong association identified between rare (allele frequency <1/10 000) nonsynonymous monoallelic *GP1BB* variants and macrothrombocytopenia, with a family history suggestive of autosomal dominant inheritance. Eight probands with *GP1BB* variants were identified from the discovery cohort; they did not have other genetic variants previously implicated in macrothrombocytopenia. In the validation cohorts, 10 further *GP1BB* variants were identified, for a total of 18 probands (27 total cases), with 9 distinct *GP1BB* variants across cohorts.

Patients with *GP1BB* variants had platelet counts ranging from 47 to 172×10^9/L (mean, 108×10^9/L) and increased platelet volumes (10.7-14.3 fL; mean, 12.74 fL). Macrothrombocytopenia was defined as a platelet count below 150×10^9/L and mean platelet volume above 12 fL. Patients with platelet anisocytosis with a subset of abnormally large platelets were also considered