potential patient population for this treatment to under 2% of those with AML. Moreover, therapies based on TCRs are potentially vulnerable to immune evasion tactics, such as the reduction of target antigens or the genes of MHC classes I and II. Despite these obstacles, T cells that target the $IDH2^{R140Q}$ mutation hold substantial promise as an innovative treatment strategy for AML, potentially revolutionizing the way the disease is combated.[7]

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LYMPHOID NEOPLASIA

Comment on Abdelbaky et al, page 1752

New signature predicts MBL-to-CLL progression

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In this issue of *Blood*, Abdelbaky and colleagues report the development of a combined epigenetic and immunogenetic signature to study individuals with high-count monoclonal B-cell lymphocytosis (HC-MBL).[1] Using this signature, they define a new high-risk group with significantly increased likelihood of progression to chronic lymphocytic leukemia (CLL) compared with low-risk individuals.

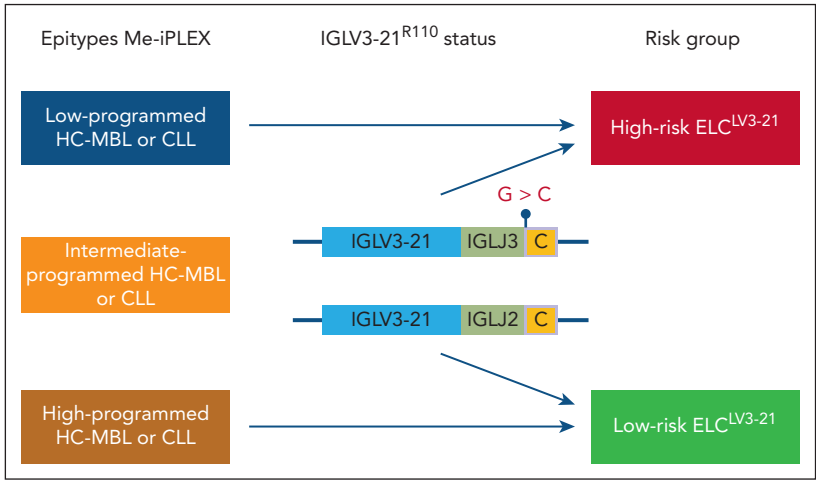
Twenty-five years ago, 2 seminal articles were published in *Blood*, fundamentally altering the landscape of CLL research.[2,3] These studies demonstrated that patients with CLL with somatically hypermutated immunoglobulin heavy variable (IGHV) genes (M-CLL; 60%-70% of patients) experienced better outcomes than patients with unmutated IGHV genes (U-CLL; 30%-40% of patients). Over time, the IGHV gene mutational status has been established as a robust prognostic and predictive marker. As a result, contemporary guidelines recommend IGHV gene analysis for all patients before initiating treatment.

DNA methylation arrays revealed early on that U-CLL and M-CLL display distinct methylation profiles. In a pivotal study from 2012, Kulis et al compared the methylation signature in CLL with different B-cell differentiation stages.[4] This led to the identification of 2 major "epitypes," one resembling naive B cells, largely corresponding to U-CLL (termed naive B-cell-like CLL), and the other more similar to memory B cells, overlapping with M-CLL (designated memory B-cell-like CLL). An intermediate epitype (i-CLL) with an intermediate methylation pattern and a corresponding intermediate outcome was also identified. In a similar study, based on the level of DNA methylation programming, Oakes et al also identified 3 prognostic subgroups, which were named the high-programmed (HP), intermediate-programmed (IP), and low-programmed (LP) epitypes.[5]

Further characterization of the intermediate epitype revealed an enrichment of CLL cases with borderline IGHV mutational status (97%-97.9% identity) or *SF3B1* mutations or belonging to stereotyped subset 2.[6,7] Subsequently, Maity et al identified a critical somatic mutation (G>C) in the IGLV3-21 gene at position R110 in subset 2, crucial for homeotypic B-cell receptor interaction.[8] Extended analysis showed that nonsubset 2 cases with the $IGLV3\text{-}21^{R110}$ mutation had a similarly poor prognosis as subset 2. Furthermore, a recent study suggested that intermediate patients can be regrouped depending on $IGLV3\text{-}21^{R110}$ status; patients with $IGLV3\text{-}21^{R110}$-mutated i-CLL demonstrated a poor outcome similar to patients with naive B-cell-like CLL, and patients with $IGLV3\text{-}21^{R110}$-wildtype i-CLL had a similar prognosis as patients with memory B-cell-like CLL.[7]

Healthy individuals may harbor clonal B-cell expansions of varying sizes, termed monoclonal B-cell lymphocytosis, a precursor condition always preceding CLL.[9] The likelihood of MBL increases with age and in patients with first-degree relatives with CLL. MBL can be categorized into low-count MBL ($<0.5 \times 10^9$ clonal B cells per L) and HC-MBL ($>0.5 \times 10^9$ to $<5 \times 10^9$ per L). Although the majority of HC-MBL cases exhibit favorable-prognostic features (IGHV-mutated and low-risk genomics), an estimated 1%-2% of individuals with HC-MBL will develop CLL requiring treatment.[1,9] Considering the relatively low proportion of HC-MBL cases developing high-risk disease, identifying new biomarkers is crucial for early-stage identification of those at high risk of progression.

In the study by Abdelbaky et al, information from DNA methylation profiling was



The combined epigenetic and immunogenetic $ELC^{LV3\text{-}21}$ signature. Using the Me-iPLEX methylation assay, HC-MBL and CLL were divided into LP, IP, and HP epitypes. The IGVL3-21^{R110} status was assessed by sequencing in IP MBL/CLL. IP cases carrying the IGVL3-21^{R110} mutation were grouped with the LP epitype into the $ELC^{LV3\text{-}21}$ high-risk group, and IGVL3-21^{R110}-wild-type IP cases were merged with the HP epitype into the $ELC^{LV3\text{-}21}$ low-risk group.

combined with the assessment of the IGLV3-21^{R110} status to create a joint epigenetic and immunogenetic signature termed the epigenetic and light chain IGLV3-21 immunoglobulin ($ELC^{LV3\text{-}21}$) signature (see figure). Using their previously developed methylation-iPLEX (Me-iPLEX) assay,[6] which interrogates the methylation status of 7 loci, the majority of HC-MBL (65%) belonged to the good-prognostic HP epitype, and the IP epitype and LP epitypes were seen in 19% and 16% of individuals, respectively. Sequencing of the λ light-chain rearrangements revealed that 19% of individuals in the IP epitype carried the IGLV3-21^{R110} mutation. Notably, individuals with the IP epitype and the IGLV3-21^{R110} mutation had a similar time to CLL progression and time to first treatment (TTFT) as those with the LP epitype, leading to the grouping of these cases into an $ELC^{LV3\text{-}21}$ high-risk group. In multivariable analysis, including $ELC^{LV3\text{-}21}$ and IGHV mutation status, only the $ELC^{LV3\text{-}21}$ signature remained significant. Similarly, when including CLL-international prognostic index (IPI) and $ELC^{LV3\text{-}21}$, only $ELC^{LV3\text{-}21}$ retained its status as an independent risk factor.

Applying the $ELC^{LV3\text{-}21}$ model to an independent CLL cohort, the $ELC^{LV3\text{-}21}$ high-risk signature was again associated with inferior TTFT and overall survival (OS). In multivariable analysis, including IGHV mutation status, genomic aberrations, and CLL-IPI, $ELC^{LV3\text{-}21}$ maintained significance for TTFT, whereas the IGHV status no longer held significance. Importantly, patients with $ELC^{LV3\text{-}21}$ high-risk HC-MBL had shorter TTFT and OS than patients at low risk of developing CLL, more akin to patients at high risk of developing CLL.

In summary, the authors have identified a new signature to identify individuals with HC-MBL with a particularly high risk of progression to CLL, who also appear to have a shorter OS. Although this new prognostic signature is promising, further validation in independent data sets is crucial, especially considering the relatively low number of $ELC^{LV3\text{-}21}$ high-risk IP individuals included. An in-depth analysis comparing the benefit of the $ELC^{LV3\text{-}21}$ grouping versus only performing IGHV/IGLV gene sequencing is also critical. Moreover, given the diverse treatment regimens received by patients in the collected cohorts over the long time span of the study, the OS data may not be reflective of current treatment practice and might therefore be a less definite finding. Therefore, assessing the impact of the $ELC^{LV3\text{-}21}$ signature in more homogeneously treated cohorts, in particular in relation to targeted therapies such as Bruton tyrosine kinase and B-cell lymphoma 2 inhibitors, is essential.

From a methodological perspective, the authors assert that the Me-iPLEX is a rapid and cost-effective test. However, this assay is not widely used and would need standardization to be implemented in routine diagnostics. Given that other epityping assays include different loci, collaborative efforts among key stakeholders to define the relevant epitypes and nomenclature would be highly beneficial. As the authors point out, their approach can be adapted to next-generation sequencing-based assays, which would be an important development. In fact, using capture-based panel sequencing, it is possible to include epigenetic marks, along with assessment of oncogenic mutations. Although IGHV gene sequencing is performed in routine diagnostics, primarily using Sanger sequencing, the assessment of the IGLV3-21^{R110} mutation status is currently not part of routine practice. Nevertheless, it would be relatively straightforward to use targeted assays to detect the IGLV3-21^{R110} mutation or to incorporate this feature in a gene panel design.[10] Alternative approaches, such as long-read sequencing, enabling simultaneous analysis of genomic aberrations, and DNA methylation profiles, could also be considered.

Once validated, the $ELC^{LV3\text{-}21}$ signature would be expected to become a highly relevant and useful tool for identifying individuals with a very low risk of CLL progression, as well as high-risk individuals with MBL requiring closer monitoring.

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THROMBOSIS AND HEMOSTASIS

Comment on Dou et al, page 1758

LNKing eosinophilia and atherothrombosis

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In this issue of *Blood*, Dou et al[1] reveal a decisive role for hematopoietic lymphocyte adapter protein (LNK) deficiency in promoting eosinophilia, systemic eosinophil activation, and arterial thrombus formation. The authors use pharmacological and genetic eosinophil depletion to reveal that eosinophil-specific *LNK* deficiency exacerbates arterial thrombus formation through reciprocal eosinophil-neutrophil activation and extracellular trap (ET) formation.

Eosinophils protect the host against parasitic infections, but eosinophilia and hyperactivation of circulating eosinophils, as observed in autoimmune diseases like eosinophilic granulomatosis with polyangiitis, are associated with collateral self-damage. This is, at least in part, driven by a prothrombotic state, including clinically relevant increases in myocardial infarction and stroke.[2] Moreover, eosinophils are enriched in arterial thrombi from patients with stroke and myocardial infarction. Recent studies have highlighted mechanistic details into how eosinophils modulate cardiovascular disease including thrombosis and associated inflammation: although eosinophils have been shown to aggravate the formation of experimental atherosclerotic lesions and arterial thrombus formation,[3-5] they also have been shown to have beneficial effects on myocardial remodeling in the setting of chronic coronary artery ligation in a mouse model of myocardial infarction.[6] The effect of eosinophils may be different in the setting of myocardial ischemia-reperfusion injury. This dichotomous role of eosinophils in cardiovascular disease warrants further investigation in a context-dependent manner.

SH2B3/LNK is a negative regulator of Janus kinase (JAK)/signal transducer and activator of transcription proteins (STAT) signaling. Carriers of a single nucleotide polymorphism with functional loss of *SH2B3/LNK* have an increased risk for developing myeloproliferative disorders and are often identified due to elevated leukocyte counts with prominent eosinophilia. Importantly, carriers also suffer from an increased risk of cardiovascular disease, including myocardial infarction.[2] However, the functional link between eosinophilia in LNK-deficient individuals and the observed increases in thrombotic events are not wholly understood.

Mimicking the genetic background of T-allele carriers with LNK loss of function, Dou et al show that hematopoietic LNK deficiency increases peripheral eosinophil counts and eosinophil activation under steady-state conditions. The eosinophilia and eosinophil activation phenotypes become even more pronounced during metabolic disarray or during a chronic inflammatory state as induced by high-fat diet. Further, unleashed JAK/STAT signaling in response to interleukin-5 (IL-5) renders LNK-deficient mice more prone to forming eosinophil extracellular traps (EETs). Next, the authors used an elegant combination of genetic and pharmacological eosinophil ablation strategies, including both well-defined models such as anti-Siglec F antibody injection and ΔdblGata1 mice. To dissect the specific contribution of LNK deficiency in eosinophils to arterial thrombosis in this setting, an eoCre-Lnk$^{fl/fl}$ mouse was generated. In all of the models, eosinophil depletion consistently alleviated arterial thrombus formation in ferric chloride-induced arterial thrombosis.

An interesting finding is that in addition to increased EET formation, infiltrating neutrophils and neutrophil extracellular traps (NETs) were also frequently observed in *Lnk*-deficient mice. In line with this, the authors found that in response to eosinophil depletion, thrombus infiltration of both eosinophils and neutrophils was markedly reduced. Further, both EET and NET formation was alleviated following eosinophil depletion, pointing toward reciprocal activation loops of neutrophils and eosinophils in *Lnk*-deficient thrombi. Mechanistic in vitro experiments confirmed that *Lnk*-deficient eosinophils potently induced NET formation. On a cellular level, oxidized phospholipids (OxPL) generated by activated *Lnk*-deficient eosinophils promoted neutrophil activation in a platelet-activating factor receptor (PAFR)-dependent manner, leading to enhanced neutrophil (trans) migration and exacerbating NET formation (see figure). This observation is in line with previous work from the same authors that highlights neutrophil activation and enhanced NET formation through platelet-derived OxPL-mediated PAFR signaling.[7] However, the reciprocal communication between eosinophils and neutrophils is still not completely understood, but it is a relevant issue beyond cardiovascular diseases.

Finally, the authors used human-induced pluripotent stem cells carrying wild-type or mutated *LNK* alleles to generate eosinophils with or without functional LNK, emphasizing the translational relevance of their findings. Indeed,