

function, whereas STAT5B is critical for growth hormone-regulated functions.<sup>7</sup> STAT5B also plays a dominant role in the development and function of lymphocytes.<sup>8</sup> Unique functions of STAT5A vs STAT5B correlate with tissue-specific differences in their relative levels of expression.<sup>7,8</sup> Intriguingly, Kollmann et al found that STAT5A and STAT5B were expressed at similar levels in HSCs and that stimulation of HSCs with growth factors mostly activated STAT5B, and oncogenes preferentially activated STAT5B in hematopoietic malignancies.<sup>1</sup> STAT5A and STAT5B differ primarily in their Src-homology 2 (SH2) domains, which bind specific phosphotyrosine-containing motifs in the receptors with which they associate.<sup>2</sup> However, SH2 domain dissimilarities between STAT5A and STAT5B cannot account for the selective activation of STAT5B in HSCs because growth factor stimulation activates STAT5A and STAT5B in megakaryocytes.<sup>1</sup> Differences in the nature/extent of posttranslational modifications of STAT5A and STAT5B and/or the presence of specific regulators in HSCs and LSCs could impact the receptor interactions or activation states of STAT5A or STAT5B. These findings suggest that selective activation of STAT5B underlies its unique and dominant role in self-renewal of HSCs and LSCs. Proteomic analysis might help to further elucidate the molecular mechanism underlying selective activation of STAT5B in these cells.

The different transcriptional signatures of STAT5A and STAT5B further complicate understanding of the dominant role of STAT5B in HSCs and LSCs. Selective activation of “quiescence” genes by STAT5B could explain why STAT5B, but not STAT5A, is able to drive self-renewal and quiescence. Dissimilarities located in the transactivation domains of STAT5A and STAT5B might contribute to the distinct transcriptional signatures of STAT5B in HSCs and LSCs.<sup>2</sup> Thus, the STAT5B transactivation domain might specifically recruit a unique partner in HSCs and LSCs to facilitate its promoter binding and/or transcriptional activation of “quiescence” genes, which may also be influenced by the distinct epigenetic landscape of HSCs and LSCs. Future proteomic and epigenetic analyses of HSCs and LSCs might help to reveal the molecular mechanism by which STAT5B, but not STAT5A, activates “quiescence” genes in these cells.

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## LYMPHOID NEOPLASIA

Comment on Kittai et al, page 2372

# The more complex, the worse outcome in CLL

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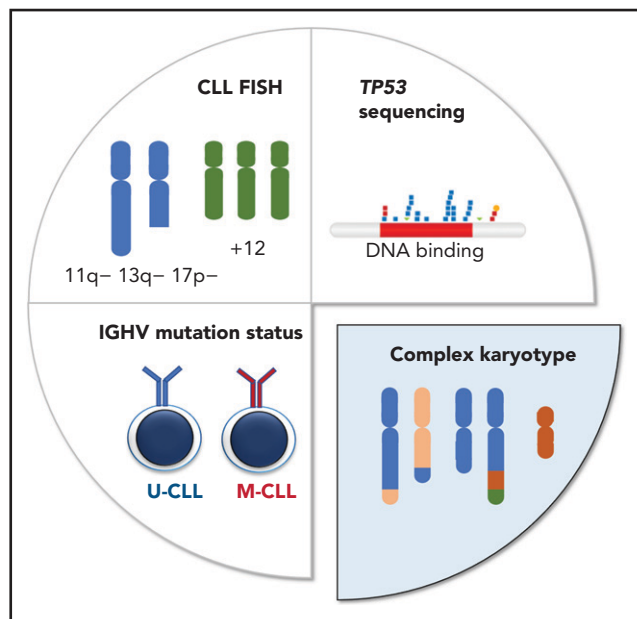
**In this issue of *Blood*, Kittai and colleagues investigate the impact of a complex karyotype on outcome in a large cohort of patients with chronic lymphocytic leukemia (CLL) treated with the BTK inhibitor ibrutinib.<sup>1</sup> As in prior studies, they confirm that a complex karyotype, defined as  $\geq 3$  or  $\geq 5$  chromosomal alterations, is a high-risk marker in ibrutinib-treated patients. They propose using karyotypic complexity as a continuous variable for predicting outcome, as increasing numbers of aberrations correlated with decreasing survival.**

By the early 1990s, Juliusson et al reported that the higher the number of chromosomal aberrations detected, the worse the outcome in patients with CLL.<sup>2</sup> This finding was confirmed in studies applying genomic arrays, where increasing genomic complexity was associated with shorter time to first treatment and overall survival (OS).<sup>3</sup> Because of the inherent difficulties to generate metaphase chromosomes for cytogenetic analysis in CLL, it was not until newer culturing protocols that included CpG and IL2 were introduced that most CLL samples could be karyotyped.<sup>4</sup> In a series of studies, complex karyotype, defined as  $\geq 3$  chromosomal alterations, was found to be a high-risk factor<sup>4</sup>; also, the presence of unbalanced structural aberrations was linked to a more dismal prognosis

In a recent study published in *Blood*, Balias et al investigated the impact of a

complex karyotype in more than 5200 patients with CLL.<sup>5</sup> In this cohort, they found that for patients without *TP53* aberrations, a complex karyotype was associated with high-risk disease if  $\geq 5$  chromosomal aberrations were present. On the other hand, if a patient carried a *TP53* aberration [ie, del(17p) and/or *TP53* mutation], the association with a worse outcome was already reached, if the patient had 3 or more alterations. Although few patients were treated with newer agents in their retrospective cohort study, the presence of a complex karyotype has been shown to be a high-risk factor in patients treated with BTK or BCL2 inhibitors, albeit mostly in smaller patient series.<sup>6</sup>

In the current study, Kittai et al explored the impact of complex karyotypes in a large, single institution cohort (n = 456), including both treatment naive (22%)



Different diagnostic methods are applied to determine clinically relevant genetic markers in CLL. Fluorescence in situ hybridization (FISH) is performed to detect the deletions of 11q, 13q, and 17p, as well as trisomy 12, whereas the IGHV and TP53 mutation status is determined by sequencing (Sanger sequencing and/or NGS). Karyotypic complexity is determined by cytogenetic analysis, counting both numerical and structural chromosomal aberrations.

and relapsed or refractory (78%) patients, treated with single-agent ibrutinib or ibrutinib in combination with an anti-CD20 monoclonal antibody. In addition to confirming the impact of a complex karyotype, defined as either  $\geq 3$  or  $\geq 5$  cytogenetic abnormalities, on outcome, they observed that patients with an increasing number of alterations, from 0, 1 to 2, 3 to 4, 5 to 9, 10 to 14, and  $\geq 15$  aberrations, had progressively worse survival curves. Hence, they assessed whether karyotypic complexity, included as a continuous variable, had an impact on outcome. Their analysis showed that accumulating karyotypic complexity was an independent variable associated with both inferior progression-free survival and OS. Furthermore, they had access to multiple cytogenetic analyses from a subset of patients progressing while on ibrutinib therapy (ie, at baseline and relapse) and demonstrate that the presence of karyotypic evolution at progression was linked to a worse outcome (see figure).

Today, different genetic analyses are performed to identify high-risk patients (see figure) and the present study adds further support to the clinical relevance of identifying patients with a complex karyotype. Moreover, Kittai et al show the importance of performing reanalysis at disease progression. As they rightly point out, there is a lack of consistency in the protocols used

for cytogenetic analysis in CLL when the more recent culturing conditions are used. Hence, it is important to standardize protocols and even perform a multicenter evaluation to determine the reproducibility of cytogenetic analysis in CLL.

Another option would be to examine genomic arrays or perform whole-genome sequencing (WGS) to determine the degree of genomic complexity. In a recent multicenter effort using genomic arrays, the presence of  $\geq 5$  or more genomic aberrations was linked to high-risk disease.<sup>7</sup> In fact, centers are already using genomic arrays in routine diagnostics to identify recurrent aberrations in CLL and can, at the same time, detect genomic complexity (but not translocations). The other alternative would be WGS, which has the potential, not only to determine single mutations, copy-number aberrations, and structural aberrations, but also to identify more complex markers, such as complex karyotype and IGHV gene mutation status. Although initiatives are ongoing in acute leukemia to introduce WGS instead of cytogenetic/molecular analyses, the cost of sequencing must decline further for it to be a realistic option in CLL diagnostics.

One important caveat with the current study concerns the lack of TP53 mutation status in the cohort. We know that patients

with del(17p) plus TP53 mutation comprise 60% of patients with TP53 aberrations, whereas another 30% of patients have only TP53 mutations.<sup>8</sup> In other words, a proportion of patients included in this cohort most likely carried TP53 mutations that were undetected. Considering the tight link between genomic complexity and TP53 aberrations, this information would have been valuable. In addition, we know that patients may harbor minor subclones with TP53 mutations, detected only by next-generation sequencing (NGS)-based assays, that are similarly linked to a poor response to therapy, at least to chemoimmunotherapy.<sup>9</sup> In future studies, it is important to conclusively analyze the TP53 gene, using targeted NGS and including other genes linked to genomic instability (eg, ATM and SETD2) or with prognostic impact in CLL. In recently developed, broader NGS panels, it is also possible to include a copy-number backbone to detect larger genomic aberrations, in addition to small mutations.<sup>10</sup>

In summary, the results of Kittai et al underscore the clinical relevance of increasing karyotypic complexity in patients with CLL treated with ibrutinib. In the coming years, we should standardize protocols for cytogenetic analysis or other methods selected to identify genomic complexity and define how increasing complexity should be measured. If we decide to use complex karyotype as a continuous variable, what number of aberrations should be included for each unit increase in karyotypic complexity? Do all types of alterations have the same clinical impact? Finally, we must discuss whether NGS-based technologies could represent an alternative approach to low-resolution cytogenetics for identifying complex karyotype in CLL.

*Conflict-of-interest disclosure:* R.R. has received honoraria from AbbVie, AstraZeneca, Janssen, Illumina, and Roche. ■

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## PLATELETS AND THROMBOPOIESIS

Comment on Lee-Sundlov et al, page 2408

# pDC as a modulator of platelet production

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**In this issue of *Blood*, Lee-Sundlov et al<sup>1</sup> demonstrate a novel surveillance mechanism of megakaryocyte (MK) sialylation by plasmacytoid dendritic cell (pDC)-like cells and its regulatory effects on platelet production via type I interferon (IFN-I) signaling.**

Desialylation has been recognized as a mechanism for platelet clearance in various conditions, including infection and immune thrombocytopenia (ITP), and plays a role in the removal of aged platelets.<sup>2</sup> However, it remains unclear whether, and if so how, MK desialylation affects thrombopoiesis. The study by Lee-Sundlov et al identified a novel surveillance system of MK sialylation status by pDC-like immune cells, leading to inhibition of platelet production from MKs. Targeted deletion of O-glycan sialyltransferase (St3gal1), specifically in MK lineage (St3gal1<sup>MK-/-</sup>), generated a mouse model with increased Thomsen-Friedenreich (TF) antigen expression on MKs. TF antigen, which is normally masked by terminal sialylation, becomes exposed when St3gal1 is deleted. The St3gal1<sup>MK-/-</sup> mice had thrombocytopenia with platelet counts at ~50% of the control mice. Interestingly, thrombocytopenia in St3gal1<sup>MK-/-</sup> mice was reversed

by treatment with dexamethasone or targeted deletion of Jak3, suggesting an immune-mediated component. Antibody-mediated cell depletion studies and RNASeq identified unique pDC subtypes with increased transcripts of immunoglobulin rearrangement genes specifically in St3gal1<sup>MK-/-</sup> mice. pDC clusters identified in St3gal1<sup>MK-/-</sup> bone marrow (BM) also showed enrichment in IFN-I gene sets. The authors further showed that thrombocytopenia in St3gal1<sup>MK-/-</sup> mice could be restored or ameliorated by treatment with antibodies against Siglec (sialic acid-binding immunoglobulin-like lectin) H.<sup>3</sup> By coculturing pDCs with St3gal1<sup>MK-/-</sup> MKs, it was shown that pDCs inhibit thrombopoiesis through secretion of IFN-I and potentially through involvement of Siglec H (see figure). Based on these results, the authors concluded that the sialic acid moiety of MKs regulates platelet production via immune

cells, mainly CD4<sup>+</sup> pDC-like immune cells in the BM.

The most important finding of this article is the recognition of desialylated MK by immune cells, specifically those with a pDC-like signature. The regulatory effect of these cells on platelet production was elegantly demonstrated by an ex vivo coculture study. Further studies are needed to prove this hypothesis by depleting pDCs in vivo. pDCs express endosomal Toll-like receptor (TLR) 7/9, which senses microbial or self-DNA/RNA, promoting secretion of large quantities of IFN-I. In this study, enhanced IFN-I secretion was noted when pDCs were cocultured with St3gal1<sup>MK-/-</sup>. Blockade of IFN-I ameliorated thrombocytopenia in St3gal1<sup>MK-/-</sup> mice. Of note, enhanced colocalization of CD4<sup>+</sup> cells with MKs was observed in the St3gal1<sup>MK-/-</sup> BM. Thus, it is intriguing to speculate that pDCs recognize and ingest a part of the desialylated MKs. Internalization of ingested MK fragments and trafficking to endosomes may contribute to TLR activation and IFN-I secretion. In this scenario, ingested desialylated MKs may also help potentiate antigen-specific T-cell responses, bridging innate and adoptive immunity in cases of ITP. In the normal state of sialylated O-glycan on MKs, Siglec H may inhibit IFN-I secretion. The contribution of Siglec H needs to be further investigated using Siglec H knockout mice. It is not yet known which component of MKs activates TLRs (TLR7 and/or TLR9) in pDCs to activate IFN-I signaling. Although the major agonists for TLR7 are pathogen-derived single-stranded RNAs, host-derived RNAs (eg, microRNAs [miRNAs] and transfer RNAs [tRNAs]) have also been known to serve as endogenous agonists to activate endosomal TLRs. Thus, it is feasible to speculate that MK RNAs, including miRNA and tRNA, activate TLR7 in pDCs.<sup>4</sup> Alternatively, mitochondrial DNA of MKs might stimulate TLR9.<sup>5</sup> Further investigations are necessary to test these hypotheses.

This study also identified increased anti-TF antigen antibodies in pediatric patients with ITP, suggesting pDC-mediated inhibition of thrombopoiesis as a part of the mechanism inducing thrombocytopenia. Although the platelet antigen is not determined in the patients studied in this article, it is well established that binding of the anti-GPIIb $\alpha$  antibody causes desialylation of platelet GPIIb $\alpha$ .<sup>6</sup> Thus, it is likely