The key message of the report by Guolla et al, despite the known limitations of systematic review meta-analysis, is that, in high-resourced countries, VCR/steroid pulses during maintenance therapy of both newly diagnosed patients with most favorable risk characteristics and likely also of first relapse standard-risk children should be either definitively abandoned or their frequency at least decreased. The situation may be different in those parts of the world with limited resources, where intensive therapy in the first year after diagnosis and the necessary supportive care to be used for preventing infectious complications are either not available or not feasible. In this particular context, the inclusion of VCR/steroid pulses during maintenance therapy may compensate for the lack of intensive therapy.[10] However, it cannot be ignored that the VCR/steroid pulses during maintenance can cause severe, sometimes fatal, infections, mainly of either viral or fungal origins, especially with prolonged dexamethasone pulses of 2 weeks. In addition, it is well known that glucocorticoids may cause other significant toxicities, including increased emotional lability, disruptive behaviors leading to missed days of school, myalgias, myopathies, hyperglycemia, osteonecrosis, obesity, metabolic sequelae, and adrenal axis suppression. VCR administration may also result in declines in fine motor and sensory-perceptual performance. Thus, even in low- to medium-income countries, all that glitters may not be gold.

*Conflict-of-interest disclosure: The author declares no competing financial interests.* ■

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**LYMPHOID NEOPLASIA**

*Comment on Tsagiopoulou et al, page 2955*

# Richter transformation: epigenetics to blame?

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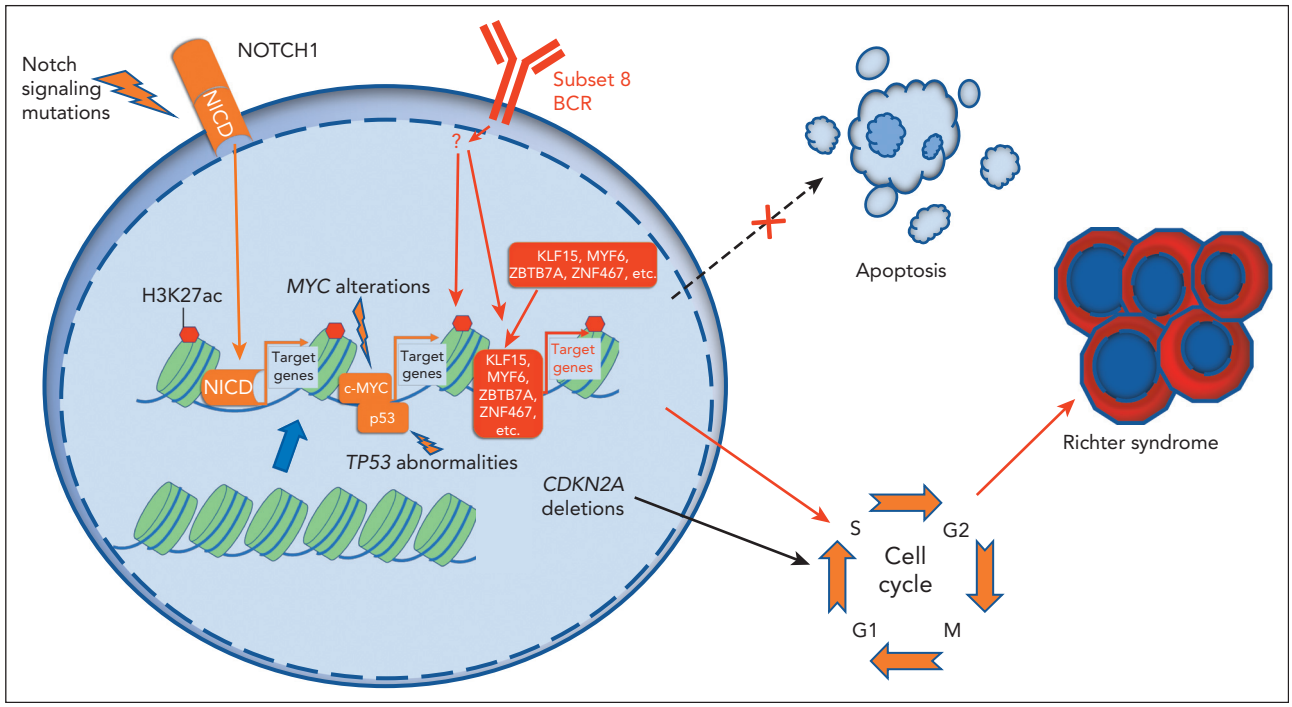
**In this issue of *Blood*, Tsagiopoulou et al[1] report that B-cell receptor (BCR) immunoglobulin stereotyped subset 8 of chronic lymphocytic leukemia (CLL) is characterized by a distinct chromatin activation profile bearing similarities with that found in Richter syndrome (RS). This observation is particularly intriguing given the fact that patients with subset 8 are a CLL subgroup at high risk of Richter transformation and that up to now a mechanistic explanation for this transformation propensity has remained elusive.[2,3] The article from Tsagiopoulou et al resolves important aspects of this enigma. The identified upregulated genes in patients with subset 8 should help us better understand the aggressive clinical behavior of this peculiar CLL variant, including its predisposition to progress to RS.**

Patients with CLL may express (quasi)-identical, ie, "stereotyped," BCR immunoglobulins, which allows grouping of patients into subsets. Stereotyped BCRs not only suggest a role for (auto)antigenic drive in the pathogenesis of CLL but also have important clinical implications, as some of the subsets have distinct biological and clinical characteristics and differing outcomes.[4] The most notable examples are stereotyped subsets 2 and 8, both associated with aggressive disease. In the case of subset 2, the aggressive behavior is driven by a single point mutation (R110) that confers the ability for autonomous signaling to BCRs that use light chains encoded by the alleles *IGLV3-21*01* and *IGLV3-21*04*.[5,6] This finding led to the definition of a broader subset 2L that encompasses subset 2 cases plus all other IGLV3-21$^{R110}$-expressing CLL cases regardless of immunoglobulin heavy-chain variable (IGHV) mutational status.[5] Interestingly, this IGLV3-21$^{R110}$-expressing CLL subset is also characterized by an increased frequency of *SF3B1* and *ATM* mutations, as well as by an intermediate DNA methylation signature, placing it epigenetically between the naïve-like and memory-like CLL subtypes.[6] However, there is no detailed understanding of the implications of a BCR composed of IGHV4-39 and IGKV1(D)-39 chains that define subset 8.

RS is defined as an aggressive lymphoma arising in the context of CLL. It most commonly has a histology that resembles diffuse large B-cell lymphoma (DLBCL) and predominantly (in approximately 80% of cases) expresses unmutated IGHV genes. RS can be clonally related to the antecedent CLL or represent a new clone, ie, (independent) secondary de novo DLBCL. A number of risk factors for RS have been uncovered, eg, unmutated IGHV and especially usage of IGHV4-39 (hence the connection with CLL subset 8), del(17p), activating *NOTCH1* mutations, *MYC* amplifications, loss or inactivation of *TP53* and/or *CDKN2A/B*, and dysregulation of the MAPK pathway (see figure).[7,8] Nevertheless, we are still far from a thorough understanding of the interplay of the factors driving the pathogenesis of the condition. This lack of understanding is also reflected in the unacceptably poor outcomes with median overall survival times of <12 months. Only recently has it become possible to use epigenetic signatures and gene expression profiles to determine whether RS is clonally related to the underlying CLL, even without the need for a matched sample from the CLL stage.[9] These techniques are expected to be incorporated into the treatment decision-making process as clonally unrelated RS is generally sensitive to standard chemotherapy regimens. Clonally related RS, on the other hand, is characterized by global DNA hypomethylation and subsequent dysregulation of EZH2, Wnt, PI3K/AKT, and IGFR1 pathways.[9] These changes can potentially be responsible for the chemotherapy resistance and the very poor prognosis of this RS subtype but may also be exploited as potential treatment targets.

Given the above, the work of Tsagiopoulou et al provides valuable new insights. It builds on previous seminal work, namely the delineation of the reference epigenome and the regulatory chromatin landscape of CLL, with its 2 major clinico-biological subtypes M-CLL and U-CLL (CLL with mutated or unmutated IGHV).[10] This previous work also identified specific chromatin regulation patterns in CLL cases with trisomy 12 or with *MYD88* mutations. Now, Tsagiopoulou et al delve into the chromatin regulation of stereotyped CLL subsets. A limitation of their work is that it evaluated only H3K27 acetylation as a marker of chromatin activation and did not perform full characterization of the chromatin landscape. Nevertheless, the authors could identify a remarkably distinct chromatin activation profile in CLL subset 8 compared with other stereotyped or nonstereotyped CLL cases. Importantly, the subset 8 signature also differed from the typical signature of CLL with trisomy 12, although the majority of subset 8 cases carry trisomy 12. The authors identified robust differentially acetylated regions in subset 8 compared with nonsubset U-CLL cases and separated them into 6 subclusters based on differing acetylation patterns among CLL subgroups and normal B-cell populations. They could demonstrate that hyperacetylated regions in subset 8 had higher positive correlation with gene expression and that the H3K27 acetylation signature of subset 8 overlaps considerably with the published H3K27 acetylation profile of nonsubset 8 U-CLL cases that progressed to RS. Next, they identified transcription factor binding sites that were enriched in de novo acetylated regions in subset 8 and showed that the respective transcription factors are overexpressed, eg, KLF15, MYF6, ZBTB7A, and ZNF467. Accordingly, this correlated with overexpression of target genes, among them known cancer-associated genes like *NDUFA4L2, ZDHHC19, TRAF2, CLIC3, FBXW5,* and *CCDC183*.




Various factors contribute to Richter transformation, including aberrant NOTCH1 signaling, increased c-MYC activity, and deletions and mutations of *CDKN2A* and *TP53*, as well as CLL subset 8, characterized by a specific chromatin activation pattern and transcription factor activity. CDKN2A, cyclin-dependent kinase inhibitor 2A; H3K27ac, histone 3 lysine 27 acetylation; KLF15, Krüppel-like factor 15; MYF6, myogenic factor 6; NICD, Notch intracellular domain; ZBTB7A, zinc finger and BTB domain-containing protein 7A; ZNF467, zinc finger protein 467.

The hypothesis that the propensity of subset 8 CLL to undergo Richter transformation is due to the epigenetic derepression of a particular set of genes caused by overactivation of a limited set of transcription factors is intriguing; however, important questions remain to be answered. Further research is necessary to elucidate the mechanistic connection between the BCR of subset 8 and the implicated transcription factors. This might help to identify druggable targets that could be exploited to improve the dismal clinical prognosis of patients with subset 8 and to potentially prevent Richter transformation. The current study did not find examples of distinct chromatin regulation in other CLL subsets, but this may be due to the relatively small sample sizes and the resulting low statistical power. Future studies with more patients and broadened scope by including cases from rarer CLL subsets might yield additional insights. In any case, Tsagiopoulou et al have to be applauded for their remarkable achievement outlining how a comprehensive "omics" approach linking immunogenetic features with (epi) genomic and transcriptional characterization can be instrumental in illuminating complex diseases mechanisms.

*Conflict-of-interest disclosure: The authors declare no competing financial interests.* ■

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**PLATELETS AND THROMBOPOIESIS**

*Comment on Kaiser et al, page 2973*

# The hemorrhage risk of dasatinib therapy

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**In this issue of *Blood*, Kaiser et al[1] pinpoint the glycoprotein IIb (GPIIB)/G$\alpha_{13}$/Src tyrosine kinase (c-Src)/14-3-3ζ signaling axis as a requirement for platelet migration and shed light on how the antineoplastic oral tyrosine kinase inhibitor dasatinib increases the risk of inflammation-associated mucosal hemorrhage.**

Platelets are highly dynamic, migratory anucleate blood cells that not only are involved in blood clot formation but also play a key role in first-line immune responses. To accomplish both functions, platelets restructure their cytoskeleton when they adhere to matrix molecules following vascular injury.[2] This phenomenon mainly involves myosin heavy chain 9 (MYH9) and actin-related protein complex 2/3 (Arp2/3).[3,4] So far, the signaling pathways that regulate platelet migration up- and downstream of these mechanisms have remained elusive. In this issue of *Blood*, Kaiser and colleagues reveal how the sarcoma family kinase c-Src and the Src family kinase-binding protein 14-3-3ζ, situated downstream of the fibrinogen receptor GPIIBIIIA, coupled to G$\alpha_{13}$, is required for platelet polarization and migration.[1] In addition to further defining this downstream signaling pathway, Kaiser et al elucidate an important side effect of the antineoplastic oral tyrosine kinase inhibitor dasatinib. Dasatinib is an inhibitor of B-cell receptor-ABL-tyrosine kinase and of the sarcoma family kinase c-Src, used clinically to treat chronic myeloid leukemia (CML) and Philadelphia chromosome-positive acute lymphoblastic leukemia (ALL). They found that dasatinib-treatment causes mucosal bleeding through the blockade of platelet migration.

The fibrin(ogen) receptor GPIIBIIIA regulates platelet adherence to extracellular matrices. Kaiser and colleagues found that both genetic and pharmacologic targeting of the Arp2/3 complex abrogates the polarized platelet phenotype and the ability of platelets to form lamellipodia and migrate on albumin/fibrin(ogen) hybrid matrices, highlighting the importance of Arp2/3 for platelet polarization and migration.[5] Intriguingly, although platelet size and circularity was reduced, inhibition of Arp2/3 did not affect clot retraction. Previous work by Gaertner and coworkers has demonstrated the critical involvement of MYH9 in platelet migration.[6] In this issue of *Blood*, Kaiser et al show that coordinated myosin II function is a requirement for motility (retraction of lamellipodia) but is not necessary for platelet polarization, which