4 confirmed infections per child, showed malaria risk was significantly associated with achieving MTD, absolute neutrophil count (ANC), hemoglobin, and splenomegaly. Age, season, MTD does, hemoglobin F, α-thalassemia, and G6PD deficiency had no effect. In multivariate regression analysis of confirmed malaria infections, ANC values < 3.0 × 10^9/L were associated with lower malaria incidence. Compared with nonpalpable, splenomegaly < 5 cm was associated with higher malaria risk.

Are the observed reductions in malaria infections accurate, and are they truly associated with HU therapy? The authors cannot definitively exclude longitudinal or reporting bias in an open label trial such as REACH. Nevertheless, on the backdrop of the findings of NOHARM, a double-blind, placebo-controlled trial that reported ~30% reduction in malaria infections in children on HU, the observed association with lower malaria incidence in the REACH cohort is likely accurate. NOHARM reported on ~100 patient-years in each arm (HU vs placebo) in a single site with low malaria infection rate. In contrast, REACH reports on over 3300 patient years of HU treatment with high baseline rates of malaria infection.

Univariate and multivariate analysis of confirmed malaria infections identified ANC and splenomegaly as the 2 most important variables affecting malaria risk. How can these be explained? The authors offer 2 possible explanations for the ANC effect. ANC values may have a direct effect on decreased malaria risk through reduced adhesion, inflammation, and endothelial activation; or they simply reflect that higher HU levels have antimalarial benefits. HU inhibits *Plasmodium falciparum* growth and prevents cerebral malaria from *Plasmodium berghei* in mice, at concentrations attainable in vivo with HU treatment in SCA.[8] In patients with untreated SCA, the spleen is packed with sickled erythrocytes, impairing its ability to phagocytose parasitized erythrocytes.[9] HU, by reducing sickling, can, in part, restore this essential antimalarial function of the spleen, thereby reducing clinical malaria infection. This brings us to the association of palpable splenomegaly < 5 cm with increased malaria incidence. The authors' explanation that the spleen could potentially serve as a reservoir for *P falciparum* is not supported by direct evidence. Another consideration is that splenomegaly in SCA is probably a sign of more active hemolysis, and this may predispose to clinical malaria episodes.

Efforts to combat malaria including use of insecticide-treated nets, vector control, chemoprevention, and effective treatment of clinical malaria have reduced malaria mortality rates worldwide from 30 per 100 000 population in 2000 to 13 per 100 000 in 2019.[10] Although the incidence of malaria in SCA is not actually increased, the risk of death from clinical malaria is reported to be higher in children with SCA than other children. It is surprising, therefore, that malaria prevention practice was not consistent across the study sites, a reflection of the lack of consensus among public health authorities in SSA. This needs to be addressed. The emergence of new malaria vaccines will help strengthen malaria prevention efforts in SSA.

The research goal to better understand the clinical significance of the association between HU and lower malaria risk is worthwhile. What is more, this finding together with the proven clinical and laboratory benefits of HU therapy should encourage development of strategies to make HU widely accessible to children with SCA in SSA where the disease burden is heaviest.

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

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https://doi.org/10.1182/blood.2022018873

LYMPHOID NEOPLASIA

Comment on Stachelscheid et al, page 1425

TCL1A expression promotes aggressive biology in CLL

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In this issue of *Blood*, Stachelscheid et al[1] report a novel nuclear TCL1A-CDC20 axis that signals DNA damage-prone cell cycle transition and genomic instability, thereby accelerating aggressive chronic lymphocytic leukemia (CLL).

TCL1A is a proto-oncogene first identified in T-cell prolymphocytic leukemia. Upregulation of TCL1A via a gene rearrangement bringing *TCL1* (on chromosome 14q31.2) under the influence of *TCR* (T-cell receptor) enhancer has

been identified as a characteristic of the disease.[2,3] The gene product is frequently overexpressed in many types of T- and B-cell lymphomas, and increased levels of TCL1A correlate with aggressive clinical behavior and poor prognosis in T- and B-cell lymphoma. Its expression is physiologically restricted to early developmental stage B and T cells and embryonic tissues.[4] Genomic rearrangements or gain-of-function mutations involving the *TCL1A* locus are virtually absent in B-cell tumors.[5,6] Accordingly, the molecular mechanism(s) and signaling pathway(s) by which TCL1A mediates oncogenicity in B-cell neoplasms are not well understood. Notably, transgenic mice expressing *TCL1A* in B cells (ie, *Eμ-TCL1A* or *pEμ-B29TCL1A*) develop CLL or germinal center–derived B-cell lymphoma, including Burkitt lymphoma (BL), follicular lymphoma (FL), and diffuse large B-cell lymphoma (DLBCL), suggesting unknown mechanism(s) and pathway(s) contributing to lymphomagenesis.[7] Stachelscheid et al describe the pleiotropic effect of TCL1A as a major factor in the pathogenesis of CLL through acceleration of premature and DNA-damage–prone cell cycle transition causing aneuploidy, impaired DNA-damage repair, and increased cell survival signaling as well as apoptotic resistance.

The expression pattern of TCL1A, subcellular localization, and interactome may determine its physiological or tumorigenic properties. In this regard, the signaling consequences of subcellular site-specific TCL1A have not been fully explored. TCL1A encodes a 14 kDa nonenzymatic protein, which is functionally required to form homodimers that bind with AKT as a cofactor and enhances its kinase activity as well as promotes its nuclear transport.[1,3] Constitutively active myristoylated AKT in murine B cells does not appear to recapitulate the oncogenic effect of TCL1A. Therefore, Stachelscheid et al investigated the role of the nuclear TCL1A in CLL pathogenesis. Because TCL1A itself does not have enzymatic activity or DNA-binding capability, it can be hypothesized that it forms multiprotein complexes to exert its effects.

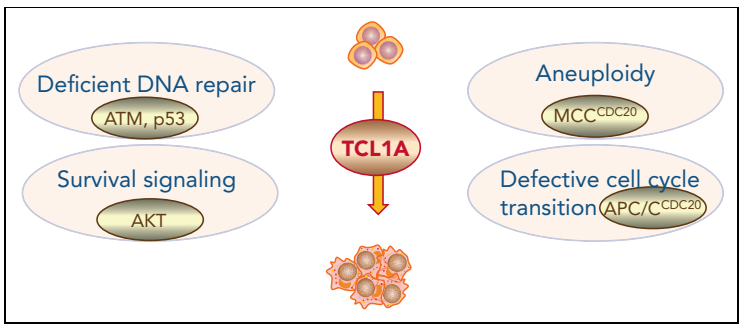
Stachelscheid et al uses various proteogenomic approaches to identify the effectors and critical pathways perturbed by TCL1A to promote CLL growth. Further elegant and robust in silico, in vitro, and in vivo experimental approaches including multiple cell lines and transgenic murine models demonstrate that expression of TCL1A promotes premature and defective mitotic checkpoint protein complex composition by directly binding to CDC20. This disrupts the interaction of CDC20 with MAD2 and PLK1 in the spindle assembly checkpoint, leading to accelerated cell cycle transition and aneuploidy. The role of nuclear TCL1A and its effectors and pathways that are deregulated in lymphomagenesis are poorly understood. Stachelscheid et al demonstrate the signaling pathways (cell cycle transition, chromosomal segregation, DNA damage response, and apoptosis) affected and cell cycle modulators recruited by nuclear TCL1A to promote its oncogenicity. The study also demonstrates that the expression of nuclear localized TCL1A in an in vivo model promotes aggressive biologic behavior of CLL and significantly shorter survival compared with wild-type or membrane-targeted TCL1A.

Stachelscheid et al further show that in primary human CLL cells, high levels of TCL1A expression are inversely correlated with reduced levels of phospho-activated and cleaved ATM upon genotoxic stress. B cells from *Eμ-TCL1A* mice show reduction of p53 and accumulation of DNA double-strand breaks compared with wild-type control mice, suggesting that TCL1A promotes DNA damage and hinders the DNA repair response. However, the role of TCL1A in regulating DNA damage response needs further exploration to understand the mechanisms involved and assess the potential for therapeutic targeting.

Extensive genomic analyses of CLL published to date have not documented genomic lesions (mutations or copy number variations) of *CDC20*. However, bioinformatic analysis of publicly available databases by the authors demonstrates that transcript levels of *CDC20* are significantly reduced in CLL compared with the levels in other B-cell neoplasms. They show that lower levels of TCL1A protein were observed in more aggressive CLL. Furthermore, low expression of *CDC20* was directly proportional to high genomic instability. Downregulation of *CDC20* in the *Eμ-TCL1A* CLL model further accelerated leukemic progression and increased aneuploidy.

The role of TCL1A in impaired DNA damage repair pathway warrants further investigation. The results presented by Stachelscheid et al expand our understanding of the multifaceted mechanisms used by nuclear TCL1A to promote defective and accelerated cell cycle transition, aneuploidy, and impaired DNA damage response that collectively contribute to CLL pathogenesis (See figure). Considering the important insights offered by this investigation of TCL1A and its contribution to pathogenesis of CLL, it is important to identify whether similar mechanisms are used by TCL1A in other B-cell lymphomas such as BL, FL, and DLBCL. Furthermore, it is critical to determine the mechanisms involved in downregulation of CDC20 in aggressive CLL to exploit this avenue for therapeutic intervention.

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



Signaling pathways regulated by TCL1A in CLL. TCL1A functions as a pleiotropic adapter molecule regulating multiple signaling pathways (deficient DNA repair, increased survival, apoptotic resistance, aneuploidy, and defective cell cycle transition) resulting in genomic instability that contributes to pathogenesis of CLL. APC/C, anaphase-promoting complex; MMC, mitotic checkpoint complex.

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https://doi.org/10.1182/blood.2022018435

MYELOID NEOPLASIA

Comment on Li et al, page 1442

Context is key for FLT3-ITD

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In this issue of *Blood*, Li et al[1] report age and co-mutation contexts wherein FLT3 internal tandem duplication (ITD) orchestrates unique transcriptional and epigenetic programs to deliver distinct functional outputs from myeloid progenitor cells.

Constitutive activating *FLT3-ITD* mutations are common in both adult and pediatric acute myeloid leukemia (AML). With the advent of FLT3-ITD directed tyrosine kinase inhibitors (TKI) over the past two decades the field has shifted its focus toward understanding FLT3-ITD signaling in the context of these FLT3-target inhibitors, with less effort aimed toward discovery and elucidation of FLT3-ITD leukemogenic mechanisms. Previous work demonstrated that knock-in of the human *ITD* mutation into the endogenous mouse *Flt3* gene was not sufficient to produce AML.[2,3] In patients with AML, regardless of age, *FLT3-ITD* mutations occur relatively late in AML pathogenesis and are observed in combination with a variety of mutational partners. Indeed, studies demonstrate that *FLT3-ITD* cooperates with *DNMT3A*, *TET2*, *RUNX1*, *NPM1c*, *MLL-PTD*, *NUP98-HOXD13*, and *CBFB-MYH11* mutations to produce AML in mice.[4-10] Despite the growing number of bona fide cooperating mutations leading to AML pathogenesis with FLT3-ITD, several questions remain. How this mutation contributes to profoundly different diseases in children and adults remains elusive. How FLT3-ITD signaling output changes as it is introduced with different co-occurring mutations remains vastly understudied. Whether the mutations that are coincident with *FLT3-ITD*, or the age of the patient, or both, are more important for specific disease characteristics, is still not clear. FLT3-ITD's ability to enhance cell proliferation via STAT/extracellular signal-regulated kinase activation in leukemogenesis is well known, and this, coupled with continued improvements in potency and efficacy of FLT3 TKIs, has cast a shadow over identifying more intricate, context-specific functions of FLT3-ITD. Li et al took a completely new approach to understanding novel FLT3-ITD biology by comparing its role in models of pediatric vs adult AML, and in doing so, begin to answer the open questions lurking in the shadows.

Driver gene alterations that co-occur with *FLT3-ITD* tend to be more age specific with chromosomal rearrangements, including *NUP98-t* and *MLL-t*, more common in pediatric AML, and single-gene mutations, such as *DNMT3A* and *RUNX1* loss-of-function mutations, more common in adult AML. Based on coincidence in human AML, Li et al selected previously characterized mouse models of *Flt3-ITD*, harboring either *NUP98-HOXD13* or *RUNX1* deletion (*RUNX1*^{MUT}) as representative of pediatric and adult coincident mutations, respectively. To level the playing field when making comparisons across these two different AML models, several key variables including the age of mice and the premalignant cell type were carefully controlled, and heterogeneity of the cell population was accounted for by single-cell RNA sequencing. This allowed direct comparison of Flt3-ITD's contribution to the transcriptionally and immunophenotypically defined cell states in pediatric *NUP98-HOXD13* vs adult *RUNX1*^{MUT} contexts. In young mice, Li et al found that transcriptional reprogramming occurred earlier in *Flt3-ITD* multipotent progenitors (MPPs) coexpressing the fusion oncoprotein NUP98-HOXD13 (see figure panel A) and later in *Flt3-ITD/RUNX1* co-mutant MPPs (see figure panel B). Despite both sets of mice having the same constitutively active Flt3-ITD mutation, very little overlap was observed in their leukemogenic gene expression profiles. These uniquely coordinated expression profiles also distinguished older mice with more advanced disease, as well as pediatric vs adult patients with AML. At the single-cell level, Flt3-ITD caused expansion of a granulocyte/monocyte progenitor (GMP)–like population and emergence of a new MPP-like population. The addition of *RUNX1*^{MUT} co-mutation primarily enlarged these populations. Conversely, NUP98-HOXD13 drove development of a totally new and unique MPP-like population, which was also dominant with co-mutation of *Flt3-ITD*. Thus, *Flt3-ITD/RUNX1*^{MUT} and *Flt3-ITD/NUP98-HOXD13* each produce transcriptionally divergent cell states by distinct mechanisms (see figure). These novel insights demonstrate that FLT3-ITD is much more dynamic than previously thought. Moving forward, we should broaden how, and what, we consider as roles of FLT3-ITD in AML pathogenesis, keeping in mind that mutational and developmental contexts are key.

Critically, studies by Li et al identified a novel context-specific dependency of *Flt3-ITD/NUP98-HOXD13* co-mutant MPPs on type 1 interferon (see figure panel A). *Flt3-ITD/NUP98-HOXD13* but not *Flt3-ITD/RUNX1* MPPs were sensitive to deletion of *Ifnar1*, resulting in