response were performed after 56 and 140 days of therapy and then every 84 days. The primary endpoint of the study was assessment of clinical benefit rate (CBR), defined as complete and partial responses, or stable disease that lasted at least 6 months.

Patients were assigned and evaluated in 3 cohorts: cohort 1 included those with known activating JAK and/or STAT mutations; cohort 2 included those with functional activation of JAK/STAT by demonstration of ≥30% pSTAT3 3 expression in tumor cells by immunohistochemistry; and cohort 3, which those with neither genetic or phenotypic evidence of JAK/STAT activation or insufficient tissue for this assessment. Ruxolitinib activity was found to be greater in those patients with PTCL with JAK/STAT activating mutations or active signaling, with cohort 1 having a CBR of 53%; cohort 2 having a CRB of 45%; and cohort 3 having a CRB of 13%. The median time to best response was 6.3 months. Adverse events were consistent with known side effects of this drug and primarily involved cytopenias.

Although an overall CBR of 35% is likely commensurate (although many may agree it is also commiserate) with other approved agents, 8 exceptional responders were identified with responses >1 year. These included 4 of the 5 patients with T-large granular lymphocytosis (T-LGL), the CBR of which was 80%. Of the 8 patients with T-prolymphocytic leukemia (T-PLL), CBR was 50%, all in patients with evidence of JAK/STAT activation. In contrast, only 1 of 7 patients with mycosis fungoides experienced a response to this treatment.

From this study, we learned that an already available oral JAK inhibitor was active against various subtypes of PTCL with acceptable toxicity most often when JAK/STAT pathway activation could be demonstrated. Although lack of JAK/STAT activation appears to predict a reduced response to ruxolitinib in PTCL, the presence of activation was not a guarantee of response, and the lack of activation did not assure there would be no response. This was clearly demonstrated in some cases of T-LGL.

The responses seen in the small number of patients with T-PLL and T-LGL are particularly encouraging. Here, we have 2 T-lymphocytic entities characterized by circulation of malignant cells, cytopenias, and splenomegaly. T-PLL is an aggressive disorder with very limited treatment options. T-LGL is an indolent disorder with treatment options that are successful in approximately half of cases. Of interest, in T-PLL, up to 76% of cases have mutations in JAK1, 2, or 3 or STAT3 or 5B; in LGL, up to 40% of cases have mutations. Although 60% of the patients with LGL are symptomatic, STAT3 mutations have been associated with increased symptoms and shorter time to treatment failure.[4-7]

We look forward to studies that would specifically address these 2 neglected subtypes of PTCL with either expansion of single-agent investigation and/or combination with other pathway inhibitors, such as bcl-2 inhibitors or Pi3 kinase inhibitors. We also anticipate further characterization of the role that JAK/STAT inhibition may play in the treatment of the larger group of patients diagnosed with non–mycosis fungoides PTCL who need improved outcomes.

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MYELOID NEOPLASIA

Comment on Sheng et al, page 2838

Ticket to divide: m^{6}A reader YTHDC1 drives acute myeloid leukemia proliferation

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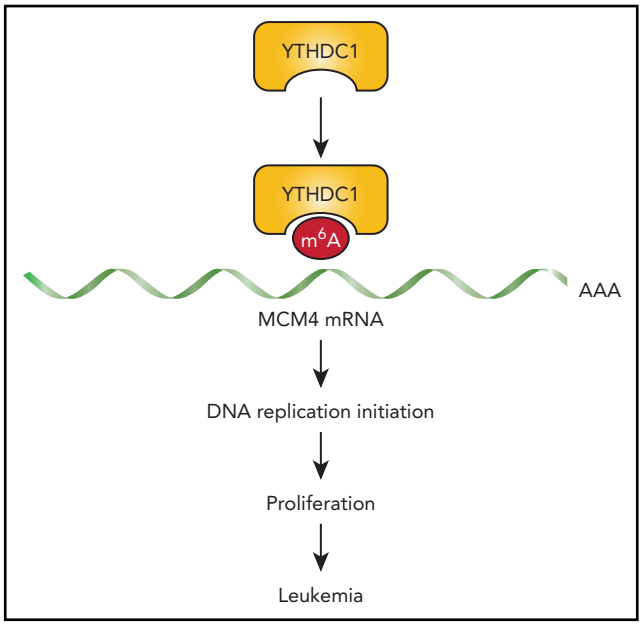
In this issue of *Blood*, Sheng et al show that the N^6-methyladenosine (m^{6}A) messenger RNA (mRNA) reader protein YTH domain containing 1 (YTHDC1) promotes leukemic cell proliferation through stabilizing transcriptions of DNA replication initiation complex subunit MCM4.[1]

The central dogma of biology is that DNA is transcribed to RNA, which is then translated into protein. Each step of the process is highly regulated by discrete modifications to the target molecule and associated factors. These modifications impart changes in the target molecule so profound to its function that related mutations account for most oncogenic drivers. The field of cell biology has been broadly focused on two-thirds (DNA and protein) of the central dogma trifecta. We still need to understand how modifications to RNA contribute to disease and development.

RNA modifications affect the stability, localization, and translation efficiency of the molecule. The m^{6}A modification cooccurs with transcription and occurs within a DRACH (A/G/U; G/A; A; C; A/C/U) consensus sequence.[2] Broadly speaking, the



m^{6}A reader protein YTHDC1 promotes leukemogenesis by stabilizing m^{6}A-marked MCM4 mRNA. YTHDC1 is highly expressed in malignant hematopoietic cells, which contributes to preservation of MCM4 transcripts. This leads to stabilization of the replication initiation complex through adequate levels of MCM4, which in turn supports the rapid proliferation of malignant hematopoietic cells.

METTL family of proteins are responsible for writing the mark,[3] YTH domain-containing proteins such as YTHDC1 are responsible for reading the mark,[4] and erasers such as FTO and ALKBH5 are responsible for removing the mark.[5,6] In the past few years, several studies have highlighted the importance of these marking pathways in leukemogenesis. For example, 1 study showed that METTL3 (m^{6}A writer) cooperates with CEBPZ to bind promoters of key acute myeloid leukemia (AML) survival genes and promote their translation,[7] and other studies showed that m^{6}A erasers FTO and ALKBH5 are necessary for AML development through regulation of RARA, MYC, and CEBPA.[8,9]

YTHDC1 is localized to cell cycle-associated YT bodies, commonly known as nuclear speckles, and is primarily responsible for facilitating the cytoplasmic export of m^{6}A-marked mRNA. Although YTHDC1 has been shown to preferentially bind noncoding RNAs such as XIST,[9] there are many mRNAs that are exported from the nucleus without YTHDC1 involvement. This suggests there are additional levels of regulation that remain to be uncovered, and studies like the one by Sheng et al present evidence for a novel role for YTHDC1 in promoting the stability of mRNA transcripts.

Sheng et al report the observation that YTHDC1 is broadly upregulated in AML samples compared with healthy hematopoietic tissues, and selective depletion of YTHDC1 through RNA interference in leukemic cell lines leads to growth arrest and loss of colony formation. They then confirm the intrinsic requirement of YTHDC1 to AML cell growth and survival with an exhaustive examination of many retrovirally expressed oncogene models including MLL-AF9, AML1-ETO9a, HOXA9, and PML-RARα. In each case, elevated YTHDC1 expression was required for growth and colony formation in vitro and disease development in vivo. Haploinsufficiency significantly impaired leukemic stem cell function in vitro and in vivo. This finding contrasted with the effects of YTHDC1 reduction in healthy hematopoiesis, in which there was no discernable phenotype in YTHDC1 haploinsufficient animals.

The most salient discovery in this work is the finding that YTHDC1 does not necessarily drive the nuclear export of key transcripts, but instead predominantly stabilizes the transcripts of genes related to cell cycle. This was surprising, as previous accounts of YTHCD1 function in other cell biology models primarily attribute YTHDC1 interactions to nuclear export.[10] Using RNA sequencing to identify changes in transcript levels following YTHDC1 knockdown in the MOLM13 AML cell line, Sheng et al identified several components of the DNA replication initiation complex with reduced transcripts following YTHDC1 knockdown including members of the minichromosome maintenance (MCM) family MCM2/4/5, the anaphase promoting complex, and the chromatin assembly factor 1a. However, as these transcript levels could be changing because of cell cycle, Sheng et al used the RNA-binding protein immunoprecipitation assay to show their direct interaction of YTHDC1 was responsible for their stability. Knockdown of YTHDC1 in AML cell lines led to DNA damage and cell-cycle arrest, which the authors determined was due to defective replication initiation. In support of their findings, they found reexpression of MCM4 in YTHDC1 knockdown cells was sufficient to reverse the phenotype. The cell growth and colony formation defects were seen most profoundly in several primary patient AML samples when compared with the relatively little effects on healthy CD34$^+$ peripheral blood stem cells from healthy donors.

In summary, Sheng et al show us that YTHDC1 is a key leukemogenesis gene. They propose YTHDC1 reads the m^{6}A mark on target transcripts (with the focus on MCM4 in this study) promoting its stability. In the case of AML, the transcript targets of YTHDC1 are broadly associated with the cell cycle, which results in supporting proliferation of the malignant clone. Acute leukemia-initiating cells capitalize on this activity because of their comparatively higher expression of YTHDC1 (see figure).

This study highlights the importance of mRNA reader proteins in the pathobiology of AML. And like all good studies, this one leaves us with many more questions. Some questions include exploring the basic biology into how YTHDC1 expression is regulated. Is there a leukemia-specific program that is promoting the upregulation of YTHDC1 in the malignant context that is different from healthy hematopoiesis? Downstream of YTHDC1, how is the protein promoting the stability of MCM4 in AML? Is it through preventing degradation by the nuclear PAXT complex, or are there other mechanisms? There are molecules identified that are directed toward inhibiting RNA methylation, but most inhibit either the writing

or the erasing of the m^{6}A mark. Could there be a way to target mRNA readers like YTHDC1 in a way that would selectively affect leukemic cells and spare healthy hematopoietic cells?

YTHDC1 is a conductor that allows AML to expand and make a mess on the metaphorical train of the bone marrow. Studies like this from Sheng et al have demonstrated that we may be able invalidate its ticket by targeting RNA methylation.

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THROMBOSIS AND HEMOSTASIS

Comment on Zwagemaker et al, page 2853

Can we do something about ICH in hemophilia?

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Hemophilia is one of the diseases considered under the rubric of "benign hematology"; however, hematologists should never consider this severe bleeding disorder as anything but a life-threatening disease that, short of death, can result in debilitating and permanent sequelae. In this issue of *Blood*, the exceptionally well-done meta-analysis performed by Zwagemaker et al[1] clearly demonstrates that intracranial hemorrhage (ICH) is much more common in persons of any age with hemophilia than the general population, although the incidence rate is strikingly high in children, especially neonates. Among nonneonate children (ie, children >1 month of age), the highest rates of ICH occur in the first few years of life and often at ages before prophylactic therapy is typically initiated. Importantly, ICH was demonstrated to be associated with increased mortality. In my 20+ years of caring for many patients with hemophilia, I have cared for several dozen who suffered an ICH, many of whom suffered permanent neurologic damage.

The strengths of the report are the number of studies reviewed and the sheer number of total patients included in those studies, which span many decades. Although period bias is sometimes an issue with studies that span such a long period of time, given that this study reports on an epidemiologic manifestation of hemophilia that should be static and for which specific (to ICH) preventive measures still do not exist, the fact that many decades of studies have been reviewed is a strength.

Thus, this confirmatory meta-analysis removes any question about the high incidence of ICH in persons with hemophilia and adds strength to the notion that this is largely a pediatric problem and moreover one that mostly occurs in the first few years of life. What is one to do with such important information regarding a potentially devastating complication? Of course, prevention of ICH would be ideal, but how could these data be used to alter the current paradigm of treatment? To be sure, prophylactic therapy with factor concentrates is the established standard of care for patients with severe hemophilia and many patients with moderate hemophilia; however, the current approach even in resource-rich countries is to start prophylaxis ~1 to 2 years of age before or shortly after the first joint bleed. Although long-term studies have demonstrated this approach to be effective at preventing permanent joint damage,[2] more recent data suggest that this protection may not be as robust as we had thought.[3]

Although prophylaxis has been used for decades, it requires repeated IV infusions of factor concentrates, which carry with them a significant treatment burden especially in young children such that often the initiation of prophylaxis is delayed as much as possible. As such, the notion of using prophylaxis to prevent ICH has never taken hold despite the potentially devastating outcomes. Recently, emicizumab, a bispecific monoclonal antibody that mimics the function of factor VIII has become available and has been demonstrated to be very effective at bleed prevention.[4,5] Unlike factor concentrates, this medication is given subcutaneously and less frequently than factor therapy, easing the treatment burden. Importantly, it has also made it feasible to initiate prophylaxis at a much younger age, even in the neonatal period. The question is not whether one can administer emicizumab to neonates and young infants, but rather should this become the standard approach. To be clear, there are very limited data on the use of this agent in patients <2 years of age, and in particular,