Restrictive transfusion thresholds, as confirmed by the current study, result in fewer patients transfused and less blood use and, thus, reduced costs and fewer transfusion-associated adverse events. However, hemoglobin may not be the best indicator of the need for transfusion. A review of RBC transfusion effects in critically ill patients revealed that patients with abnormal tissue oxygenation prior to transfusion oxygenation improved significantly posttransfusion, suggesting that tissue oxygenation may be a better indicator of transfusion needs than hemoglobin.[7] Another review suggests that intraoperative cerebral oxygen desaturation may result in postoperative cognitive dysfunction.[8] The primary reason to transfuse is to increase oxygen delivery; the short- and long-term effects of tissue oxygenation remain largely unknown.

Most randomized clinical trials regarding RBC transfusion have looked at 30-day mortality as their clinical outcome. The question remains "Is this the optimal clinical end point?" The current study highlights that other clinically meaningful outcomes may be of use.[1] Additionally, individuals with the low hemoglobin trigger had not recovered their baseline hemoglobin at 30 days. A recent retrospective cohort study evaluating postdischarge outcomes at 6 months demonstrated an increase in anemia with decreased RBC transfusion from implemented patient blood management programs. This anemia was not accompanied by an increase in RBC use, rehospitalization, or mortality,[9] highlighting the lack of anemia management postdischarge. Anemia has consequences, especially for patients with comorbidities, which may be the cause of the higher 90-day morbidity in this study.[10] Therefore, it is important to ask whether morbidity is the appropriate outcome for short-term treatment of transfusion, rather than a more comprehensive evaluation of efficacy, and whether transfusion thresholds should be more integrated into larger patient treatment plans that include hospital and postdischarge care.

In conclusion, Møller et al's feasibility study creates awareness of transfusion's role in patient care; how transfusion is integrated into each patient's care to optimize long-term outcomes remains an area for study. We need to keep learning about different patient population needs, the appropriate indications for transfusion, and how to support these patients in the months following their event.

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LYMPHOID NEOPLASIA

Comment on Ghia et al, page 2651

Hedgehog activation in CLL

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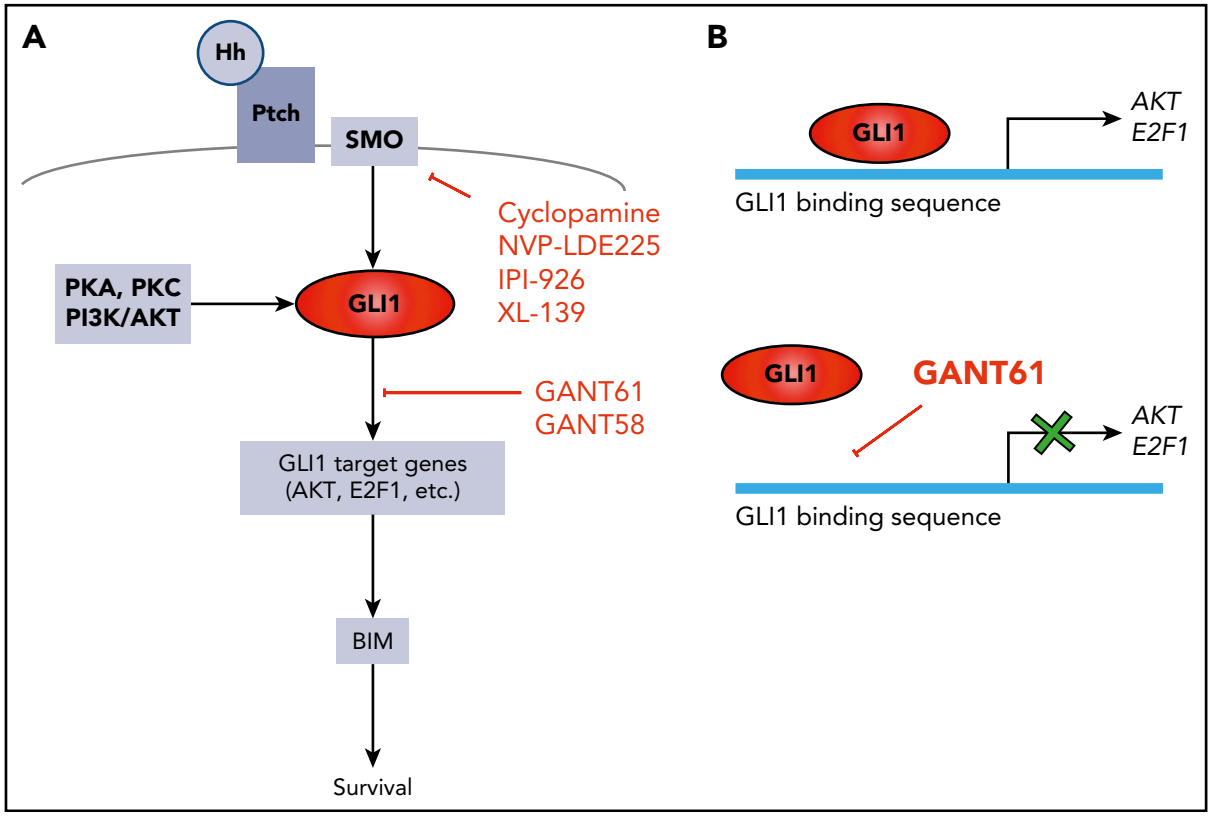
In this issue of *Blood*, Ghia et al observe that a proportion of patients with chronic lymphocytic leukemia (CLL) harbor mutations in genes involved in the Hedgehog (Hh) pathway resulting in expression of glioma-associated oncogene homolog 1 (GLI1). They also find that GLI1 is expressed in CLL cells without evidence of Hh mutations and that the high GLI1 levels correlate with disease progression.[1]

A remarkable feature of CLL is the extremely variable clinical behavior, which is known to be influenced by environmental interactions and by genetic changes. There are 2 types of genetic changes affecting CLL. One type is somatic hypermutations of the B-cell receptor (BCR) immunoglobulin gene variable (IGV) region. These mutations are naturally acquired in the B cell of origin and are preserved in the entire CLL clone after transformation. CLL patients with unmutated tumor IGV (U-CLL), of pregerminal center (GC) origin, have a more rapid disease progression than CLL patients with mutated tumor IGV (M-CLL), of post-GC origin.[2-4] Variability of outcome likely reflects a variable grade of antigen-driven BCR anergy, which is particularly prominent in M-CLL cells.[5] The second type is tumor lesions of genes involved in relevant signaling/metabolic pathways.[6] These pathways include those associated with BCR signaling, NOTCH1 signaling, inflammation, WNT signaling, chromatin modification, response to DNA damage, cell cycle control, and RNA processing.[6]

By focusing their attention on genes not involved in any of these known pathways, Ghia et al have identified a new set of recurrently mutated genes associated with the Hh signaling pathway. This pathway is very important for development, proliferation, and differentiation of mammalian cells. It is activated by the



Hedgehog pathway targets for potential therapeutic attack. (A) GLI1 activation is central in the Hh pathway. In the absence of mutations, canonical GLI1 activation occurs following binding of Hh protein to Ptch receptor and consequent activation of SMO. GLI1 can also be induced by PKA, PKC, or PI3K/AKT (SMO-independent GLI1 activation). Hh signaling can be inhibited by constructs that target SMO or by inhibitors of GLI1-induced transcription. (B) GANT61 is a GLI1 antagonist that inhibits GLI1-induced transcription by binding to the GLI1 consensus sequence in the promoters of the target genes.

binding of Sonic Hh (SHh), Desert Hh (DHh), or Indian Hh (IHh) ligands to Patched1 or Patched2 (PTCH1-2) receptors, eventually resulting in the activation of GLI1 transcription factor by Smoothened (SMO) (see figure). However, other ligand-independent mechanisms can lead to GLI1 activation. Gene mutations resulting in activation of GLI1 have been described in a variety of cancers in which the Hh pathway is important,[7] whereas information on the Hh pathway in CLL has remained circumstantial.[8,9] In their article, Ghia et al document mutations of Hh pathway-associated genes in 11% of CLL cells. The acquisition of mutations in any of these genes, including the epigenetic regulators EZH2 and CREBBP, associates with expression of GLI1 and predicts for relatively short time to first treatment, implying that activation of Hh signaling has a critical role for disease progression in CLL.

The importance of Hh pathway activation in CLL is further highlighted by some additional observations. One is that almost 40% of CLL cells that do not harbor Hh pathway gene mutations also express GLI1 and that GLI1 expression then seems to inform disease progression independently of Hh mutations. This is particularly evident in M-CLL, whereas other genetic lesions associated with more rapid outcome are less frequent.[5] Another observation is that viability of GLI1$^+$ CLL cells, but not GLI1$^-$ CLL cells, is significantly reduced by GLI1-specific small interfering RNA (siRNA) or by the GANT61 small molecule inhibitor of GLI1. Inhibition by either siRNA or GANT61 leads to reduced expression of GLI1 gene targets E2F1 and AKT1 and ultimately of BIM.[10]

Other Hh pathway inhibitors, including SMO inhibitors, have been proposed for therapeutically attacking cancer cells. However, SMO inhibitors would be able to block ligand-dependent Hh pathway activation, but they would still be ineffective in CLL cells that harbor GLI1 proactivating mutations of SMO or downstream molecules.[9] The attraction of inhibitors such as GANT61 is that they may successfully operate in all the GLI1$^+$ CLL cells by blocking either ligand-dependent or ligand-independent (by proactivating gene mutations) Hh pathways. The data from the Ghia et al study suggest that an approach with molecules targeting GLI1 may not be particularly toxic, because it does not seem to affect GLI1$^-$ cells.

These new results indicate that Hh seems to be another critical pathway that needs further investigation in CLL to help us better understand pathogenesis of a large subset of CLL cells (~40%). This may be one of the subsets in which we will need to assess new molecules for therapeutic attack, when the currently available precision therapies, such as BCR-associated inhibitors or BH3 mimetics will fail to overcome the adaptability of CLL tumor cells for survival.

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

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MYELOID NEOPLASIA

Comment on Pecquet et al, page 2669

Mutated *CALR*: tails from the crypt

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The discovery by exome sequencing that calreticulin (*CALR*) gene mutations are important gain-of-function myeloproliferative neoplasm (MPN) driver mutations[1,2] was as inexplicable as it was unexpected. In this issue of *Blood*, Pecquet et al[3] report that mutated CALR behaves like a rogue chaperone, usurping the role of JAK2 and promiscuously transporting immature or traffic-defective thrombopoietin receptors (TpoRs) to the cell surface from the endoplasmic reticulum (ER) (see figure).

The MPNs, polycythemia vera, essential thrombocytosis, and primary myelofibrosis, are clonal hematopoietic stem cell (HSC) disorders because all MPN driver mutations are expressed in HSCs. MPN driver mutations directly or indirectly activate JAK2, the cognate tyrosine kinase of the erythropoietin receptor (EpoR) and the TpoR (also referred to as MPL). JAK2 can also be used by the granulocyte colony-stimulating factor (G-CSF) receptor, which accounts for the shared clinical features of the MPNs.

JAK2, however, is not only a tyrosine kinase, it is a chaperone for the EpoR and the TpoR. Specifically, with the TpoR, JAK2 enhances its stability, its cell surface presence, and its total cellular concentration.[4] The EpoR and TpoR also differ in other important ways. EpoRs are transported to the cell surface fully mature; TpoRs are transported to the cell surface in immature and mature conformations, both of which engage in signal transduction.

EpoRs are downregulated from the cell surface and degraded after signal transduction by proteasomal and lysosomal pathways. TpoRs recycle to the cell surface after signal transduction and downregulation, presumably because they are essential for HSC maintenance, as well as for hematopoietic progenitor cell proliferation. TpoRs also have a reduplicated cytokine receptor homology domain (CRHD), where thrombopoietin (Tpo) and mutated CALR bind, and which is a hot spot for inherited mutations causing thrombocytosis or thrombocytopenia. Because TpoR is the only hematopoietic growth factor receptor in HSCs, MPNs are functionally thrombopoietin receptor disorders.

Calreticulin is a multifunctional soluble ER-resident protein that is responsible for folding and glycosylation of nascent glycoproteins and ER calcium homeostasis; although wild-type CALR has functions outside the ER,[5] it is not a protein chaperone and does not bind wild-type TpoR or TpoR mutants. Structurally, CALR has an N-terminal ER-localizing signal sequence, an N-terminal lectin-(glycan) binding domain, a P domain with high-affinity Ca^{2+} binding sites, and a negatively charged C-terminal tail with low-affinity Ca^{2+} binding sites and a KDEL ER-retention sequence.

CALR driver mutations, all in exon 9, include a 52-base deletion (designated type 1) and a 5-base insertion (designated type 2), both of which cause a +1-base frameshift, resulting in a positively charged C-terminal tail (shorter in the type 1 mutation) lacking the KDEL ER-retention sequence.[1,2]

Previous studies demonstrated that the lectin-binding domain and the neomorphic positively charged C-terminal tail of mutated CALR are essential for TpoR binding and activation[6,7]; that mutated CALR must bind to the TpoR distal extracellular CRHD as a homomultimer for JAK2 activation[8]; that mutated CALR binds strongly to the TpoR, weakly to the G-CSF receptor, and not at all to the EpoR[9]; and, finally, that cell surface expression of the mutated CALR–TpoR complex is mandatory for TpoR activation and JAK2 signaling[6] (see figure). By itself, mutated CALR has no oncogenic or paracrine activity.

Pecquet et al now demonstrate that the TpoR contains a hydrophobic patch in its distal CRHD, which is essential for mutated CALR binding and is absent in the EpoR, explaining the lack of EpoR activation by the mutant protein. They also demonstrate that mutant CALR can transport incompletely glycosylated wild-type TpoR from the ER through the Golgi apparatus to the cell surface, as well as rescue from ER retention the TpoR MPL^{R102P}, which causes congenital amegakaryocytic thrombocytopenia (CAMT), as well as other ER traffic-defective TpoRs.

Importantly, wild-type TpoR bound to mutated CALR could still respond to Tpo, the plasma level of which is elevated in the MPN, whereas the TpoR MPL^{R102P}, once rescued, could respond to a Tpo mimetic.

The report by Pecquet and colleagues suggests, first, that attention currently