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**MYELOID NEOPLASIA**

Comment on Schlenk et al, page 3441

**Allelic ratio: a marker of clonal dominance**

Soheil Meshinchi  
FRED HUTCHINSON CANCER RESEARCH INSTITUTE

In this issue of Blood, Schlenk et al provide compelling data on the prognostic implications of FMS-like tyrosine kinase 3 (FLT3)/internal tandem duplication allelic ratio (ITD-AR), as well as its impact on response to allogeneic stem cell transplantation.1

They provide detailed analysis of data from a large cohort of patients with FLT3-ITD treated in 4 multicenter German-Austrian Acute Myeloid Leukemia (AML) Study Group (AMLSG) trials and correlate the variation in ITD-AR with response to induction chemotherapy and clinical outcome. Given the significant variation in ITD-AR and lack of a preestablished biological threshold, the authors establish a statistically defined cut-point for clinically meaningful ITD-AR of 0.51 for further correlation with clinical outcome. It is important to note that this ITD-AR is similar to those previously defined clinically defined thresholds.2 They demonstrate that those with high ITD-AR have a significantly worse complete remission (CR) rate and correspondingly poor survival and relapse. They further demonstrate that the adverse outcome of high ITD-AR is abrogated by stem cell transplantation. In contrast to patients with high ITD-AR, there appears to be no distinction between those with low ITD-AR and those without FLT3-ITD (FLT3 wild type [FLT3/WT]) in terms of overall outcome or response to hematopoietic stem cell transplantation (HSCT).

To discuss the role of varying ITD-AR in AML, one must address the underlying mechanism for such a wide variation in AR (ranging from 0.01 to >100), which is the ultimate representation of the enormous complexity and genomic heterogeneity of FLT3-ITD-positive AML, as multiple factors contribute to this variation in ITD-AR. The most obvious of these factors is that ITD-AR is a representation of the state of clonal dominance (or lack thereof) of the mutation, where in those with high ITD-AR, FLT3-ITD is the dominant lesion and is present in the majority or all of the leukemic cells. In contrast, in those with low ITD-AR, FLT3-ITD is present in a minor subclone within the bulk leukemic population. Single cell genotyping of leukemic cells from patients with varying ITD-AR established the genomic heterogeneity in FLT3-ITD AML, demonstrating that FLT3-ITD may be present in a major (high ITD-AR) or minor (low ITD-AR) subset of leukemic cells, and defined an association of ITD-AR with the proportion of individual cells that harbor FLT3-ITD and contribute to the final ITD-AR value.3 ITD-AR is further impacted by the associated copy-neutral loss of heterozygosity (LOH) of chromosome 13q (13q CN-LOH) in patients with FLT3-ITD. This genomic variant is the result of a homologous recombination-mediated process where the WT allele is replaced by the allele with FLT3-ITD, resulting in loss of heterozygosity (without copy number loss), leading to evolution of homozygous ITD. Single cell genotyping further demonstrated that in patients with FLT3-ITD, leukemic cells are composed of a mixture of homozygous FLT3-ITD, heterozygous FLT3-ITD, and FLT3/WT, with a final value of ITD-AR determined by the proportion of each genotypic subset.4 The fact that 13q CN-LOH is uniquely observed in the setting of FLT3-ITD may suggest a causal association between FLT3-ITD and evolution of this unique genomic event.

Ultimately, ITD-AR represents the extent of the clonal dominance of FLT3-ITD (with contribution of 13q CN-LOH), where in those with high ITD-AR, FLT3-ITD is present in the majority of the leukemic cells and likely the dominant and driving genomic event. In contrast, in those with low ITD-AR, where FLT3-ITD is present in the minority of leukemic cells, FLT3-ITD is unlikely to be the driving event and contribute to leukemic relapse. The clinical impact of AR has been observed in other mutations by demonstration of the association of the AR of KIT and CBL mutations with outcome.5 These data suggest that we may need to alter our approach to evaluation of diagnostic mutations in AML and determine not only what the mutation is, but whether the mutation is a dominant, driving lesion or a minor variant. An additional layer of data that substantiates the significance of mutation burden at diagnosis is the fact that the majority of diagnostic mutations with low allele fraction (low AR) are not detected at relapse, thus questioning their clinical significance.5

Demonstration of the clinical significance of FLT3-ITD led to the evaluation of HSCT in this high-risk cohort. Initial studies demonstrated that HSCT abrogates the...
adverse prognostic implications of FLT3-ITD, although the role of HSCT, specifically in those with high ITD-AR, was not established. Recent trials have incorporated diagnostic FLT3-ITD in risk-based therapy allocation, where those with FLT3-ITD, especially those with high ITD-AR, are allocated to the high-risk therapeutic arm and receive HSCT in first CR from the most suitable donors. The article by Schlenk et al has adequate power to evaluate the efficacy of HSCT in those with high ITD-AR. They demonstrate that those with high ITD-AR, where FLT3-ITD is the dominant lesion, benefit from HSCT, whereas those with low AR (alternate dominant mutation) show no such benefit, and clinically behave similarly to those without FLT3-ITD. It also highlights the fact that leukemic cells with FLT3-ITD may be especially susceptible to the allogeic effect of the HSCT and allocation of patients with FLT3-ITD patients to HSCT must be more precise and be based on the ITD mutation load and not on the mere presence of the mutation with low AR. Further, one must keep in mind that any prognostic factor is highly dependent on the therapeutic intervention, and the significance of prognostic biomarkers may change with changing therapies. One example is the recent data that patients with FLT3-ITD who received gemtuzumab ozogamicin (GO) may have a more favorable outcome. This observed improvement in outcome with GO raises the caveat that prognostic significance of FLT3-ITD and ITD-AR in patients treated with GO should be evaluated separately from the non-GO recipients. Although the mechanism by which GO mediates improved outcome in patients with FLT3-ITD is not known, it is possible that enhanced response to GO and improved outcome with HSCT may provide therapeutic options in addition to, and in combination with, tyrosine kinase inhibitors and may provide viable targeted options in this select cohort of patients. Further, as those with low ITD-AR behave as FLT3/WT, perhaps only those with high ITD-AR should be targeted for such FLT3-ITD-directed therapies.

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Comment on Rug et al, page 3459

A traffic jam to reduce morbidity in malaria

Hans-Peter Beck

SWISS TROPICAL AND PUBLIC HEALTH INSTITUTE; UNIVERSITY OF BASEL

In this issue of Blood, Rug et al report that genetic disruption of a single malaria parasite protein disturbs recruitment of host actin and protein trafficking, thus disabling parasite adherence to the host endothelial lining, which is the major cause of malaria pathology.

Malaria caused by Plasmodium falciparum still elicits a substantial burden of disease mainly in sub-Saharan countries, where malaria is still endemic despite many efforts to curb the disease. P. falciparum is an aggressive invader of cells, beginning with the invasion of hepatocytes upon the infectious bite of an Anopheles mosquito. Subsequently, after a massive cellular amplification, the P. falciparum blood forms emerge and invade mature erythrocytes. These blood forms can cause severe anemia, but the true damages inflicted by this deadly disease come from structural modifications of the infected erythrocyte caused by the parasite.

It has long been known that the parasite exports a massive number of proteins into the host cell cytosol and beyond, but there has been little evidence of how this works (see figure). In their paper, Rug and colleagues’ share information on the function of exported proteins of P. falciparum and their involvement in the remodeling of the host cell.

Why is this important? Parasite-induced modifications change the form and shape of the infected erythrocyte; they also change both the osmotic regulation of the host cell and the membrane rigidity. Most importantly, the induced modifications convey the ability of infected erythrocytes to adhere to the endothelial lining of the capillaries. Most of this adherence is mediated by a single parasite protein called P. falciparum erythrocyte membrane protein 1 (PfEMP1). This molecule, encoded by a variable parasite gene family, is embedded into the erythrocyte membrane and conveys adherence to a variety of endothelial receptors. This cytoadherence protects the parasite from splenic clearance and is considered the major virulence factor in malaria tropica. Therefore, understanding how PfEMP1 migrates to the surface of the infected cell is extremely important and may lead to