

of relapsed CLL is a landmark event that heralds a new era of targeted therapies for the management of this disease. The drug is generally well tolerated with mostly mild side effects that frequently resolve despite continuation of therapy.⁶ The responses observed are primarily sustained partial responses with occasional complete responses, which appear to be independent of conventional prognostic markers. Similar results were also seen in the current study. However, progression-free survival (PFS) has been shown to be inferior in patients with deletion 17p or 11q.⁶ Interestingly, the redistribution lymphocytosis and its persistence in some patients also do not appear to correlate with PFS.⁷ In addition, minimal residual disease negative state, which could potentially be used as a surrogate for PFS, is rare with single-agent use.⁶

A small percentage of patients treated with ibrutinib develop progressive disease or resistance to therapy, the mechanisms of which are currently being investigated and can at least be partially attributed to specific mutations in the BTK protein itself or downstream targets. With the expected rapid increase in the use of ibrutinib, similar well-designed trials are desperately needed to study not only the mechanisms of action and resistance, but also to obtain better understanding of its off-target effects. Studying the various disease compartments simultaneously will also enable us to evaluate the differential effects of these agents and tumor escape mechanisms, and will enable us to devise rational combination strategies that will hopefully result in the eventual cure of CLL.

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● ● ● MYELOID NEOPLASIA

Comment on Taskesen et al, page 3327

Splicing factor mutations in AML

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In this issue of *Blood*, Taskesen et al propose a novel subset of myeloid neoplasms that encompasses splicing factor (SF) mutation-positive refractory anemia with excess blasts (RAEB) and acute myeloid leukemia (AML), which is composed of 2 molecularly and clinically distinct subgroups.¹

Myeloid neoplasms comprise a wide variety of clinically and pathologically distinct entities commonly characterized by neoplastic proliferation of myeloid lineage cells with deregulated differentiation.² Although straightforward for typical cases, distinction among different subtypes of myeloid neoplasms may not be unambiguous, complicating the diagnosis of and the therapeutic choice in borderline cases. According to the current World Health Organization (WHO) classification system, for example, distinction between AML with low blast counts (AML-LBC) and the RAEB subtype of myelodysplastic syndromes (MDS) is made only by the presence or absence of $\geq 20\%$ bone marrow blasts.² However, these criteria, based on the blast counts, would lead to a diagnosis of AML for some patients who might be biologically/clinically more properly classified as RAEB or vice versa. In this regard, during the past 10 years, our knowledge about the molecular pathogenesis of myeloid neoplasms has been substantially improved by the identification of major driver mutations causally related to these neoplasms,³⁻⁶ which may help better understanding, classification, diagnosis, and management of different entities of myeloid neoplasms.

Taskesen et al focused on pre-messenger RNA SF mutations and tested their hypothesis that SF mutations could define a novel subset of myeloid neoplasm that includes SF mutation-positive RAEB and AML as well as AML-LBC. SF mutations are a novel class

of driver mutations recently identified through whole exome sequencing of myeloid dysplasia.^{7,8} Affecting at least 8 different components of the pre-messenger RNA splicing machinery, including SF3B1, SRSF2, U2AF35, ZRSR2, U2AF65, SF1, SF3A1, and PRPF40B, SF mutations represent among the most frequent genetic lesions in MDS (45% to 85% of MDS depending on their subtypes) and other related myeloid neoplasms with myelodysplasia but much less common (5% to 10%) in de novo AML and classical myeloproliferative neoplasms.^{3,7} In the current study, authors investigated 47 RAEB, 29 AML-LBC, and 325 other AML patients for predominant hot-spot mutations in SF3B1, U2AF35, and SRSF2, and found that patients with RAEB and AML-LBC showed significantly higher SF mutation rates compared with other AML and, except for white cell counts and bone marrow blast counts, shared a highly similar clinical, cytological, and molecular profile. Also, SF-mutated patients among RAEB, AML-LBC, and other AML categories had similar clinical phenotypes, including lower blast counts, older age, lower white cell counts, and higher erythroblast counts in bone marrow compared with SF-unmutated cases, indicating that SF-mutated cases comprised a distinct entity among MDS/AML. In accordance with this, a hierarchical clustering of AML/AML-LBC/RAEB cases based on combined, but not separate, gene expression (GEP) and DNA-methylation profiles (DMP) identified

2 distinct clusters (#3 and #11) highly enriched for SF mutations and RAEB/AML-LBC phenotypes with significantly lower bone marrow blast counts, together with known clusters characterized by the presence of t(8;21), inv(16), t(15;17), and *CEBPA* mutations.^{9,10} Interestingly, whole exome sequencing of 14 cases with SF mutations within the 2 clusters revealed mutations in 3 genes implicated in RNA splicing. Among the 2 clusters, #11 was characterized by an erythroid signature with higher erythroblast percentages and differentially expressed or hypomethylated genes involved in erythroid development, whereas cluster #3 was significantly enriched for *NRAS/KRAS* mutations and poor overall survivals compared with other patients.

Although the conclusions need to be validated further in independent studies with more comprehensive detection of SF mutations, not just hot-spot mutations, the present study points to an intriguing possibility that SF mutations could override the conventional separation between AML and MDS and help to define novel biological subtypes of myeloid malignancies for better understanding and management of AML/MDS. However, the biological basis for the SF-mutated AML is still unclear, as is the reason why the 2 SF-mutated clusters are identified only through combined GEP and DMP analysis. Finally, and more importantly, the impact of the SF-mutated AML or the novel clusters identified through GEP/DMP profiling on the choice of therapies and patients' outcomes should be clarified before these subtypes are found to be relevant to clinical practice.

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● ● ● RED CELLS, IRON, & ERYTHROPOIESIS

Comment on Atkinson et al, page 3221

The iron fist: malaria and hepcidin

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In this issue of *Blood*, Atkinson and colleagues document dynamic fluctuations in plasma concentrations of hepcidin, the master regulator of iron homeostasis, in African children during malaria transmission season and further show that low hepcidin levels and iron deficiency are more prevalent at the end of the malaria season, implying that iron therapy may be most beneficial at this time.¹

Our understanding of iron homeostasis in humans has made huge strides in the last decade, and Atkinson and colleagues have now brought the new knowledge to bear on the troubling clinical problem of iron deficiency in malaria.¹ Iron deficiency with or without anemia commonly accompanies malaria, especially in cases with a high parasite burden, and is too often seen in African children. More than 250 million episodes of febrile illness due to malaria occur each year in sub-Saharan Africa. Although iron supplements are known to promote their development, young children have been denied this benefit because of the fear that an elevated iron concentration could increase their susceptibility to malaria and other infectious diseases.^{2,3}

Recent studies on iron homeostasis have shown us that iron absorption and recycling are tightly regulated by plasma hepcidin levels which are themselves affected by infection, inflammation, and iron loading, as well as by iron deficiency and ineffective erythropoiesis.^{4,5} High levels of hepcidin suppress iron absorption whereas low hepcidin levels have the contrary effect. It has been found, moreover, that hepcidin levels rise following infection by malaria parasites, which makes it unlikely that iron supplements would afford any significant benefit in treatment of iron deficiency in malaria at the point in the

infectious disease cycle.^{6,7} So far, so good, but these discoveries raise new and deeper questions, and until now there has been no data on fluctuations of hepcidin levels and iron status in any sizeable cohort of children during the malaria season. It is in this context that the findings of the present study, showing that plasma iron concentration and hepcidin levels in children in 2 different parts of Africa vary with time through the malaria season, should be seen. That hepcidin levels are low and prevalence of iron deficiency is high at the end of the malaria season suggests that iron therapy will be most beneficial for these children when administered during this time window, so as to minimize the adverse effects of iron on malaria victims, while delivering at the same time optimal correction of iron deficiency through increased iron absorption, by reason of the low hepcidin levels.

The work of Atkinson et al represents a start toward taming both the “iron fist” and the complex problem of disentangling the many factors contributing to the regulation of hepcidin levels in malaria-endemic areas of Africa. These factors include such imponderables as nutritional deficiencies, the exact role of iron in malaria pathogenesis, and the role of parasites and inflammation themselves on iron regulation, recycling, and bioavailability. Thus, many questions remain. The authors have restricted