IMiDs, principally thalidomide and its derivatives lenalidomide and pomalidomide, have received approval by the US Food and Drug Administration to treat patients with multiple myeloma or mantle cell lymphoma. IMiDs bind to cereblon (CRBN), which is involved in the formation of a ubiquitination ligase complex, and further promote the selective degradation of specific substrates, notably the Ikaros family transcription factor IKZF1.[4] The IKZF family of transcription factors plays a crucial role in the differentiation and development of immune cell populations.[5] Mutations and copy number alterations affecting the *IKZF1* locus have been reported in several hematologic malignancies, including myeloproliferative neoplasms[6] and B-cell[7] and T-cell acute lymphoblastic leukemia.[8] However, our understanding of the role and function of IKZF1 in TCLs is very limited. Even though *IKZF1* has been identified as a vulnerability in some TCL-derived cell lines by CRISPR/Cas9 screens,[9] suggesting that IMiDs should be able to target IKZF1 in TCLs, IMiDs have shown disappointing activity in TCLs.[10] There are still gaps in understanding the mechanisms underlying TCL resistance against treatment with IMiDs.

In the article by Wu et al, elegant and robust in vitro and in vivo experimental approaches in multiple TCL lines and patient-derived xenografts demonstrated that selective resistance to IMiDs in TCLs involves codependence of IKZF1 and ZFP91. They also addressed the question of whether resistance to IMiDs is simply a result of pharmacologic failure or a lack of dependence on the identified target proteins IKZF1 and ZFP91. They found a close correlation between the CRBN expression levels and the extent of IMiD-induced IKZF1 degradation. In addition, when CRBN overexpression was induced in IMiD-resistant TCL cells, the degree of IKZF1 and ZFP91 degradation was markedly increased, and the cells were re-sensitized to treatment with IMiDs. On the basis of these results, the investigators hypothesized that limited CRBN expression reduces IMiD activity in TCLs and therefore, it is highly likely that a more potent degrader can overcome IMiD resistance in TCLs. They then explored the efficacy and clinical potential of CC-92480, an agent from a new class of CRBN E3 ligase-modulating drugs that share the same glutarimide rings with IMiDs for CRBN- and substrate-binding in IMiD-resistant TCLs in vitro and in vivo. CC-92480 was broadly effective against multiple TCL subtypes and showed higher potency than IMiDs by an ~100-fold lower concentration with selective yet robust degradation of IKZF1 and ZFP91 across IMiD-resistant TCL lines. Moreover, using preclinical models of several subtypes of TCL patient-derived xenografts, CC-92480 induced tumor regression and prolonged survival in vivo. Thus, CC-92480 overcame IMiD resistance in TCLs by targeting IKZF1 and ZFP91, which suggests that this novel class of agents shows a good therapeutic window for clinical use in patients with TCLs.

Overall, the results presented by Wu et al expand our understanding of IMiD resistance mechanisms in TCLs (see figure) and show the potential of the novel agent CC-92480 in overcoming IMiD resistance in TCLs. However, the clinical safety and efficacy of this agent is currently being investigated only in trials in multiple myelomas but not in TCLs. Considering the important insights regarding disease biology revealed by this study, more studies using IMiDs to investigate prospects for improving outcome of patients affected by TCLs are certainly warranted.

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MYELOID NEOPLASIA

Comment on Clough et al, page 2038

RNA missplicing and ring sideroblasts in MDS

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In this issue of *Blood*, Clough et al[1] report an induced pluripotent stem cell (iPSC) model of *SF3B1*-mutant MDS that recapitulates ring sideroblast formation during in vitro erythroid differentiation. Their findings show that coordinated mis-splicing of mitochondrial transporters *TMEM14C* and *ABCB7* by mutant SF3B1 sequesters iron in mitochondria, causing ring sideroblast formation.

In Ancient Greek, σιδηρος (*sideros*) means iron. In 1954, Kaplan et al used the term "sideroblasts" to define erythroblasts containing cytoplasmic iron granules stainable with the Prussian blue (or Perls) reaction, analogous to the term "siderocytes" that was already in use to designate circulating red cells containing

blue-staining granules.[2] Using the electron microscope, Bessis and Breton-Gorius found that these granules were aggregates of ferritin.[3] In normal individuals, approximately one-third of bone marrow erythroblasts contain 1 to 3 Perls-positive granules in their cytoplasm, representing endosomes filled with excess iron not used for heme synthesis (siderosomes). These normal erythroblasts are defined as "ferritin sideroblasts."

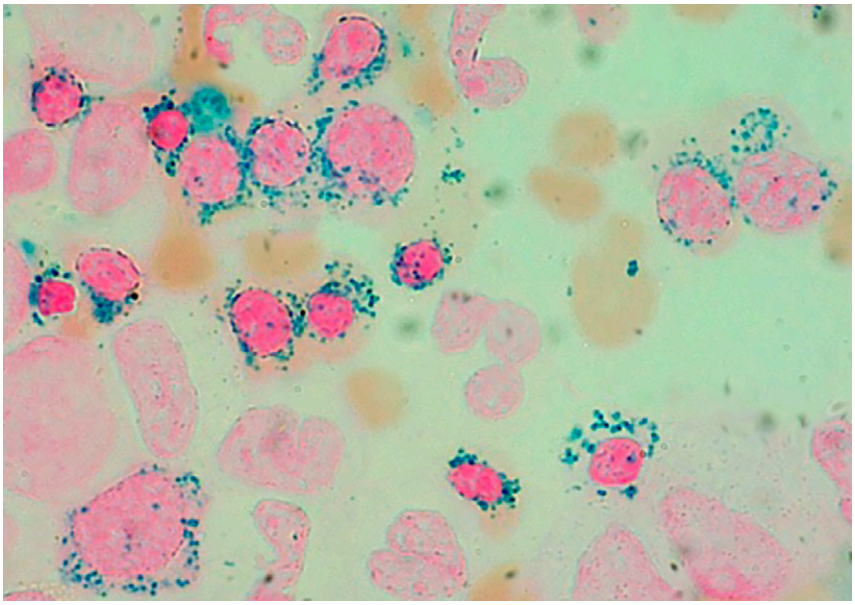
The term "ringed (or ring) sideroblasts" was coined by Bowman in 1961 to define abnormal sideroblasts that have exceptional accumulations of iron-positive granules surrounding the nucleus like a ring.[4] Bowman described 2 categories of patients with ring sideroblasts: (1) cases of refractory normoblastic anemia with ineffective erythropoiesis, and (2) young males with hereditary, hypochromic anemia with hyperplastic, normoblastic marrow.[4] These 2 categories are now defined as myelodysplastic syndrome (MDS) with ring sideroblasts (MDS-RS), and X-linked sideroblastic anemia associated with *ALAS2* mutations, respectively. Bessis and Breton-Gorius found that in patients with ring sideroblasts, iron granules were not cytoplasmic aggregates of ferritin, but rather perinuclear mitochondria filled with iron-containing material termed "ferruginous micelles."[3] More recently, we showed that the iron deposited in perinuclear mitochondria of ring sideroblasts is present in the form of mitochondrial ferritin, a protein encoded by an intronless gene and highly expressed only in the testis under normal conditions.[5]

When, as a trainee in hematology, I started examining bone marrow aspirates from patients with refractory anemia, I found ring sideroblasts not only extremely useful for the recognition of morphologic dysplasia but also beautiful. Images like those reported in the figure reminded me of paintings of the French "*pointillisme*" (see figure). However, despite my strong interest, it took me a long time to understand their pathogenesis in MDS. In 2010, within the Chronic Myeloid Disorders Working Group, we decided to employ massively parallel sequencing technology to identify somatic mutations in MDS patients. We reasoned that ring sideroblasts represented a distinctive morphological abnormality that was likely to be underpinned by distinctive genetic lesion(s). Indeed, of the 8 patients with MDS-RS we initially examined, 6 had a somatic mutation in *SF3B1*.[6] Follow-up studies showed a close relationship between somatically acquired *SF3B1* mutation and ring sideroblasts in myeloid neoplasms.[7]

Exploiting a unique morphological abnormality was instrumental in defining a new disease paradigm, which opened novel avenues of research. In a subgroup of MDS-RS patients with a somatic *SF3B1* mutation, this was the only genetic lesion found. Therefore, we asked a fundamental question: how does a somatic mutation in a splicing factor gene result in a clinical phenotype. Precursor messenger RNA splicing is catalyzed by the spliceosome, a macromolecule composed of small nuclear RNAs associated with proteins, with ~100 000 spliceosomes in every human cell. As the *SF3B1* mutation is heterozygous in MDS-RS, hematopoietic cells contain both normal and SF3B1-mutant spliceosomes, approximately in the same proportions. In turn, this means that about half of splicing events are run by normal spliceosomes, whereas the remaining half are controlled by spliceosomes with a mutant SF3B1 splicing factor. To understand the consequences of this dual RNA splicing, we performed transcriptomic analyses of bone marrow cells from MDS patients.[8] *SF3B1* mutation was associated with aberrant 3′ splice-site selection, degradation of mis-spliced transcripts because of a premature termination codon (nonsense-mediated decay), and reduced production of canonical transcripts. Three of mis-spliced genes (*ABCB7*, *PPOX*, and *TMEM14C*) were involved with heme biosynthesis and mitochondrial iron metabolism. This suggested that the defective production of the proteins encoded by these genes played a role in ring sideroblast formation, but there was no convincing experimental proof of this hypothesis.[9]

In their elegant studies, Clough et al show first that iPSC-derived MDS-RS progenitors efficiently form ring sideroblasts in late-stage erythroblasts. Then, using deep RNA-seq, they studied mutant *SF3B1* mis-splicing during iPSC erythroid differentiation. Approximately 100 genes were mis-spliced throughout erythroid differentiation, and aberrant transcripts included *TMEM14C*, *PPOX*, and *ABCB7*, resulting in a significant reduction of protein expression. Whereas functional rescue of PPOX had no effect on ring sideroblast formation, that of TMEM14C and ABCB7 markedly reduced this process; of note, combined TMEM14C/ABCB7 rescue nearly abolished ring sideroblast formation. Thus, coordinated mis-splicing of *TMEM14C* and *ABCB7* causes ring sideroblast formation in *SF3B1*-mutant MDS.

This study clarifies the pathogenesis of the striking morphologic abnormality of



Prussian blue staining of a bone marrow smear from a patient with MDS-RS (Division of Hematology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy). Ring sideroblasts include both polychromatic and orthochromatic erythroblasts. Original magnification, ×1250.

patients with refractory normoblastic anemia that Bowman described and named 60 years ago.[4] It represents a prototype of the functional studies that should be conducted in splicing factor mutant-neoplasms to understand how abnormal splicing results in abnormal cell differentiation and maturation. Ideally, these studies should be aimed not only at deciphering disease pathogenesis but also at developing novel effective treatments.

SF3B1-mutant MDS is a distinct disease subtype, mainly characterized by ineffective erythropoiesis and a relatively indolent clinical course.[10] The major factor in the pathogenesis of anemia in *SF3B1*-mutant MDS is represented by the apoptosis of late-stage erythroblasts, that is, polychromatic and orthochromatic erythroblasts. As these erythroid precursors are also characterized by ring sideroblast formation, a causal relationship between mitochondrial iron overload and increased propensity to apoptosis is likely. However, this relationship needs to be verified experimentally, and this might be the next task of the Doulatov laboratory in Seattle. Patients with MDS-RS may respond to luspatercept with the abolition of their transfusion requirement, but how this compound targets ineffective erythropoiesis in this MDS subtype has never been definitely documented. Verifying how luspatercept improves red cell production in MDS-RS is therefore important. However, the approach described by Clough et al might also lead to the identification of new targets and the discovery of novel drugs.

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TRANSPLANTATION

Comment on Albert et al, page 2066

Towards a standard of care in transplant for WAS

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In this issue of *Blood*, Albert et al[1] continue the tradition established in 1968 of punctuating the timeline of hematopoietic transplant with important advances in the treatment of this rare nonmalignant disorder. In their article, they demonstrate that, even in the absence of prospective trials, uniform adoption of a limited number of regimens combined with the use of novel composite end points allows a meaningful analysis that establishes a standard of care for these boys.

Wiskott-Aldrich syndrome (WAS) was first identified in 1936 and affects just 1 in ~100 000 males born worldwide, causing bleeding, immune deficiency, and eczema, in addition to a predisposition to autoimmunity and malignancy. The identification of the *WAS* gene in 1994 quickly led to the realization that a range of disease severity was related to mutations in the *WAS* gene and that disease severity could be predicted by protein expression.[2] For some boys with WAS, prognosis based on phenotype can be made at the time of diagnosis. Although advances in diagnosis and supportive care have improved the outcome for boys not undergoing definitive therapy, especially those with less severe disease, patients with severe disease continue to have a poor chance of surviving into the third decade.[2] Stem cell transplantation has long been studied as a definitive therapy for WAS. One of the first allogeneic hematopoietic stem cell transplants (HSCTs) was performed in 1968 in a boy with WAS.[3] This child had only partial correction of disease. Subsequent studies showed that myeloablation, initially with total body irradiation[4] and shortly thereafter with busulfan,[5] was associated with durable and complete chimerism and improved outcomes. Correction of the WAS phenotype was one of the first targets of ex vivo gene therapy; however, unfortunately, it was also one of the first examples of the potential genotoxicity of γ-retroviral vector therapies.[6]

Cooperative groups have performed retrospective analyses for this rare genetic disorder, documenting serial improvements in transplant outcomes, especially those using alternative