Unlocking the dysplasia puzzle

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In this issue of Blood, Zhou and colleagues describe preclinical results in vitro and in vivo, showing constitutive TGF-β activation in MDS and enhanced hematopoiesis following inhibition of TBRI kinase in MDS.

These investigators have previously shown that p38 MAP kinase regulates stem cell apoptosis in human hematopoietic failure. Moreover, a small-molecule inhibitor, SCIO-463 of the p38 MAP kinase pathway, improves hematopoiesis in myelodysplastic syndrome (MDS) progenitors in vitro. The elegant experiments by Zhou et al extend their study of TGF-β signaling in MDS and show the constitutive downstream activation of smad2 in MDS bone marrow precursors and its overexpression in MDS-derived CD34+ cells. Suppression of TGF-β activity by shRNA down-regulation of TGF-β receptor I kinase (TBRI) as well as pharmacologic inhibition of TBRI (alk5) by a small-molecule inhibitor (SD-208) leads to a reversal of this TGF-β–mediated inhibition of hematopoiesis in MDS. Furthermore, SD-208 treatment alleviates anemia and stimulates hematopoiesis in vivo in a novel murine model of bone marrow failure generated by the constitutive hepatic expression of TGF-β1. Finally, the enhancement of hematopoiesis seen in several MDS subtypes exposed in vitro to SD-208 underscores the importance of TBRI as a potential therapeutic target in low-risk MDS.

Stem cell apoptosis in MDS, illustrated in the figure, is a facet of the heterogeneity of this disease and the interplay of various mechanisms affecting the marrow microenvironment as well as progenitor proliferation and apoptosis. Ineffective hematopoiesis in MDS may be intrinsic to dysregulated gene expression as well as resulting from dysfunction of cell-to-cell contacts within the stromal microenvironment. TGF-β over-activation in MDS leads to altered stromal cytokine expression with decreased IL-7 and decreased B-cell proliferation and enhanced IL-1β and TNFα associated with increased stromal IL-6, IL-8, and IL-32 expression. These proinflammatory cytokines are associated with natural killer cell dysfunction and may lead to programmed cell death of all hematopoietic cell lineages via autophagy or apoptosis.

Our understanding of the molecular pathobiology of MDS and its progression to acute myeloid leukemia (AML) has been made possible by advances in unraveling the molecular underpinnings of acute and chronic leukemias and myeloproliferative syndromes. Intrinsic stem and progenitor cell abnormalities in MDS may be attributed to altered DNA methylation and gene silencing. This affects specific hematopoietic lineages, such as Survivin in erythropoiesis and WT-1 and CHK2 during granulopoiesis, as well as structural genetic alterations. While this report does not address the importance of gene silencing and altered differentiation programs in MDS, it nevertheless offers a clear rationale to test compounds such as SD-208 in all phases of MDS. Correlative studies will be needed to determine whether inhibition of TBRI can alter cytokine, chemokine, and oncogene expression profiles in this disease.

Currently approved treatments in MDS are now directed at intermediate and high-risk patients and include immunomodulators, such as lenalidomide and hypomethylating agents 5-azacytidine and decitabine. Patients with low-grade MDS in the future can look forward to these novel hematopoietic enhancing treatment modalities, which provide an alternative to supportive care, transfusions, and growth factors. Finally,
since reactive oxygen species can damage stem cell DNA and may accelerate clonal evolution from MDS to AML, it remains to be seen if the early use of SD-208 and SCIO-463 small-molecule inhibitors of TBRI and p38 MAPK will result in delayed disease progression and improved survival in MDS.

**Conflict-of-interest disclosure:** The author declares no competing financial interests.

**REFERENCES**


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**Comment on Rimsza et al, page 3425**

**Blocks to paraffin get the CHOP, +R**

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In this issue of Blood, Rimsza and colleagues present an embarrassment of riches. They report the use of a multiplex quantitative nucleic acid protection assay to measure, in FFPET, prognostic genes for DLBCL in patients treated with rituximab. Their approach overcomes 3 significant problems in the field, namely the difficulty of working with FFPET, the requirement for a simple method for clinical measurement of prognostic genes, and the need for a prognostic gene signature applicable to patients treated in the rituximab era.

Microarray gene expression profiling has resulted in an exponential increase in the understanding of cancer. It has also identified a plethora of putative novel diagnostic and prognostic biomarkers. These are increasingly important as traditional markers, either clinical or cellular, are beginning to lose discriminatory power, especially in the emerging era of personalized medicine. However, almost all diagnostic material is in the form of formalin-fixed paraffin embedded tissue (FFPET), in which gene expression profiling is compromised by RNA degradation and cross-linking, and this has hindered translation of microarray-identified biomarkers to clinical practice. Several groups have sought to overcome this, either by use of modified RNA extraction and labeling for microarray analysis, by measurement of candidate prognostic genes by real-time PCR or by bead-based approaches, such as the DASL platform. These studies demonstrate the feasibility of gene expression profiling in FFPET but admit reduced sensitivity compared with fresh/frozen tissue, though Malumbres and colleagues reported successful measurement of a 6-gene model in FFPET in diffuse large B-cell lymphoma (DLBCL) patients treated with R-CHOP. Additionally, while real-time PCR is possible in FFPET, there remains a need for multiplex measurement of several genes, particularly as the power of prognostic gene signatures is often based on more than one gene. Furthermore, identification of specific prognostic groups often suggests new drug targets, increasing the importance of FFPET-based studies capable of extending the range and depth of clinical material for translational study.

The biology and behavior of DLBCL is highly heterogeneous. Several gene expression profiling studies have provided greater prediction of disease behavior, separating DLBCL into at least 2 distinct molecular subtypes: a favorable germinal center B-cell type (76% 5-year survival) and an unfavorable activated B-cell type (16% 5-year survival). Rituximab has dramatically altered outcome in DLBCL and its introduction has been contemporaneous with these studies. Therefore, their results must be tempered by their analysis of patients treated in the pre-rituximab era, with some reports of loss of prognostic distinction between germinal center (GC) and non-GC types in patients treated with rituximab. Monti et al addressed this problem by unsupervised analysis of samples of DLBCL from untreated patients to determine an a priori biological signature predictive of new therapeutic targets, demonstrating the importance of identifying a prognostic gene signature in patients treated with rituximab.

The paper by Rimsza et al uses an elegant novel methodology to solve, at a single stroke, the multiple problems outlined above. The
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