data from sub–Saharan Africa for some of these interventions, including penicillin prophylaxis, immunizations, and antimalarials. They provide credible evidence from studies in the United States and western Europe to support their assertion that further clinical efficacy trials in Africa may be unnecessary to proceed with implementation of these measures. Are there additional screening studies or interventions that are feasible? Could hydroxyurea reduce vaso-occlusive pain episodes and acute chest syndrome in Africa while also reducing early mortality as it has in the United States?[5] There are limited data on safe blood banking in Africa, yet transfusions have been an effective intervention for numerous SCA acute and chronic complications.[6] Stem cell transplantation and other curative therapies are appealing as definitive treatments, however, donor availability and high costs appear to put these interventions out of reach for now.

Although the clinical and public health aspects of SCA are foremost, enhanced research partnerships could also profoundly impact SCA in Africa. Many fundamental observations of SCA have stemmed from research in Africa, including the relative protection of sickle cell trait against malaria, and the identification of β-globin haplotypes from the multiple geographic loci from which the sickle mutation originated. Given the high burden of disease in African countries, opportunities for collaborative research in Africa could enhance our understanding of genotype–phenotype relationships, identify additional genetic modifiers, and reduce sickle cell–related mortality with early detection and improvements in care.

This review is timely in that it highlights several of the American Society of Hematology (ASH) Sickle Cell Research Priorities, and supports the ASH Sickle Cell Disease Call to Action as well as initiatives by the National Heart, Lung, and Blood Institute to address global issues in SCA. Understanding the opportunities as outlined in this manuscript is a critical step in making a profound impact on conquering SCA worldwide.

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● ● ● **CLINICAL TRIALS AND OBSERVATIONS**

Comment on Abu Zaid et al, page 162

Transplant biomarkers ready for the clinic?

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Biomarkers promise to refine the prediction of allogeneic stem cell transplantation (SCT) outcomes. In this issue of *Blood*, a phase 3 clinical trial reported by Abu Zaid et al brings us closer to routine biological profiling of major complications that occur after allogeneic SCT.[1]

In the last few years, pioneering studies, notably by Paczesny and colleagues, have discovered a range of molecules that can be assayed in plasma, which has proven to be strongly related to some key transplant complications that define transplant survival.[2]
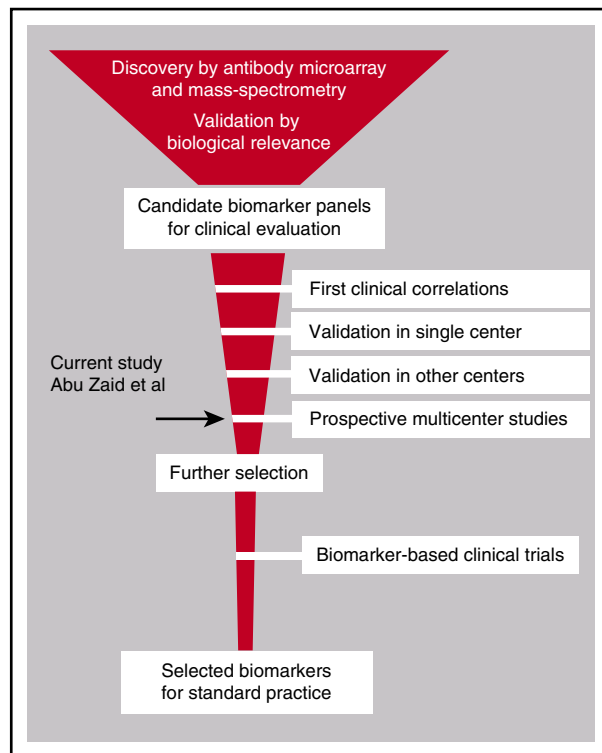
Predictive biomarkers of stem cell transplant outcome: The road from biomarker discovery to general clinical application through stepwise clinical validation.

To reach this point basic research has been required to identify potential biomarkers in an unbiased manner, together with rigorous correlative statistics to find strong and true associations between biomarker variations and posttransplant events, such as the overall outcome (survival or death) and specific complications, such as graft-versus-host disease (GVHD). Initially, biomarker research focused on acute GVHD. First, an unsupervised and giant array of markers was pared down to a shortlist of a dozen or so candidates strongly consistent with acute GVHD outcome. Subsequently, the most reliable and strongly correlative markers were selected. Validation of candidate biomarkers required correlative analysis of biomarkers with large groups of patients developing training sets and subsequent validation sets. Initial trials were performed within a single institute.[3] Over time, new and better biomarkers were discovered, and further studies have validated some key biomarkers: suppression of tumorigenicity-2 (ST2) as a predictor for acute GVHD, and in particular, steroid refractory GVHD and nonrelapse mortality (NRM),[4] Reg3α as a predictor of gastrointestinal GVHD,[5] chemokine (C-X-C motif) ligand 9 (CXCL9), associated with chronic GVHD,[6] and L-ficolin in association with sinusoidal obstruction syndrome/veno-occlusive disease (SOS/VOD).[7]

Although many factors are already known to determine transplant outcome (age, comorbidity, donor-recipient compatibility, and investigator-determined issues, such as choice of conditioning regimen and posttransplant GVHD prophylaxis), they are incomplete guides for predicting outcomes. Biomarkers promise to further refine our ability to determine the likely outcome of the individual patient, making possible the prevention of complications by individualizing the treatment approach.[2] For this to become a reality, it has to be demonstrated that biomarkers are robust and sure predictors of outcomes across a variety of transplant approaches performed in any center (see figure). In this issue, Paczesny's group has moved the field closer to this goal. Collaborating with the Blood and Marrow Transplant Clinical Trials Network, they prospectively measured a set of previously validated biomarkers in patients participating in a multicenter trial comparing GVHD prophylaxis with tacrolimus and sirolimus vs

tacrolimus and methotrexate. The study involved 304 patients transplanted in 23 US transplant centers.[8] Blood samples for biomarker analysis were collected at fixed time points between days 28 and 365 after SCT in 211 individuals. Critically, the trial found no significant difference in GVHD between the 2 study arms, although there was slightly more SOS/VOD in the tacrolimus/sirolimus set. After multivariate analysis in a proportional hazards model with time-dependent coordinates, they identified 4 biomarkers associated with outcome high day 28 ST2 and Tim3 correlating with NRM and survival, low L-ficolin correlating with SOS/VOD, and high CXCL9 correlating with chronic GVHD.

The predictive value of a biomarker is dependent upon the quality of the statistical analysis, the reliability of the clinical readout, and above all, the relevance of the biomarker to the biological process underlying the clinical event. This study followed the required norms of statistical evaluation involving a sufficiently large, prospectively studied, patient cohort. Multivariate analysis and allocation of proportional hazards ensured the identification of independent determinants of outcome. The study was conducted under the rigor of a well-organized clinical trial. The biological relevance of the winning markers is also a strength, and the mechanisms are discussed in the paper: ST2 in plasma is the soluble form of the interleukin-33 (IL-33) receptor, acting as a decoy receptor for IL-33 and preventing the binding of IL-33 to T cells. The observation by this group that an antibody to ST2 prevents GVHD in a mouse model strongly links this molecule with the GVHD process.[9] Tim-3 is present on activated T cells as well as in a soluble decoy form. Plasma Tim3 can limit the interaction of cellular Tim3 and its ligand, blocking its regulatory role in cytotoxic T-cell function. The role of CXCL9 as a gatekeeper for tissue distribution of alloreactive T cells in chronic GVHD is supported by the high levels of CXCL9 seen in oral, ocular, and mucosal chronic GVHD. Low levels of L-ficolin correlate with diminished hepatic clearance of mitochondria. However, the relationship of L-ficolin with the underlying mechanism of SOS/VOD is unclear.

The design and execution of this study incorporated several limitations. First, not all the patients in the clinical trial had biomarkers measured. Furthermore, the choice of the day 28 collection of the first sample for analysis

eliminated any possibility of exploring acute GVHD prediction, because a third of the patients had already developed GVHD by this time. Although the results were validated across different GVHD prophylaxis protocols, the study groups were otherwise uniformly treated. It has yet to be determined if biomarker predictions can span SCT given for a variety of disease indications with diverse donor types and different strengths of conditioning regimens. Furthermore, no study has yet combined NRM predictors with relapse predictors (notably sensitive molecular analysis of residual disease) to provide a comprehensive biological profiling of all the determinants of posttransplant disease-free survival where the indication is for malignant disease.

How will biomarkers change the way we do transplants in the future? Apollo gave Cassandra the unenviable gift of divining the future, with the proviso that nobody would believe her. To avoid Cassandra's fate, biomarker prediction must be proven to work in diverse transplant conditions, before it can achieve general acceptance. Furthermore, without the ability to modify outcomes, the precise determination of fate is neither a gift to the patient nor to their physician. How much we can control the destiny of the transplant will depend upon the outcome of further trials where biomarkers are used to make decisions between treatments designed to avoid, for example, acute or chronic GVHD or SOS/VOD. There is still a long way to go, but this paper is a sound basis for new trials designed to further extend biomarkers to larger and more diverse transplant populations and to explore ways to modify predicted outcomes by individually directed treatment approaches.

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

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● ● ● **HEMATOPOIESIS AND STEM CELLS** ▬▬▬▬

Comment on Iwamura et al, page 171

Microbes prevent HSPCs from "NOD"-ing off

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In this issue of *Blood*, Iwamura et al investigate the impact of microbes on the hematopoietic stem and precursor cell (HSPC) compartment. Using a mouse model and in vitro experiments, the authors demonstrate that the microbiota induces NOD1 in mesenchymal stem cells (MSCs), and that this, subsequently, induces proliferation within the HSPC compartment.[1]

Mammals and microbes have coevolved for ~200 million years, since the origin of mammals in the late Triassic period. This ancient relationship is largely believed to be mutualistic. Common parlance has favored referring to the majority of the microbes in the gut as "commensal," which suggests that the microorganisms benefit from receiving nutrients in the human niche without affecting their host. However, a growing body of evidence suggests that the relationship is "symbiotic," where both parties of the mutual relationship actively affect one another, most often in beneficial ways. A compelling argument in support of the symbiosis model is the finding that proper immunological development and competence are impaired in the absence of microbes. Although this phenomenon has been long acknowledged, the mechanisms underlying this relationship have been elusive.

Animals can be delivered and reared in so-called "germ-free" environments, where important microbiota niches such as the gastrointestinal system remain free of detectable microorganisms. Such germ-free animals have widespread deficits in immune development. These aberrancies, which are nicely reviewed by Round and Mazmanian,[2] are most prominent in the intestinal immune compartment. One of the earliest specific

examples of such a microbe-immune cell relationship was demonstrated by Littman and colleagues in 2009, where the introduction of segmented filamentous bacteria into germ-free mice induced the production of Th-17 cells.[3]

More recently, a phenomenon of hematopoietic cell compartment alterations has been noticed in these extensively studied germ-free animal models. Several immunologic cell types are impacted by exposure to microbes, including myeloid, natural killer T cells (NKTs), and monocytes. Myeloid cell progenitors in germ-free mice are lower in number and in differentiation potential. The finding of a lower number of myeloid cell progenitors in germ-free mice extends to those progenitors that derive from the yolk sac as well as the bone marrow. Microbially triggered granulopoiesis is at least partially mediated by interactions between microbial molecules such as lipopolysaccharides and toll-like receptors (TLRs), such as TLR4. These receptors, along with adapter molecules such as TRIF and Myd88, appear to be required for microbiota-driven myelopoiesis.[4] A recent article in this journal, by Hergott et al, showed that intestinal microbes control neutrophil and monocyte turnover in a NOD1/NOD1L–dependent manner.[5] NOD1, an intracellular

pattern recognition receptor, when bound by the NOD1 ligand D-glutamyl-*meso*-diaminopimelic acid, activates the downstream transcription factor NF-κB to modulate both innate and adaptive arms of the immune system.

Consequences of depressed myeloid cell development in the germ-free setting include impaired innate immunity to gut pathogens, such as *Listeria* species. Although resistance against *Listeria* infection can be partially rescued by reintroduction of a balanced gut microbiota, it is possible that long-term defects of immunity persist even after a brief, early period of exposure to impaired immunity. The groups of Kasper and Blumberg found that early life exposure to microbes was critical for invariant NKT function. Invariant NKTs localized to the colonic lamina propria and lung in animals that were born germ free and thus were devoid of microbial exposures in the neonatal time period. Exposing these animals to microbes later in life was insufficient to drive redistribution of these invariant NKTs. Thus, it was concluded that there is an age dependence of microbially mediated immune "training."[6]

Although there is strong evidence for tissue-level lymphoid and peripheral myeloid cell proliferation in response to microbial signals, predominantly through the TLR pathway, to date, there have been limited data on how microbes may impact the production and proliferation of hematopoietic progenitors. A significant conceptual step forward is made by the contribution of Iwamura et al in this issue. Building off the observation that naive Myd88 and TRIF–deficient mice have unaltered HSPC composition, the authors posit that alternative bacterial sensors may play a role in hematopoiesis. Using a germ-free mouse model, the authors demonstrate that hematopoietic stem cells are decreased in number compared with the amounts found in specific pathogen–free (SPF) mice. The administration of NOD1 ligand is sufficient to rescue HSPC numbers to levels found in SPF mice, and this effect is shown to be NOD1 dependent. Interestingly, although NOD1 is expressed in both HSPCs and MSCs, it is the NOD1 in MSCs that appears to be critical for effects on the HSPC compartment. The supernatant from NOD1L-treated MSCs is sufficient to recapitulate the effect on HSPCs, and specific MSC-secreted factors are suggested as potential mediators of this effect. Thus, the authors are able to show, for the first time, that MSCs mediate microbiota-induced