recognized and correlate with a milder phenotype. This supports the emerging concept that monoallelic KLF1 mutations can play a modulatory role in hemoglobinopathies.

β-thalassemia is a quantitative globin disorder that results from decreased levels of β-chain synthesis.2,3 The uncoupled α-chains form insoluble aggregates leading to ineffective erythropoiesis and shortened red cell survival. Iron overload from increased absorption and red cell transfusions contributes to end-organ damage. Globally, it is estimated that 1% to 5% of people are carriers, with certain geographical areas exhibiting a greater prevalence.4 The large clinical and hematologic variability can be partly accounted for by the milder phenotype seen in patients with a concomitant α-thalassemia mutation or with a compensatory increase in levels of the normally repressed γ-globin chain (hereditary persistence of fetal hemoglobin [Hbf]), leading to clinically significant levels of Hbf.5 Although some of the hereditary persistence of Hbf mutations map to the globin locus, a significant focus of recent small- and large-scale genetic studies has been to identify nonlinked loci that achieve the same result.6

KLF1 (formerly known as EKLF) is an erythroid-enriched transcription factor that plays a critical global role in red cell gene regulation.5 Among its targets are genes within the β-like globin locus, where it directly (adult β-) and indirectly (fetal γ-) regulates the β-like globin switch during mammalian ontogeny.6 Although ablation of KLF1 is lethal, haploinsufficiency is benign but leads to altered gene expression of specific targets that are highly sensitive to KLF1 levels, such as the La antigen. Of relevance to the present study, another sensitive target is the Bgl11a gene, a repressor of γ-globin expression,7 whose levels are decreased such that Hbf levels increase when KLF1 expression is lowered because of monoallelic disruption.8

The article focuses on the significant prevalence of KLF1 mutations and the evidence that these are linked to amelioration of the severity of β-thalassemia in those regions with high incidence; in the present case, the southern China provinces of Guangxi and Guangdong. Several aspects of this analysis are notable. Although there have been reports of KLF1 mutation occurring in patients with hemoglobinopathies, this is the first large population study to address how common this finding is. As a result, a major strength of the study is the large number of individuals surveyed (~5000 total). This enabled significant conclusions to be made from a comparison of β-thalassemia endemic region (~3800) vs non–thalassemia region (~1200) samples, primarily that the KLF1 mutation prevalence was dramatically higher in the endemic samples. These numbers also allowed a comparison to be made of the median time to first transfusion between cohorts to show that patients with KLF1 mutations were significantly favorably affected. Perhaps surprisingly, patients with KLF1 mutations had a stronger ameliorative effect on severity than mutations within the β-locus, the HB51L-MYB intergenic region, or in BGL11A. This resulted in patients who, although genetically ββ-thalassemia homozygous (or compound ββ-thalassemia heterozygotes) and therefore expected to have a thalassemia major phenotype, exhibited only a mild β-thalassemia intermedia phenotype and were largely transfusion-independent. Strikingly, 20% of nonthalassemic subjects with elevated HbA2 and Hbf levels harbored KLF1 mutations.

Previously identified and novel KLF1 coding variants are described, and it is of interest that almost all mutations gave rise to truncation variants or were within the KLF1 zinc finger region, thus rendering 1 allele not expressed or functionally inactive.5

The present analyses strongly suggest that elevation of Hbf and HbA2 levels, coupled with a decrease in CD44 expression, can be used as a basis to screen for KLF1 mutation. Identification of KLF1 mutations in individuals with β-thalassemia mutations can now be used along with other currently known predictors of disease severity to address prognosis and inform genetic counseling. Further, these types of analyses could well be directed at sickle cell disease patients, as a corollary to the present study is that monoallelic KLF1 mutations may also ameliorate the phenotypic severity in that population.9 In addition, including more of the KLF1 promoter region and introns in the analysis could also provide an additional source of mutation discovery relevant to alteration of expression.10

As with the present impressive study, it would be most optimal to characterize a large population of carefully characterized individuals.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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Comment on Hazenberg and Spits, page 700, and on Munneke et al, page 812

Innate protection from graft-versus-host disease

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In this issue of Blood, Hazenberg and Spits provide a detailed overview of human innate lymphoid cell (ILC) subsets and their development and distribution
ILCs are a diverse family of lymphocytes whose ability to influence tissue homeostasis makes them prime candidates to regulate human disease. However, most of our knowledge on ILCs still stems from studies in experimental mouse models.

Hazenberg and Spits elegantly explain that human ILCs exist as functionally distinct subsets that mirror T helper cell specification. In short, ILC1 secrete interferon-γ (IFN-γ) and are involved in intestinal inflammation, ILC2 function during type 2 immune responses to extracellular pathogens and helminth infection through production of interleukin-13 (IL-13) and IL-5 and were implicated in tissue repair in the lung, and ILC3 that produce IL-22 and IL-17 are important in defense against enteric pathogens by activating epithelial cells. The major difference with T helper cells is that ILCs lack an antigen receptor and are activated either by cytokines or by microbial patterns recognized through Toll-like receptors. ILCs are present in the human intestine and skin, where they can be activated by commensal microbiotas. ILCs can have either an activated or naïve phenotype. Patients with mostly activated ILCs had a significantly lower incidence of GVHD after transplant.

In an elegant study, Munneke et al now show that, in acute myeloid leukemia patients, ILC numbers in the peripheral blood are strongly reduced following induction therapy prior to HSCT. Even so, natural cytotoxicity receptor (NCR)+ ILCs, which have been linked to intestinal homeostasis in animal models, are absent from the circulation in healthy controls but appear in patients after induction therapy. This suggests that the increase in this population might be driven by signals related to tissue damage or the conditioning regimen, a notion that is supported by experimental data in a mouse model of HSCT.

More importantly, the authors noted a clear dichotomy in the ILCs present after induction therapy. In a subgroup of patients, ILCs had an activated phenotype and expressed molecules involved in homing to the intestine. In other patients, ILCs appeared mostly naïve and lacked expression of these homing integrins. Interestingly, the group of patients that had activated ILCs prior to transplantation were less likely to develop GVHD after HSCT (see figure). These data suggest that activation of ILCs as a result of pretransplant induction therapy might minimize subsequent GVHD development.

After transplantation, ILC recovery was slow when compared with neutrophils and monocytes. At 7 weeks posttransplant, all host ILCs had disappeared and were replaced by donor-derived cells. This notwithstanding, the composition of the ILC subsets was still altered compared with healthy controls and the characteristic presence of the tissue-damage related NCR+ ILCs noted after induction therapy was still apparent.

Future studies will have to focus on determining whether the correlation found by Munneke et al between ILC activation status and absence of GVHD reflects a protective effect of ILCs. If ILCs are capable of protecting from GVHD development, it is likely that this will be through their ability to enhance tissue repair, as was shown for experimental models of influenza-induced lung damage. Reduced tissue damage would restrict bacterial translocation and innate immune activation, dampening GVHD development at a very early stage. If so, this would allow for the design of new therapeutic strategies to limit GVHD development.
strategies to prevent GVHD by activating ILC in situ or, more likely, through exogenous administration of ILC-derived molecules involved in mucosal healing. Alternatively, ILC activation status, or the levels of signature cytokines related to this activation by these cells, might be able to serve as a predictor for the development of acute GVHD.

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Comment on Bertaina et al, page 822

Haplo is the new black

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In this edition of Blood, Bertaina et al report 3-year survival exceeding 90% by using haploidentical αβ+CD3+/CD19-depleted allogeneic transplantation for children with nonmalignant disorders.1

For those who lived through the early days of the development of haploidentical approaches, the notion that outcomes would become so good that one could consider using this approach relatively early in the course of these disorders (rather than as a last-ditch effort to save a child with end-stage disease) is nothing short of astonishing. In fact, there are currently so many promising haploidentical approaches that priority in the field over the next few years should be (1) testing the best ones compared with each other, (2) comparing haplo to other alternative donor sources such as cord blood, and (3) comparing haploidentical with standard approaches using matched unrelated and related donor sources.

Early progress in overcoming the major obstacle of haploidentical approaches, severe graft-versus-host disease (GVHD), came when centers developed efficient techniques for removing T cells in the graft (see figure). This elimination approach has been accomplished either by positive selection of CD34+ cells only for infusion or by negative selection, removing all CD3+ cells from the graft. Although elimination of T cells to less than 10^5 per kilogram of recipient weight controlled the GVHD problem, it spawned 2 more problems: increased graft rejection and profound and prolonged posttransplant immune deficiency. Giving megadose CD34+ infusions (>10^9 cells per kilogram) partially addressed the problem, but ongoing rejection issues have led to the use of profoundly immune suppressive preparative approaches, adding to never-ending posttransplant lymphopenia. Some groups have chosen to address the immune suppression challenge with augmentation of immune recovery by multipathogen-specific T cells.3 Although this is feasible and interesting, it requires expensive cell engineering and production available only at select centers.

An explosion of translational immunologic research over the past 2 decades has resulted in a variety of very promising techniques that either regulate GVHD-causing T cells or engineer grafts designed to partially or fully overcome the GVHD-rejection-infection triad. Some studies have attempted to energize the graft to recipient tissues through T-cell costimulatory blockade.4 This approach theoretically allows grafts to retain immunity to nonanergized antigens such as infectious pathogens. Other groups are regulating GVHD by co-infusing selected or expanded T-regulatory cells along with specified doses of conventional T cells with the same end in mind.5

Bertaina et al used one of a series of variations of graft engineering that go beyond the nondiscriminating elimination of all of a given type of cell to instead selectively remove the bad cells and retain the good cells in the rich variety of cells in a graft. The first variation removed CD3+ and CD19+ cells, retaining natural killer cells, monocytes, and dendritic cells in the graft. Although immune recovery and mortality may be lower with this approach compared with earlier methods, a degree of rejection and slow immune recovery remains.6

The next generation in this technology (αβ+CD3+/CD19+ depletion used by Bertaina et al) recognizes that γδ+ CD3+ T cells do not cause GVHD and may help with immune maintenance and recovery. This takes us one step closer to removing only alloreactive T cells, leaving other cells in the graft to do their work. A similar approach designed to remove only alloreactive cells has been built on the observation that alloreactive cells have been noted to be CD45RA+; thus, depletion of these cells leaves other cells in the mix that may be important for immune function.7 A similar attempt at selectively targeting alloreactive cells uses dibromomorphadione followed by photodepletion, a method that selectively depletes alloreactive T cells while sparing T regulatory cells.8

So which of these competing methods is best? The published data are limited, and comparative data between methods is absent, but as shown by Bertaina et al, although
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