binding affinity compared with the most common alleles, providing evidence supporting the concept that the less common mutant alleles may escape from epistasis due to more modest effects on RNA binding and/or splicing.

Interestingly, it was shown that *U2AF1*^{S34} and *U2AF1*^{Q157} mutations cooccurred in myeloid malignancy patients at a significantly higher frequency than expected by chance. Single-cell DNA sequencing analysis of a double-mutant patient showed that both *U2AF1*^{S34} and *U2AF1*^{Q157} mutations were present in the same cells, suggesting potential cooperation between the 2 mutations. Furthermore, these *U2AF1* mutations were found to cooccur in *cis* with preservation of the wild-type allele, a finding in agreement with a previous study demonstrating that the expression of the wild-type *U2AF1* allele is required for survival of cells harboring a *U2AF1* mutation.[8] The analysis of further double-*U2AF1*-mutant patient samples is required to establish whether *U2AF1*^{S34} and *U2AF1*^{Q157} mutations are tolerable when cooccurring in *trans*.

This study by Taylor et al has illuminated the genetic and molecular bases for the escape of splicing factor mutations from epistasis in patients with myeloid malignancies, findings that have important clinical and therapeutic implications.

Specific mutant alleles of each splicing factor gene might have different impacts on the clinical features and/or survival of patients with myeloid malignancies. Indeed, this suggestion is supported, for example, by a recent study showing that *SF3B1*^{K666} mutations are associated with some hematological features in MDS and with shorter patient survival and increased progression to AML.[9] However, the observation by Taylor et al that SF3B1^{K666} mutations have a weaker effect on pre-mRNA splicing than SF3B1^{K700} mutations, likely resulting from distinct structural disturbances at these amino acid locations, might be expected to lead to a milder impact of *SF3B1*^{K666} mutations on patient outcome. Further studies, including functional assessment of aberrantly spliced target genes, are required to elucidate fully the effects of less common splicing factor–mutant alleles on clinical features and outcome in patients with myeloid malignancies.

The mutual exclusivity of splicing factor mutations, previous studies showing that these mutations are not tolerated in a homozygous state,[7] and the demonstration that the survival of splicing factor–mutant cells depends on presence of the wild-type allele[8] provided the rationale for the potential therapeutic use of splicing modulators in splicing factor–mutant myeloid malignancy patients. The basis of this synthetic lethality strategy is that, unlike wild-type cells, splicing factor–mutant cells would be unable to tolerate further disruption to the splicing process by pharmacological inhibition of the spliceosome.[10] The finding by Taylor et al that the most common SF3B1 and SRSF2 mutations have more prominent effects on pre-mRNA splicing and RNA-binding affinity than less common splicing factor–mutant alleles indicates that myeloid malignancy patients with *SF3B1*^{K700E} or *SRSF2*$^{P95H/L/R}$ mutations may be more susceptible to treatment with splicing modulators. Stratification of patients on the basis of specific splicing factor–mutant alleles should be considered in clinical trials involving drugs that target the spliceosome.

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

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DOI 10.1182/blood.2020008215

CLINICAL TRIALS AND OBSERVATIONS

Comment on Kornblit et al, page 1499

Safer HLA mismatch transplantation

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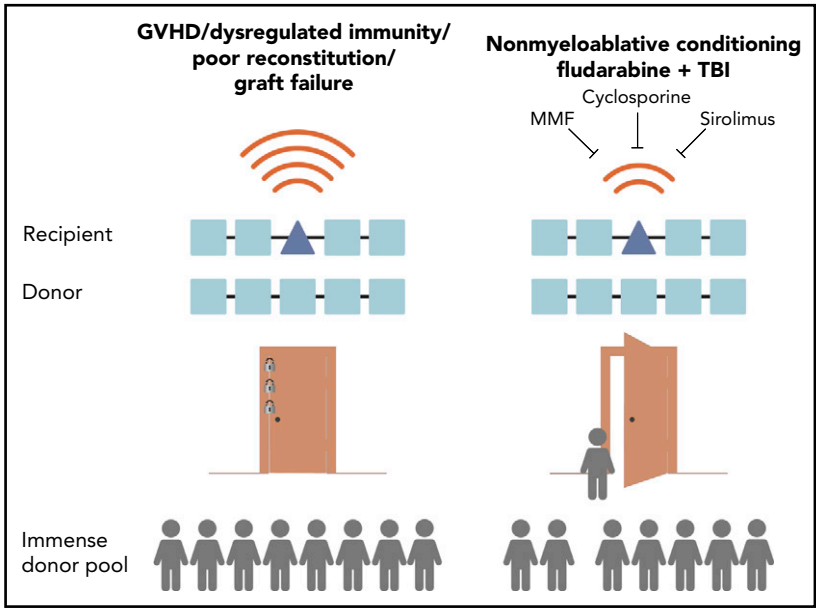
In this issue of *Blood*, Kornblit et al report on their multicenter phase 2 clinical trial (NCT01251575) investigating a new way of undertaking HLA mismatched transplantation with results that suggest a significantly improved safety profile and generally low disease relapse.[1] This protocol seems to be a promising and viable new clinical option for many patients who might not otherwise receive a transplant (see figure).

Ideally, every patient in need of a potentially curative hematopoietic stem cell transplantation would have a donor who is genetically matched to their HLAs. This has been the safest way to perform a transplantation because recipient immune



Hematopoietic stem cell transplantation between donors and recipients mismatched on 1 or more HLA alleles has historically been high risk because of complications including GVHD and dysregulated immunity. Studies of donor pools show that if HLA mismatch transplantation were safer, the vast majority of patients could receive a transplant. The Kornblit et al study of nonmyeloablative conditioning with triple-agent immunosuppression in a phase 2 clinical trial shows an improved safety profile that suggests a new option to allow more patients to receive a transplant. MMF, mycofenolate mofetil; TBI, total body irradiation.

reconstitution is generally healthier and there are fewer complications.[2]

Unfortunately, in reality most patients in need of a transplantation do not have suitable HLA-matched related siblings, and despite the impressive work of the National Marrow Donor Program, suitable HLA-matched unrelated donors cannot always be found. This is especially true for interracial or racial minority patients for whom the chances of finding a match are low—between 0% and 50%.[2] Many of these patients can receive a transplant by using cord blood or blood from family members who are half matched (haploidentical). New protocols that make use of these alternative donors have developed rapidly over the past decade and have better safety and efficacy.

Nonetheless, there are still patients whose only option is to undergo transplantation from donors who are mismatched on 1 or more HLAs. HLA mismatched transplantation has been historically fraught with a much higher and even prohibitive rate of complications. However, a few clinical studies have suggested ways in which HLA mismatched transplantation might be done more safely, and in a way that is comparable to HLA matched transplantation.[3,4] Evaluation of the unrelated donor pool has shown that if HLA mismatched transplantation were safe there would be a large untapped pool of donors available.[2]

The success of advances in unrelated donor matching and alternative donor transplantation means that fewer patients need to undergo transplantation with HLA mismatched unrelated donors, and it also means that accrual to trials can be low, which limits the size and design of trials that can be practically implemented. Kornblit et al report a well-executed trial involving 4 clinical sites. They compared their outcomes with recent historical results at these institutions which were that 69% of patients had grade 2 or greater acute graft-versus-host disease (aGVHD), a complication in which the donor immune system attacks the recipient's body; they also found a nonrelapse mortality (NRM) of 47% at 2 years.

Relatively few specific HLA mismatched transplantations are considered permissive, in that the differences in the HLAs that are specifically mismatched are less immunogenic and equivalent to HLA matches, so major efforts have been undertaken to find these combinations and use them.[5,6] However, the 77 patients enrolled in the Kornblit et al trial had nonpermissive mismatches.

These patients received fludarabine and total body irradiation as transplant conditioning and a triplet immunosuppression treatment that included mycofenolate mofetil, tacrolimus, and sirolimus. The conditioning used in the Kornblit et al trial is nonmyeloablative, meaning that it is among the least intensive regimens usually reserved for older and sicker patients, and patients would have hematologic recovery if donor cells and immunosuppression were not given then. In other clinical settings with more intensive conditioning, the combination of sirolimus and tacrolimus can increase toxicity. However, in the Kornblit et al trial, using all these agents together resulted in a significantly reduced aGVHD rate of 36% and a significantly lower NRM of 18% compared with historical controls. Given the trial participants ages and comorbidities, this would be a reasonable outcome even for HLA matched transplantation.

Although other groups have reported outcomes in HLA mismatch transplantation similar to those in HLA matched transplantation using nonmyeloablative conditioning,[3,4] the trial by Kornblit et al stands out for several reasons. First, the other protocols all used anti-thymocyte globulin (ATG) as part of the immunosuppression protocol, but this study suggests that ATG may not be necessary, perhaps because lymphodepletion is achieved by fludarabine. ATG is associated with a number of infectious disease complications. Second, the relapse and progression observed in the trial could be lower than those in other approaches, although there are many caveats to making direct comparisons.

The work of Kornblit et al also highlights that effectively managing nonmyeloablative transplantation may take years as opposed to months. For example, they observed that the rate of chronic GVHD (cGVHD) was initially low but then climbed to rates near those seen historically by 4 years. In the other approaches using ATG, the incidence rates for cGVHD may be lower over time, which suggests the possibility of using minimal-effective-dose immunosuppression or other approaches that might be worth testing with this new strategy.[3,4] Likewise, although patients may relapse after nonmyeloablative transplantation, posttransplant treatment can stimulate donor graft-versus-leukemia (GVL) and restore durable remissions, a fact that makes comparisons of relapse and disease control more complicated.

All these promising HLA mismatched transplantation strategies rely on radiation as part of conditioning. Radiation has immune-modulatory effects by eliciting apoptotic pathways and is often associated with immune tolerance. Whether radiation is needed scientifically to allow HLA mismatch transplantation has not been clinically tested. There are also newer strategies that have been successfully used preclinically in major histocompatibility mismatch transplantation, such as the use of immunoregulatory TR1 cells which is now being tested in HLA mismatched transplantation for full-intensity conditioning.[6]

Although HLA mismatched transplantation is often framed as an option of last resort, studies like that of Kornblit et al suggest that this may not be the case in the future, especially because there have been significant improvements in finding donors for all those who need transplantation.[2] Importantly, genetic mismatches could potentially be exploited to enhance GVL effects, as has been suggested for sex mismatch in the nonmyeloablative setting.[7] Likewise, a donor in a large HLA mismatch pool could be chosen on the basis of other factors that could enhance GVL, such as killer-cell immunoglobulin-like receptors.[8] More clinical trials of the same high caliber as the Kornblit et al trial are needed to advance the practical implementation of HLA mismatch transplantation so that every patient in need can receive a transplant.

Conflict-of-interest disclosure: E.M. serves as scientific advisor and is an equity holder in GigaGen Inc. and Triursus Therapeutics, and he has a sponsored research agreement with Orca Biosystems. ■

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DOI 10.1182/blood.2020006922

RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Kämmerer et al, page 1549

The role of hepcidin in fetal iron homeostasis

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In this issue of *Blood*, Kämmerer et al report that hepcidin secreted by the fetal liver has a specific role in iron homeostasis, ensuring that the fetal liver retains iron destined for hepatic erythropoiesis.[1] Surprisingly, fetal hepcidin seems to have no physiological role in regulating iron transfer from the mother to the fetus across the placenta. Why this is so is the subtext of this article and the focus of other recent analyses.[2]

Normal human pregnancy challenges the mother with a greatly increased demand for iron to support placental and fetal growth, including importantly that of the developing fetal erythron, but additional iron is also needed for expanding maternal erythropoiesis. The net effect is an ~10-fold increase in iron demand from 0.8 mg/day in the first trimester to 7.5 mg/day in the third trimester. In the second and third trimesters, the maternal iron-homeostatic system responds to this challenge by gradually decreasing the production of maternal hepcidin in the liver, resulting in very low maternal circulating hepcidin concentrations.[2] Low hepcidin concentrations allow greatly increased intestinal iron absorption when dietary iron is available and mobilization of iron from maternal stores. What causes maternal hepcidin suppression is not yet known.

The syncytiotrophoblast is the placental tissue that carries out nutrient transport from maternal blood to fetal blood and the removal of fetal waste in the opposite direction (see figure). In humans, this is a single polarized cell layer; in mice, there are 2 cellular layers that seem to be interconnected so that they function as a single layer. In both species, the syncytiotrophoblast functionally separates the maternal from the fetal milieu. Iron uptake on the maternal side is mediated by the transferrin receptor TFR1, and the iron is then exported to the fetal vasculature through ferroportin, the sole known cellular iron exporter and the molecular target of hepcidin. Hepcidin, if present at effective concentrations, regulates iron export through ferroportin by occluding this transporter and inducing its endocytosis and lysosomal proteolysis. Because placental ferroportin is localized on the fetal-facing side of the syncytiotrophoblast tissue, only fetal hepcidin has direct access to it.

Kämmerer et al used several mouse models to examine fetal iron homeostasis. In the first model, they studied wild-type fetuses of wild-type mothers, and found that as iron accumulated in the fetal liver, its hepcidin messenger RNA (mRNA) concentration increased but remained well below the already low maternal liver hepcidin mRNA concentrations, although hepcidin peptide in plasma was not measured. Nevertheless, fetal hepcidin concentrations are likely too low to affect placental ferroportin because placental ferroportin was