this great diversity and the great variation in species among human individuals, it is surprising that individual groups of bacteria, in some cases down to the genus level, are clearly associated with LGI-GVHD. The LGI has the highest bacterial density, but whether this high density is in fact relevant for LGI-GVHD is unclear. Human studies of the intestinal microbiome are limited to fecal samples; we can, therefore, not be sure about composition and activity of the microbiome along the GI tract, and whether this changes during treatment.

Bacteria metabolize intestinal nutrients and generate a great number of small molecules with largely unknown impact on the host. Short-chain fatty acids, especially butyrate, are the best investigated metabolites, and butyrate can have beneficial effects both on epithelial cells and intestinal immune cells. In the current study, the ability of the microbiome to produce butyrate correlated with better patient outcome, with different bacterial metabolic pathways linked to disease. Although we are not yet able to predict the individual risk for a patient to develop GVHD from the microbiome in a meaningful way, it is at least conceivable that we will one day arrive at this point.

Patients undergoing allo-HCT are at great risk of severe bacterial infection, requiring treatment with broad-spectrum antibiotics. Perhaps unsurprisingly, the study confirms earlier data that these antibiotics disturb the microbiome, and the authors show an association of antibiotic treatment and the risk of GVHD. The importance of antibiotics in medicine is undisputed but, in addition to the selection of resistant bacteria, microbiome toxicity has emerged as a severe side effect. In our current arsenal, there are no antibiotics that act reliably enough on pathogenic bacteria and spare the "good ones," particularly the strict anaerobes in the gut. I cannot see this happening any time soon, and it may not even be possible, but devising such a weapon would certainly be an important step in medicine.

This study contributes to the growing body of information how the microbiome maintains our health and the importance of preserving its beneficial capacity. It provides an additional rational basis for clinical trials to test if preserving or restoring a healthy microbiome alters GVHD risk and severity. Trials to find the best choice of antibiotics, tests of dietary interventions, immunomodulatory approaches, and endeavors to restore or modulate the microbiome, through fecal microbiota transplantation, are all under way.[3] As we advance in our understanding of what exactly the contribution is of the bacteria, and the fungi,[8] in our intestine that sustains our health, we are also making progress in identifying potential ways to prevent or redress microbiome disturbance. Burgos da Silva et al contribute to the ability to predict and to stratify GVHD. The accumulating evidence suggests we will soon be in a better position to prevent and to treat the disease.

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CLINICAL TRIALS AND OBSERVATIONS

Comment on Sugimoto et al, page 2398

Engineered platelets for clinical application: a step closer

Lijun Xia | Oklahoma Medical Research Foundation

In this issue of *Blood*, Sugimoto et al[1] report the result of the first clinical trial of an autologous transfusion of induced pluripotent stem cell (iPSC)-derived platelets (iPSC-PLTs) in a patient who had severe aplastic anemia but no compatible platelet donor.

Platelets are abundant, yet short-lived, blood cells. Platelets have multifaceted functions, comprising important physiological processes such as hemostasis, vascular integrity, wound healing, and immunity, as well as pathological roles such as inflammation and tumorigenesis.[2,3] Thrombocytopenia is common in many hematological diseases (eg, leukemia, aplastic anemia, and immune thrombocytopenia).[3,4] Thrombocytopenia also occurs in many other diseases and conditions such as sepsis, infectious illnesses, cancer chemotherapy, and in the recovery phases of blood stem cell transplantation.[3,5] Patients with severe thrombocytopenia, who are at a high risk of bleeding or are bleeding heavily, often require prophylactic or therapeutic platelet transfusion.[3] Approximately 2 million units (~3×10^{11} per unit) of donor-derived platelets are transfused annually in the United States.[3] However, there are numerous unsolved limitations with donor-derived platelets, including product unit quality, short shelf life, risks of product bacterial contamination, and transmission of infectious disease.[3] In

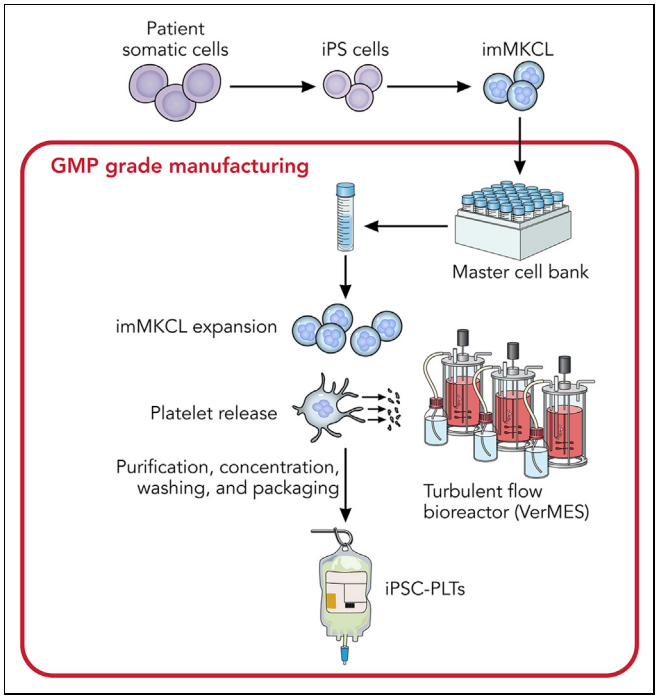

Diagram showing the large-scale production of iPSC-PLTs from imMKCLs, derived from a patient with aplastic anemia for autologous transfusion. Professional illustration by Patrick Lane, ScEYEnce Studios.

addition, platelet transfusion refractoriness, which is defined as the repeated failure to achieve the desired level of blood platelets in a patient after platelet transfusion from random donors, is also a clinical problem.[3] Alloimmune platelet transfusion refractoriness (allo-PTR) occurs in ~5% to 15% of patients who have undergone platelet transfusion due to sensitization to alloantigens, such as HLA-I and human platelet antigens (HPAs).[1] To overcome these problems, there have been many efforts in the past few decades to produce platelets in vitro or ex vivo from precursor cells for clinical application.[3,6] However, large-scale manufacturing of good manufacturing practices (GMP)–grade functional platelets ex vivo has remained an elusive goal.

In this study, the Eto group, which is an established team in the field, addresses this goal by focusing on the generation of transfusable platelets for a patient with aplastic anemia with allo-PTR with no compatible registered donor. Based on their previous innovative research,[6] the authors established immortalized megakaryocyte progenitor cell lines (imMKCLs) differentiated from induced pluripotent stem cells (iPSCs), which were generated from the patient's peripheral blood mononuclear cells (see figure).[1,7] A competent imMKCL clone was selected for the generation of a master cell bank. After biosafety validation, cells from this master cell bank were used to produce platelets (iPSC-PLTs) using their previously established turbulent flow bioreactors and a mixture of drugs to promote megakaryocyte proliferation/maturation and preserve platelet function. With this system, they successfully achieved clinical-scale manufacturing of GMP-grade autologous iPSC-PLTs for the patient.[7] The authors then conducted a first-in-human clinical trial of autologous transfusions (3 sequential escalating doses of 1×10^{10}, 3×10^{10}, and 1×10^{11}) of the iPSC-PLTs into the patient. The transfusion was well tolerated with no significant complications at 1-year follow-up. The absence of side effects is especially encouraging and paves the way for further human studies. Even though these transfused iPSC-PLTs seemed short lived (they only circulated for several hours after transfusions) with untested in vivo functionality in this patient, this study represents a significant step toward the authors' goal of applying iPSC-PLT technology to clinical use.

Although the iPSC-PLT technology has great potential, there still is a long path ahead before routine use as a source for platelet transfusion. Long production time and the high cost of producing GMP-grade functional platelets are among the major obstacles that prevent regular clinical use, even for the indication presented in this study. Therefore, the current priority should perhaps be the development of applications that cannot feasibly be achieved with the typical donor-derived platelets; for example, generating genetically modified iPSC-PLTs by deleting the main allo-PRT–causing antigens HLA-I and/or HPA for allogeneic transfusion, as suggested by the authors; generating autologous gene-corrected platelets for patients with inherited platelet disorders; or developing genetically modified autologous iPSC-PLTs to express ectopic therapeutic molecules for targeted delivery to specific sites such as tumor microenvironment for tumor therapy. With further development and suitable indications, the iPSC-PLT technology represents an innovative and promising platform to address many unmet clinical needs.

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