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Commentary

The unknown impact of conditioning on HSC engraftment and clonal dynamics

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The removal of host bone marrow (BM)-resident hematopoietic stem cells (HSCs) is a prerequisite for successful engraftment of donor cells in most allogeneic and autologous transplant settings. Conditioning regimens have become a major focus of research over the past years in an effort to reduce overall toxicity, especially for nonmalignant diseases. A plethora of agents and strategies are currently being investigated for their efficiency to deplete host HSCs at the optimal dosing to minimize genotoxicity as well as cytotoxicity and other transplant-related toxicities. While this effort is ongoing, the impact of novel conditioning regimens on the long-term engraftment of gene-modified HSCs, their contribution to the blood, and their clonal development over time are less well understood. One reason for this is the inability to reliably address these important questions in traditional mouse models. Differences in the physiology, size, and lifespan limit the ability to assess side effects or develop clinical protocols with immediate translational potential. In contrast, large animal models such as nonhuman primates (NHPs) share a close evolutionary relationship with humans, demonstrate cross-reactivity for human reagents, and permit the use of drugs, medications, and instruments also used for patients. To provide insights into the impact of conditioning, the study by Abraham et al. systematically compared the engraftment efficiency and clonal contribution of *ex vivo* barcoded HSCs after total body irradiation (TBI) with low-dose busulfan conditioning in the NHPs.[1]

Researchers from the Dunbar and Tisdale labs conditioned three primates with busulfan and transplanted *ex vivo* gene-modified CD34$^+$ hematopoietic stem and progenitor cells (HSPCs). As the expression of fluorescent markers such as green fluorescent protein (GFP) leads to the rejection of gene-modified cells in busulfan-conditioned animals,[2] the investigators in this study used a previously reported genetic barcoding strategy invisible to the immune system to track the engraftment of gene-modified cells and their contribution to the blood.[3] During the 2-year follow up, barcodes were tracked in the blood and bone marrow and the observed clonal kinetics compared with historic control animals undergoing TBI for conditioning.

Significantly lower engraftment of gene-modified cells was seen in the busulfan-conditioned animals with striking differences in the clonal dynamics compared with the TBI-conditioned NHPs. Clonal patterns in the TBI-conditioned animals established and remained stable over time,[3] while in two out of three busulfan-conditioned animals, clones disappeared in waves, and new sets of barcoded cells started to appear until long-term stability was reached. Temporal changes in the clonal composition in the busulfan-conditioned NHPs show close similarities to the clonal dynamics previously described in gene therapy patients conditioned with busulfan (and fludarabine/rituximab) demonstrating an appearance of early and late sets of clones within the first year post-transplant.[4]

The study by Abraham et al. further shows that the type and level/dosing of conditioning have a direct impact on the level of engraftment of gene-modified cells. At the same time, the type of conditioning impacted the longitudinal kinetics of HSCs' contribution. As fewer endogenous HSCs were depleted from the host using a low dose of busulfan, the time to reach clonal stability was significantly extended compared with the TBI-conditioned animals. Clonal patterns in NHPs further closely resembled previously reported data collected in patients, demonstrating the value of an NHP model to evaluate novel conditioning regimens before entering into the clinical routine.

The cytotoxicity associated with busulfan and other chemotherapy drugs is fairly nonspecific and thus affects not only HSCs but many other cells and organs. Thus, current efforts in the field focus on the development of nongenotoxic, HSC-specific agents such as specific HSC-targeting antibodies often delivered as antibody-drug conjugates (ADCs)[5] (Figure 1). Antigens currently targeted for this purpose include CD45, CD47, CD117, and others more or less broadly expressed on subsets of HSPCs.[6] Various drug conjugates are being evaluated in an effort to maximize cell-specific killing with minimal or no off target toxicity. Efforts are still in the early phases of development, and data are mostly limited to experiments in mice, with only few results in the NHP.[7] At this point, many questions remain unanswered regarding the on-target specificity and half-life of the ADCs impacting the timing and workflow of HSC transplants, as well as the efficiency and clonal dynamics of HSC engraftment. The NHP model will likely provide a transplant setting to comprehensively test and compare various antibody- and ADC-based conditioning strategies side by side with previously established methods such as TBI and busulfan. These studies will provide the necessary information on efficiency, safety, and the impact on the clonal long-term behavior of HSCs.

Instead of developing new agents for the depletion of HSCs, the Naldini lab recently reported a mobilization-based, chemotherapy-free strategy entirely relying on already existing and routinely used mobilization agents.[8] Instead of killing resident HSCs

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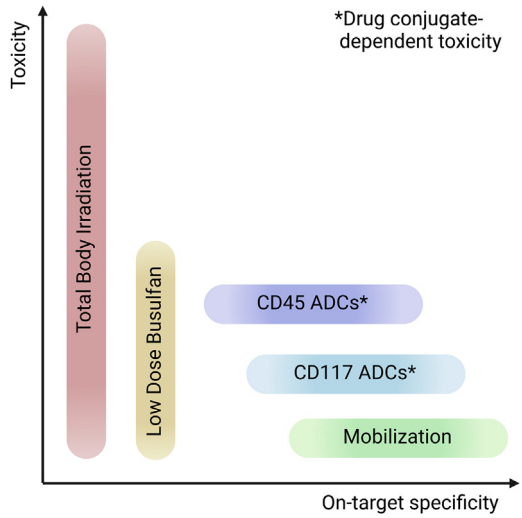

Figure 1. Toxicity vs. on-target specificity of conditioning regimens currently employed or tested for HSC gene therapy
ADC, antibody-drug conjugate.

in the bone marrow, Omer-Javed et al. demonstrated a culture-induced upregulation of CXCR4 resulting in a competitive advantage of *ex vivo* gene-modified human HSCs over AMD3100-mobilized human stem cells in the peripheral blood or NSGW41 mice to home into the BM. This competitive advantage could be further enhanced by ectopically overexpressing CXCR4 on gene-modified human HSCs. As the mobilization of HSCs with G-CSF and AMD3100 is routinely performed without known cytotoxicity or genotoxicity, this mobilization-based approach holds tremendous potential for future HSC gene therapy applications. However, little is known about the feasibility of this strategy in humans and large animals.

Results from this study by Abraham et al. highlight the importance of large-animal studies for the assessment of novel conditioning strategies and HSC gene therapy approaches regarding their safety and efficiency as well as impact on HSC engraftment, contribution, and clonal dynamics. Lower systemic toxicity of low-dose or non-genotoxic conditioning regimens may come with a trade-off that needs to be accounted for when gene-modified cells don't have a selective advantage in the host and can't reach the therapeutic threshold without any further interventions. Combinatorial approaches providing the ability to select for gene-modified cells may be needed, another level of complexity adding pressure on the clonal diversity of engrafted gene-modified HSCs. More comprehensive follow-up studies will be needed to carefully assess the feasibility and potential impact of these approaches on long-term hematopoiesis in order to provide a safe and efficient treatment for future clinical applications.

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DECLARATION OF INTERESTS

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