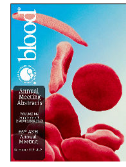
Check for updates

Blood 142 (2023) 7127–7128



The 65th ASH Annual Meeting Abstracts

ONLINE PUBLICATION ONLY

801.GENE THERAPIES

Epitope Edited Hematopoietic Stem Cells Allow Immune-Based In Vivo Selection of Genome-Engineered Cells

Gabriele Casirati, MDPhD[1], Andrea Cosentino, MD[2], Adele Mucci, PhD[3], Mohammed S Mahmoud, PhD[4], Marta Freschi, MSc[2], Jing Zeng, MD[1], Christian Brendel, PhD[1], Daniel E. Bauer, MD PhD[5], Pietro Genovese, PhD[1]

[1] Boston Children's Hospital, Boston, MA
[2] Boston Children Hospital, Boston, MA
[3] Boston Children's Hospital, Boston
[4] Fayoum University, Department of Zoology, College of Science, Fayoum, Egypt
[5] Division of Hematology/Oncology, Boston Children's Hospital, Boston, MA

Hematopoietic stem/progenitor cell (HSPC) transplantation (HSCT) offers curative options for patients affected by conditions for which the substitution of endogenous hematopoiesis with genetically corrected or healthy donor-derived cells can halt the pathogenic process. Nonetheless, the short and long-term adverse effects of genotoxic conditioning remain a substantial barrier to a wider application of HSCT and gene therapy. HSCT survivors suffer from life-long irreversible consequences, such as chronic graft-versus-host-disease (GvHD), infertility, susceptibility to viral infections, organ damage (including renal, liver and lung) and increased risk of secondary malignancies. Whereas monoclonal and toxin-conjugated antibodies (Abs) targeting HSPCs have been proposed as an alternative to chemo/radiotherapy, the mechanism of action and pharmacokinetics of these agents hamper their safe clinical use due to the risk of on target killing of transplanted HSPCs. To avoid this, the cell product is not administered at peak myeloablation to allow for washout of the conditioning agent, further impairing engraftment and therapeutic potential. Here, we show that precise editing of the targeted epitope in HSPCs can endow hematopoietic lineages with selective resistance to monoclonal Abs (mAbs) without affecting stem cell function. This strategy allows improved myeloablation - which can continue after HSCT - and progressive enrichment of gene-modified cells. We have identified amino-acid changes in the extracellular domain of KIT (CD117) that abrogate the binding of therapeutic mAbs without affecting surface expression, ligand affinity, kinase phosphorylation, downstream signaling, transcriptional and proliferative response (Fig.A). We exploited adenine base editing (BE) to introduce these mutations with high efficiency (~80%) in CD34+ HSPCs, with preservation of long-term repopulating and multilineage differentiation capacity in primary and secondary recipient mice. To ensure full preservation of KIT functionality, we performed growth analysis, RNA sequencing, and phosphoproteomic analyses of epitope-edited HSPCs stimulated with KIT ligand (stem cell factor, SCF) and observed no difference in the proliferation, transcriptional, and phosphorylation response compared to controls. *In vitro* differentiation of edited HSPCs toward myeloid, macrophage, classical dendritic, granulocytic and megakaryocytic lineages was similar to controls and did not result in counterselection of edited cells. Lineage-differentiated cells showed preserved functionality, including: reactive oxygen species production by myeloid cells, E.coli phagocytosis by macrophages, M1/M2-like macrophage polarization, phospho-flow profiling of IL4-, PMA/ionomycin-, GM-CSF-, IFN type-I-, IL-6- and LPS-stimulated myeloid cells, HLA class-II/CD86 upregulation by DCs, induction of granulocyte NETosis and generation of hyperdiploid megakaryocytes. By plating edited HSPCs with increasing concentrations of the mAb, we observed preserved expansion of edited HSPCs in response to SCF while control cells were inhibited in a dose-dependent manner. To improve HSPCs therapies for hemoglobinopathies, such as sickle cell disease, we multiplexed BE of the KIT epitope and two *BCL11A* erythroid enhancer motifs. We obtained editing efficiencies comparable to single editing and the erythroid progeny of triple-edited cells showed upregulation of HbF, as expected (~40%). In a competitive xeno-transplantation setting, with co-injection of multiplex BE and *AAVS1*-BE control CD34+ cells, mice treated with KIT mAb showed a 2-fold co-enrichment of both KIT and *BCL11A* editing when compared to mock treated controls, confirming the possibility to select gene-modified cells up to therapeutic thresholds. Similarly, mAb conditioning combined with epitope-edited grafts allowed human hematopoietic substitution in mice pre-humanized with unedited CD34+ HSPCs (89% substitution with 7.3-fold higher absolute cell counts, Fig.B), compared to AAVS1-edited controls that failed to engraft. We envision a new paradigm for non-genotoxic conditioning based on transplantation of epitope engineered HSPCs resistant to immunotherapeutic agents to achieve engraftment and in vivo selection of gene corrected HSPC with substantial advantages over alternatives in terms of safety and tolerability.

© 2023 by The American Society of Hematology

Downloaded from http://ashpublications.net/blood/article-pdf/142/Supplement 1/7127/2187746/blood-3723-main.pdf by guest on 11 June 2024

Disclosures No relevant conflicts of interest to declare.

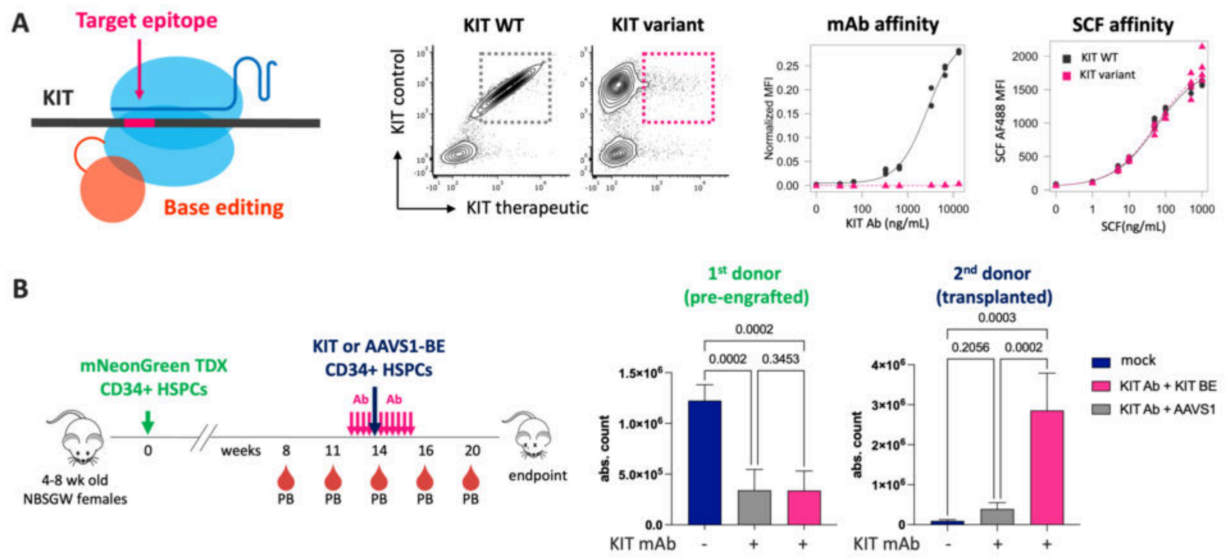


Figure 1

https://doi.org/10.1182/blood-2023-177772

Downloaded from http://ashpublications.net/blood/article-pdf/142/Supplement 1/7127/2187746/blood-3723-main.pdf by guest on 11 June 2024