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Non-Genotoxic Conditioning for Hematopoietic Stem Cell Transplant through Engineered Stem Cell Antibody Paired Evasion (ESCAPE)

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While *ex vivo* gene editing of CD34 $^{+}$ hematopoietic stem and progenitor cells (HSPCs) offers potentially curative therapies for patients with serious diseases, such as sickle cell anemia, significant autologous hematopoietic stem cell (HSC) transplant-related challenges remain, notably the requirement of myeloablative conditioning using chemotherapeutic agents such as busulfan. To address this challenge, we have developed a non-genotoxic conditioning strategy with our *E*ngineered *S*tem *C*ell *A*ntibody *P*aired *E*vasion (ESCAPE) approach where we aim to engineer the epitope of the HSC surface protein CD117 (cKIT) using base editors, in such a way that the engineered protein retains the normal CD117 receptor function, but *escapes* recognition by a cognate monoclonal antibody that recognizes the wildtype CD117 protein. The ESCAPE-CD117 edit can be combined with a therapeutic target edit to generate a multiplex-edited engineered HSC (eHSC).

We previously reported that through large parallel antibody and antigen screening efforts, we could identify mAb: edit pairs in which the engineered epitope evades mAb-mediated blocking of the WT CD117 receptor-ligand interaction while maintaining normal CD117 receptor biology and HSPC function and differentiation potential *in vitro* and *in vivo*. To further characterize binding properties of our lead CD117 variant to the natural ligand SCF, we determined the 3-dimensional structure of the receptor-ligand complex using cryo-EM. Structural overlay between engineered CD117:SCF and WT CD117: SCF complexes showed high similarity, with root mean square deviation (RMSD) of 0.848-Å, indicating that our epitope engineering did not affect the structure of CD117 nor its binding to its ligand.

We have previously demonstrated *in vivo* proof-of-concept data supporting selection of engrafted multiplex-edited eHSCs after a single dose of mAb in non-competitive and competitive transplant settings in multiple rodent xenotransplantation models. We next sought to examine whether mAb conditioning could achieve long-term, multilineage hematopoietic reconstitution of multiplex-edited eHSCs and potentially therapeutic mixed chimerism levels.

To this end, we humanized an immunocompromised mouse model with unedited mobilized peripheral blood (mPB) hCD34 $^{+}$ cells to achieve stable human bone marrow chimerism and then administered a single dose of mAb followed by a second transplant from the same donor of eHSCs harboring both ESCAPE and therapeutic edits targeting the *HBG1/2* promoter. Animals receiving CD117 mAb demonstrated significant engraftment by cells harboring our ESCAPE multiplex edits as detected by NGS of bulk BM (~50% multiplex editing) and within the sorted marrow CD34 $^{+}$ cell population (~60% multiplex editing), while the isotype control treated animals only showed 2-5% multiplex editing within the said compartments. We also isolated CD15 $^{+}$ myeloid cells and GlyA $^{+}$ erythroid cells from the bone marrow of the mAb treated animals, which also harbored >50% multiplex editing as detected by NGS. Furthermore, we could detect what is believed to be therapeutically relevant levels of HbF induction (>30%) in human GlyA $^{+}$ sorted bone marrow cells, suggesting that we could achieve therapeutic benefit with a single dose of mAb as the conditioning agent.

In conclusion, our structural data showed a high level of structural similarity between our engineered CD117 epitope and the WT protein. Our *in vitro* assays and *in vivo* rodent xenotransplantation models showed enrichment of engineered HSCs upon mAb treatment. Altogether, our data suggest the feasibility of achieving therapeutically relevant levels of mixed bone marrow chimerism with our non-genotoxic conditioning ESCAPE platform. This approach may remove the need for patients

to undergo systemic genotoxic conditioning prior to transplant and further unlock the promise of base editing therapies for the treatment of patients suffering from serious hematologic diseases.

Disclosures Mondal: *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Harmon:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Budak:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Zhang:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Wong:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Menon:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Wolin:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **White:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Musenge:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Camblin:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Coisman:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Lucini:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Bai:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Qiao:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Austin:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **DeLelys:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Kromer:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Hardy:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Law:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Manoukian:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Goldsmith:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Fallon:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Patel:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Cozier:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Lazzara:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Decker:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Jenkins:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Bohnuud:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Feliciano:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Lee:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Kopesky:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Smith:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **McDonagh:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Gaudelli:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Chu:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Hartigan:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Shankar:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Ciaramella:** *Beam Therapeutics:* Current Employment, Current equity holder in publicly-traded company.

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