Advancing a *CCR5* Gene Editing Therapy to Clinical Trial: Overcoming Viral Vector Toxicity

Background

HIV infection is currently managed by lifelong antiretroviral therapy (ART), a modality that is associated with chronic toxicity, challenging patient compliance, and comes at a significant cost over a patient's lifetime. Creating an HIV-1 infection-resistant immune system via gene editing has shown preclinical success and offers hope for a single dose therapeutic alternative.[1,2]

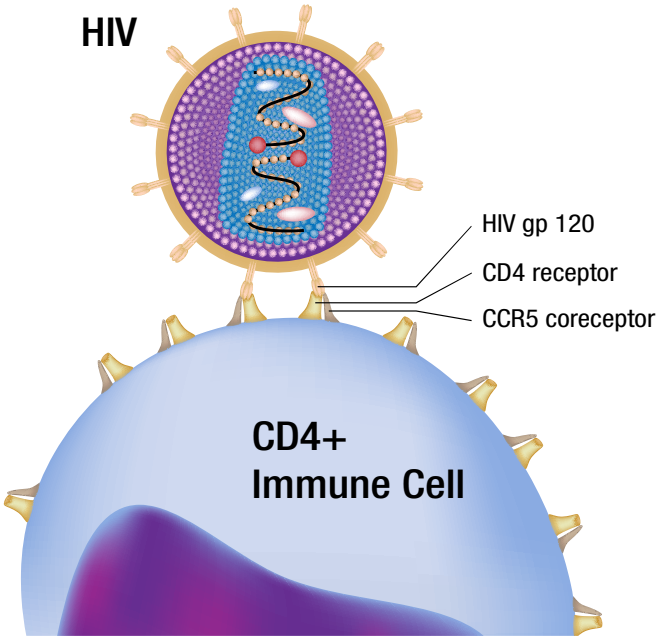
Both preclinical and clinical studies have demonstrated that blocking or mutating *CCR5*, an R5-tropic HIV-1 coreceptor — whether through small molecule inhibition, presence of a natural mutation, or therapeutic gene modification — can render cells resistant to HIV-1 infection.[3-7] In one instance, a patient was "cured" following transplant of allogeneic stem cells containing a bi-allelic *CCR5* mutation (CCR5Δ32/Δ32) with no evidence of HIV for eight years despite the halting of ART.[8,9] Allogeneic stem cell transplant as a routine HIV therapy is limited by the availability of HLA-matched CCR5Δ32/Δ32 donors and comes with a high risk of morbidity and mortality.[10]

Adoptive transfer of autologous CD4+ T cells following ZFN-mediated *CCR5* disruption (SB-728-T) was shown to be clinically safe, with cells engrafting and persisting over time.[2] The limitation of this approach, however, is that patient monocytes — a population of immune cells believed to be a key reservoir for HIV infection — maintain wild-type *CCR5*. In contract, *CCR5* gene editing of autologous HPSCs has the potential to result in HIV-1-resistant immune cells of multiple lineages, including both CD4+ T cells and monocytes, throughout the patient's lifespan.

Disruption of *CCR5* in adult mobilized CD34+ cells using ZFN in a clinical setting has been reported. Unfortunately, the cytotoxicity of the adenoviral vector used to deliver the ZFN machinery prevented its use in the intended clinical trial.[5] It was determined that to move the clinical trial forward a non-viral, clinically-feasible, and regulatory-compliant technology was necessary to deliver the ZFN gene editing machinery to HSPCs.

Aim

Conduct preclinical IND-enabling studies for the clinical-scale manufacturing of CCR5-disrupted autologous HSPCs via non-viral delivery of SB-728 mRNA using the MaxCyte GT in support of an anticipated clinical trial. Specifically, develop a process for high frequency, bi-allelic *CCR5* gene disruption with minimal manipulation and no negative impacts on cell engraftment or lineage potential, as well as assess the feasibility and reproducibility of the production process and *in vivo* safety of the final product.



CD34+ Cell Handling & Electroporation

Process Development

CD34+ cells were enriched from commercially-available, G-CSF mobilized hematopoietic progenitor cells collected by apheresis (HPC-A) followed by an overnight pre-stimulation regimen.[5]

- Stimulated CD34+ cells were resuspended in MaxCyte® Electroporation Buffer with either 0, 50, 75, or 150 mg/mL SB-728 mRNA.
- Cells were transferred to a CL1.1 processing assembly and electroporation performed using the recommended protocol on the MaxCyte GT.
- Electroporated cells were incubated for 20 minutes at 37°C, followed by overnight incubation at 30°C.[11] Cells were then incubated an additional 24 hours at 37°C prior to analysis or cryopreservation.

Engineering Runs & *In Vivo* Turmogenicity Studies

- CD34+ HSPCs were isolated by apheresis from four G-CSF mobilized, healthy donors and used for GLP qualification runs.
- Stimulated CD34+ cells were electroporated as above using 150 mg/mL clinical-grade SB-728 mRNA.
- An aliquot of cells from each lot was cryopreserved and evaluated by MiSeq deep sequencing to determine the level of *CCR5* disruption.

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- Cryopreserved cells were thawed and cultured for 24 hours in media containing a cytokine cocktail prior to intravenous injection into NOD/SCID gamma (NSG) mice for a 20-week tumorigenicity/toxicity study.

Full methods for SB-728mR manufacturing, *in vitro* assays, sequencing, and *in vivo* safety studies are detailed in *Mol. Ther. — Methods & Clinical Development, 3*, 16067, 2016.

Results

Process Development Studies: 73% Bi-allelic *CCR5* Disruption

By migrating from viral transduction to transient ZFN expression via mRNA electroporation, gene disruption rates could be maximized via mRNA concentration while minimizing off-target activity. Additionally, this eliminated the risk of adenoviral vector toxicity as noted in previous studies.[5]

Electroporated CD34+ enriched HSPCs showed viabilities of >90% with high frequency *CCR5* gene modification during initial process development.[12] Up to 73% bi-allelic gene disruption was seen, with disruption frequency correlating with the concentration of ZFN-encoding mRNA used (Table 1). The significance of such high-level bi-allelic disruption rates is highlighted by early studies that suggest bi-allelic *CCR5* disruption is required for full R5-tropic HIV resistance and thus may be essential for therapeutic efficacy.[2,3]

In addition to high-frequency gene disruption, electroporation of HSPCs, using even the highest dose of ZFN-encoding mRNA, did not negatively impact the level of engraftment or alter the lineage potential of the cells observed during a 20-week *in vivo* NSG mouse study. This suggests that engineered HSPCs can act as a long-term source of multilineage, HIV-resistant immune cells.[12]

CCR5 Gene Disruption in CD34+ Cells Following mRNA Electroporation

mRNA Concentration	50 µg/mL SB-728mR	150 µg/mL SB-728mR
Bulk Culture Disruption	41	57
Total CFU (#)	192	198
Modified CFU	55%	70%
Mono-allelic Disruption	33%	19%
Bi-allelic Disruption	22%	51%
Modified CFU with Bi-allelic Disruption	40%	73%

Table 1. Electroporated HPSCs were evaluated via Cel-1% assay for overall *CCR5* disruption in 2-week bulk cultures or individual colonies derived from 2-week CFU cultures via deep sequencing analysis. CFU = colony forming units.

Reproducible, Clinical-scale Manufacture of *CCR5*-disrupted HSPCs

Full qualification runs using the production process proposed for the anticipated clinical trial were performed to assess manufacturing feasibility, reproducibility, and donor-to-donor variability. Cells harvested from four healthy human donors were electroporated with 150 mg/mL of *CCR5*-targeted ZFN mRNA, twice the dose proposed for the clinical trial, thereby maximizing the ability to identify off-target ZFN activity and potential tumorigenic activity. Cell viability levels ranged from 82–92% with *CCR5* disruption rates >50% as determined using deep sequencing for 3 of the 4 donors.[12] Release testing for sterility, stability, and phenotypic analysis was conducted.

Engraftment Rates

HSPC transferred	No EP Controls	3 Qualification Runs
Mice with hCD45+ cells in blood	78%	88%
Mice with hCD45+ cells in bone marrow	98%	96%
CCR5 gene disruption in hCD45+ PBMC	0%	10-20%
CCR5 gene disruption in hCD45+ bone marrow	0%	18-31%

Table 2. Mice received HSPC from three independent donors either following a qualification run or non-electroporatin (EP) control cells. The presence of CD45+ cells in peripheral blood mononuclear cells and bone marrow was assessed at 4-, 12- and 22-weeks.[12] Results of 22-week analysis are reported here. *CCR5* gene disruption in hCD45+ cells was determined using MiSeq analysis.

IND-Enabling Studies: Positive Safety Profile

In vivo adoptive transfer studies using immunodeficient, NSG mice were performed using cells from the clinical-scale qualification runs to determine if any undetected genomic modifications would lead to tumor formation or toxicity upon engraftment. In pre-IND discussions with the FDA, these studies were deemed sufficient for meeting preclinical safety expectations in regard to the anticipated clinical trial. NSG mice received $1x10^6$ electroporated CD34+ cells (equivalent to a full human dose), or no electroporation cells from the same donors. At the time of engraftment, *CCR5* disruption rates ranged from 54 - 67%. Engraftment rates of the engineered HSPCs in the bone marrow and blood were similar to those of no electroporated cells over the 22-week study (Table 2). No evidence of toxicity or tumorigenicity was noted demonstrating product safety.

Initiation of Human Clinical Trial

The bi-allelic disruption efficiency, manufacturability, reproducibility, and general safety demonstrated in these studies supported initiation of a clinical trial to assess the feasibility and safety in patients with HIV-1 (Clinical Trial #: NCT02500849).

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Conclusion

These studies demonstrate how MaxCyte non-viral cell engineering technology provides the required efficiency, viability, and safety to attain the necessary therapeutic index, as well as the scalability and regulatory-compliance to rapidly navigate clinical development – all while simultaneously eliminating the cost, complexity, inconsistency, and potential toxicities of viral vectors. While this application note highlights the success of *CCR5* disruption in HSPCs as a treatment for HIV, this non-viral gene editing approach has been employed for the treatment of a growing number of indications ranging from monogenic disorders such as sickle cell disease to cancer.

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