Base Editing of Human Hematopoietic Stem and Progenitor Cells with the Pin-point™ Platform

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1 Introduction

Hematopoietic stem and progenitor cells (HSPCs) are a foundational cell type for the development of engineered therapies. Given their susceptibility to DNA damage, it is crucial to employ gene editing technologies that minimize genotoxicity. Base editors, such as our Pin-point™ platform efficiently mitigate the challenges posed by nuclease-induced double-strand breaks (DSBs), such as activation of the DNA damage response and chromosomal aberrations.

Our Pin-point™ platform is a modular base editor allowing ratio optimisation and switching out of the components for different applications[1]. It is capable of complex genetic modifications in a single intervention without relying on the introduction of DSBs as we have shown in primary human T cells[2] and iPSCs. The advanced safety profile of this technology makes it well suited to sensitive cell types such as HSPCs.

In HSPCs we used a Pin-point base editor composed of Rat APOBEC1 and SpCas9 nickase mRNAs to achieve up to 80% C to T conversion at an MHC-I chain locus with high levels of editing purity and very low incidence of indels, while retaining the most primitive HSC population. We also targeted two separate loci known to reactivate γ-globin expression and achieved a high level of base editing at both loci that corresponded with an increase in γ-globin mRNA and protein expression. Edited HSPCs retained viability, immunophenotype, and differentiation potential toward the erythroid lineage *in vitro*.

The ability to base edit HSPCs efficiently and safely, while retaining high cell viability and differentiation capability, demonstrates the strength of the Pin-point platform as a tool for the generation of advanced cell therapies using sensitive cell types.

2 The modular Pin-point base editing platform

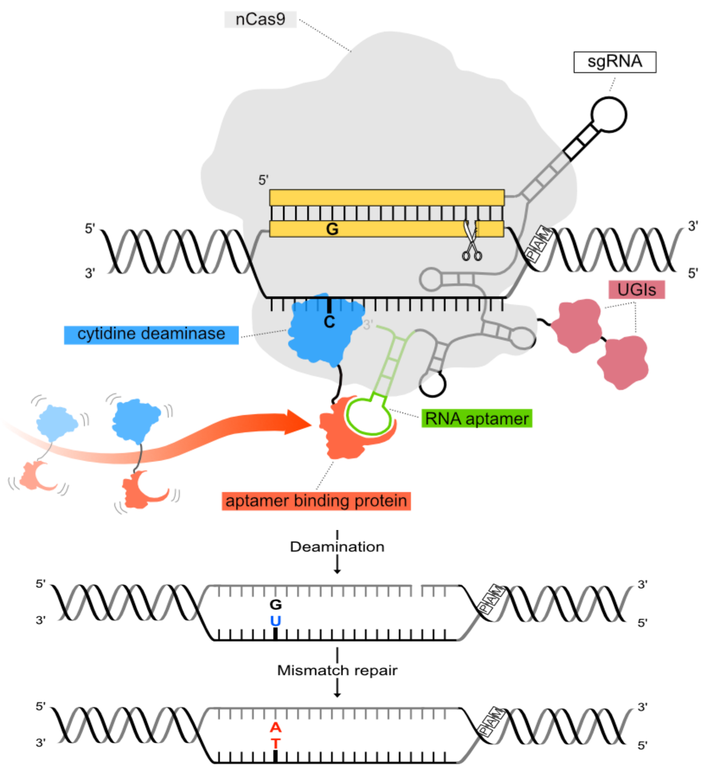


Figure 1: Schematic of one configuration of the modular Pin-point base editor system.
The Pin-point base editing platform allows precise genome modification by single nucleotide conversion. In one possible configuration of the platform, a Cas9 nickase (nCas9) is guided to the DNA target site via a guide RNA with an aptameric region engineered into the scaffold. The aptamer recruits a deaminase via fusion with an aptamer binding protein. When a cytidine deaminase is recruited, conversion of a cytidine to thymidine in the target sequence is achieved. The combination of nCas9, an aptamer binding protein fused to a deaminase, and an aptameric guide RNA efficiently base edit a DNA target of interest.

3 Cell viability and yield are retained post editing without inducing a P53 DNA damage response

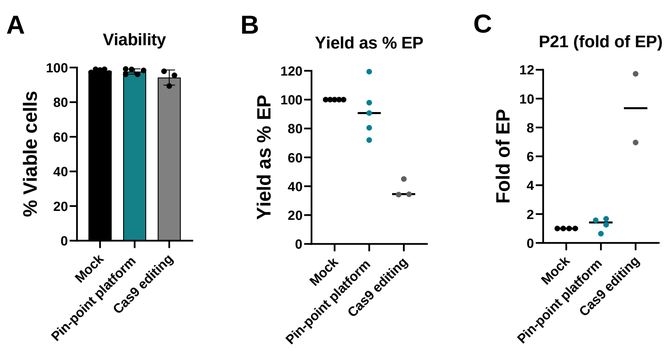


Figure 2. HSPCs were electroporated with either Pin-point mRNAs, or mRNA encoding a Cas9 gene editor, and an sgRNA targeting an MHC-I chain and cultured in StemSpan SFEM II media supplemented with StemSpan CD34 expansion supplement, SR1 and UM729. High viability (A) and cell recovery (B) were observed at day 4 after delivery of the Pin-point base editing reagents with negligible upregulation of P21 mRNA expression (C) measured by qPCR at 2 days post electroporation.

4 Highly efficient base editing achieved in HSPCs

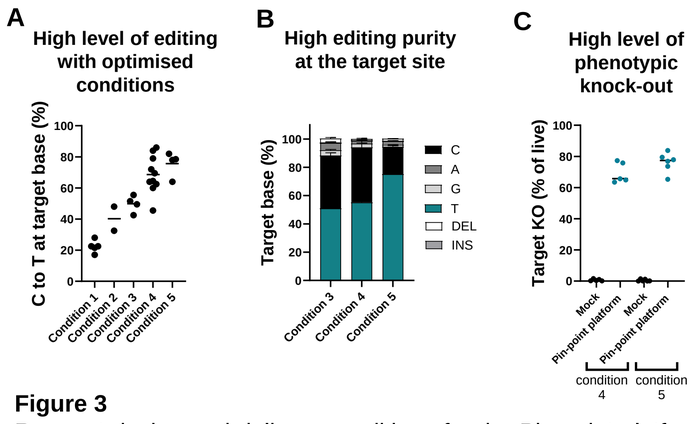


Figure 3
Reagent design and delivery conditions for the Pin-point platform were optimised to achieve high levels of base editing in mobilized peripheral blood (PB) CD34+ cells. Pin-point mRNAs (nCas9 and rAPOBEC1) and a synthetic aptameric sgRNA designed to target an MHC-I chain for gene knockout were delivered to HSPCs by electroporation. Percentages of C to T conversion in samples analysed by Sanger sequencing are reported across different conditions (A). Samples from the three best conditions were analysed by NGS and the percentage of intended (C to T) and unintended editing events (C to G, C to A and indels) at the target base are reported (B). n = 2 HSPCs donors. High levels of MHC-I chain knockout are achieved in samples analysed by flow cytometry (C). Each dot represents 1 HSPC donor (A and C).

5 HSC population is retained after editing with the Pin-point platform

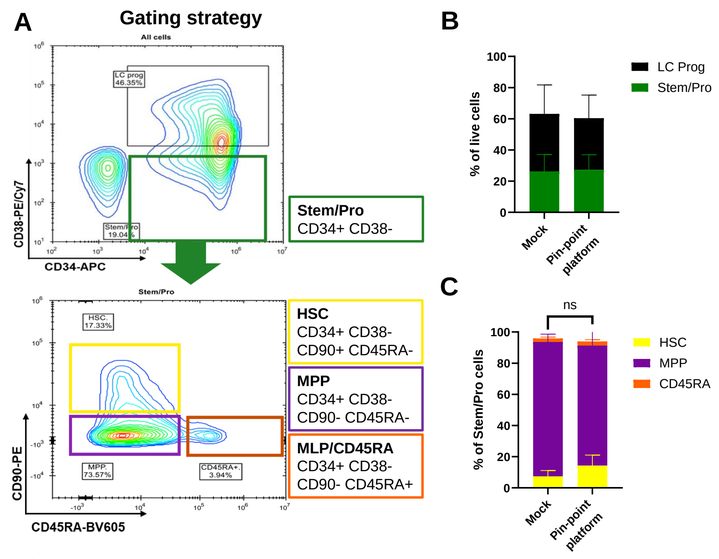


Figure 4
HSPCs were electroporated with Pin-point mRNAs and an sgRNA targeting the MHC-I chain; cultured in StemSpan SFEM II media supplemented StemSpan CD34 expansion supplement, SR1 and UM729; and immunophenotyped by flow cytometry 3-4 days after electroporation. Hematopoietic stem cells (HSC) were gated as follows (A): cells > singlets > live cells > CD34+ CD38- > CD90 vs CD45RA. The proportion of stem and progenitor cells (B) and HSCs (C) wasn't compromised by editing (2-tailed Wilcoxon non-parametric T test applied). n = 5 HSPC donors

6 Highly efficient base editing achieved in the most primitive compartment

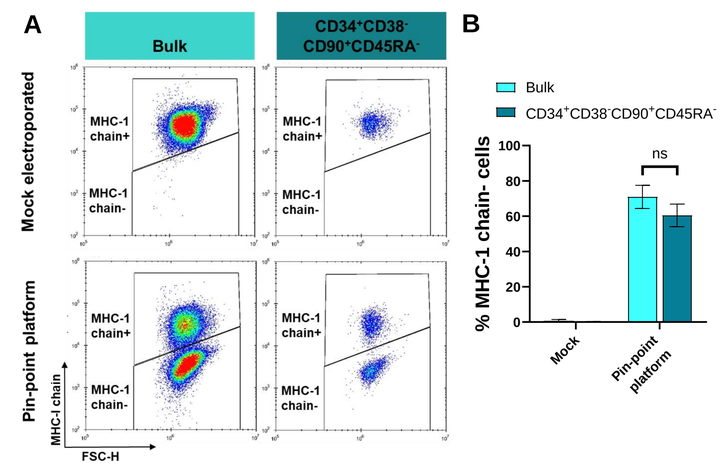
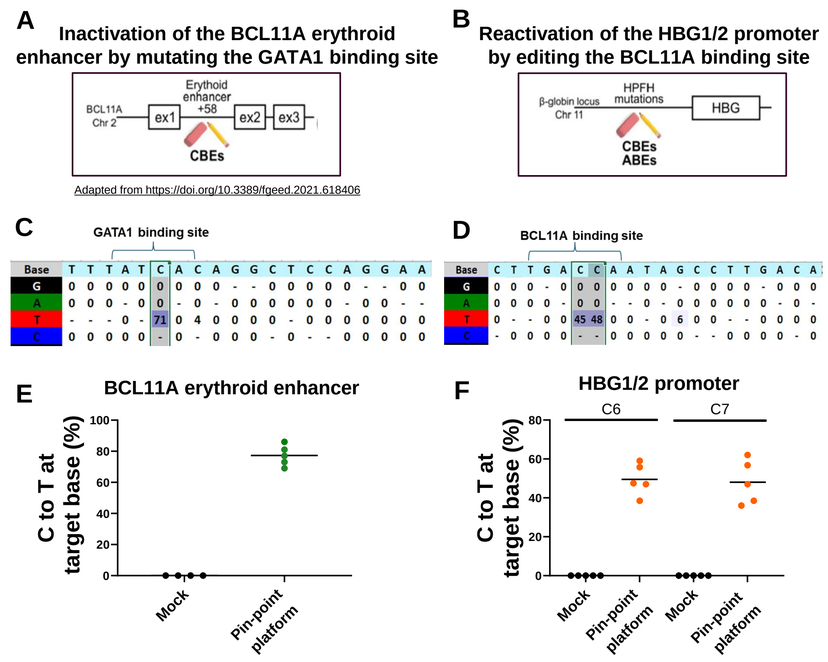


Figure 5
HSPCs were electroporated with Pin-point mRNAs and an sgRNA targeting the MHC-I chain; cultured in StemSpan SFEM II media supplemented with StemSpan CD34 expansion supplement, SR1 and UM729; and immunophenotyped by flow cytometry 3-4 days after electroporation. Hematopoietic stem cells (HSC) were gated as follows (Figure 4A): cells > singlets > live cells > CD34+ CD38- > CD90 vs CD45RA. Within the most primitive HSC population (CD34+ CD38- CD90+ CD45RA-), edited cells (MHC-I chain-) were distinguished from unedited cells (MHC-I chain+) as shown in the representative flow cytometry plots (A). 60-80% MHC-I chain KO was achieved not only in the bulk HPSC compartment, but also in the most primitive (HSC) compartment (B) n = 4; 2-tailed Wilcoxon non-parametric T test

7 Efficient base editing achieved at therapeutically relevant sites

Strategies to upregulate Fetal Hemoglobin (HbF) by base editing



Adapted from https://doi.org/10.3389/fgeed.2021.618406

Figure 6
Reactivation of fetal γ-globin (HBG1/2) expression has therapeutic benefits in patients with β-hemoglobinopathies. HBG1/2 expression can be reactivated by two main base editing strategies: 1) mutation of the erythroid enhancer of BCL11A, a transcriptional repressor of HBG1/2 (A); or 2) mutation of the binding site of the transcriptional repressor in the HBG1/2 promoter (B). The Pin-point base editing platform allows editing of the BCL11A erythroid enhancer (C) with 80% efficiency (E); and of the BCL11A binding site in the HBG1/2 promoter with 40-60% efficiency (F). n = 5 HSPC donors.

8 Editing therapeutic targets induces increased HbF expression

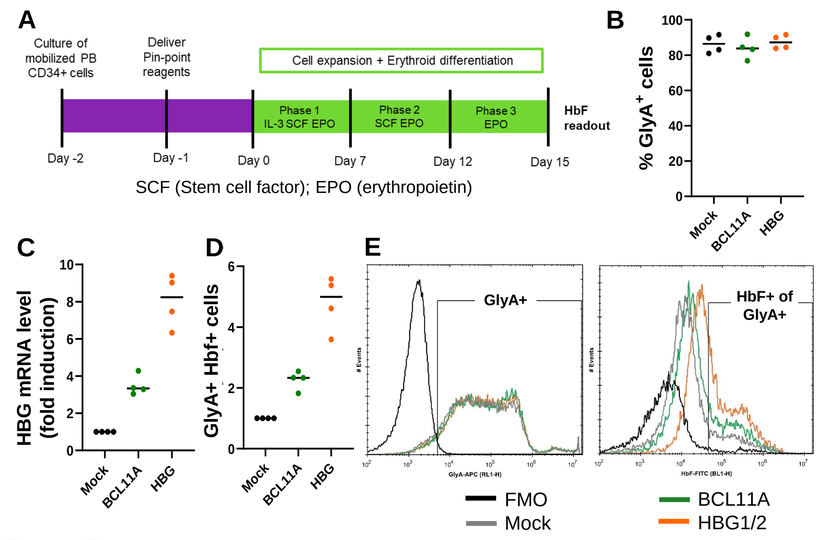


Figure 7
in vitro erythroid differentiation (A) was achieved with 80-90% efficiency using GlyA quantification by flow cytometry as a proxy for differentiation (B). Editing at both the BCL11A erythroid enhancer and the HBG1/2 promoter sites upregulated expression of HBG1/2 mRNA as measured by qPCR at day 15 of differentiation (C). This correlated with an increase in HbF levels quantified by flow cytometry (D). Upregulation of HBG mRNA and HbF expression was higher in samples edited in the HBG1/2 promoter region than samples where the BCL11A erythroid enhancer was mutated. Representative flow cytometry histograms of GlyA+ cells and GlyA+HbF+ cells are reported in (E). n = 4 HSPCs donors.

9 Summary

- Optimised reagent design and delivery conditions for the Pin-point base editing platform supports high editing efficiency with high editing purity and low incidence of indel formation in HSPCs.
- Cell viability and yield are retained post editing, without inducing a P21 DNA damage response.
- The most primitive hematopoietic stem cell (HSC) population is retained post editing with highly efficient base editing in the HSC population.
- Targeting of known regulatory sites of γ-globin with the Pin-point platform induces upregulation of HbF.

10 References

1. Collantes et al. The CRISPR Journal. DOI:10.1089/crispr.2020.003
2. Porreca et al. Mol Ther DOI: 10.1016/j.ymthe.2024.06.033