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

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1 Abstract

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 offer an efficient strategy to mitigate the challenges posed by nuclease-induced double-strand breaks (DSBs), such as activation of DNA damage response and chromosomal aberrations. We have developed the Pin-point™ platform, which enables the modular assembly of the base editor, including a DNA binding Cas and a DNA deaminase, via the interaction between an aptamer binding protein fused to the deaminase and an RNA aptamer located in the sequence-targeting guide RNA (gRNA). Allowing modifications of the DNA without relying on the introduction of DSBs, the Pin-point platform enables complex genetic modifications in a single intervention as demonstrated in primary human T cells and iPSCs, where we achieved efficient base editing at multiple sites and simultaneous targeted transgene knock-in without compromising genome integrity. The advanced safety profile of this technology makes it well suited to HSPCs. By optimising reagent design and delivery conditions of a Pin-point base editor composed of Rat APOBEC1 and SpCas9 nickase mRNAs, we achieve up to 80% C to T conversion at the B2M locus with high levels of editing purity and very low incidence of indels, an indirect measure of DSBs occurrence. Using the optimised conditions, we then targeted two separate loci known to reactivate γ-globin expression: the erythroid enhancer of the repressor BCL11A and the BCL11A binding site in the HBG promoter. We 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 Pin-point base editing platform

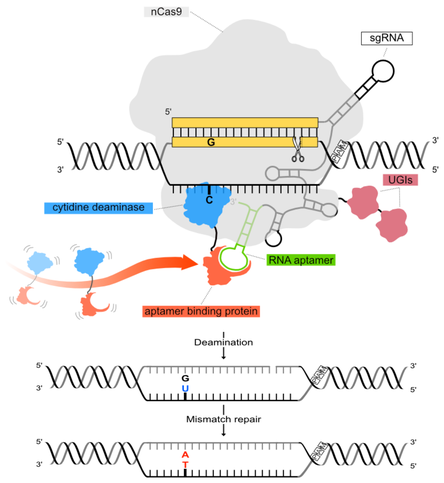

Figure 1: Schematic of one configuration of the modular Pin-point base editor system.
The Pin-point base editing platform allows precise genome modification by single nucleotide conversion. In one possible configuration of the platform, a Cas9 nickase (nCas9) is guided to the DNA target site via a guide 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 Highly efficient base editing achieved in HSPCs

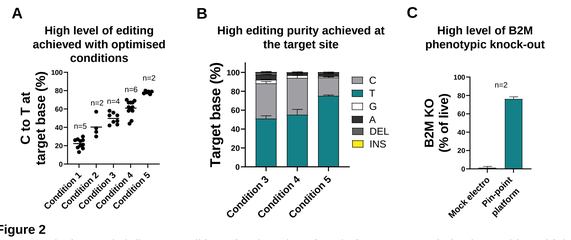

Figure 2
Reagent design and delivery conditions for the Pin-point platform were optimised to achieve high levels of base editing in mobilized peripheral blood (PB) CD34+ cells. Pin-point mRNAs (nCas9 and rAPOBEC1) and a synthetic aptameric sgRNA designed to target B2M for gene knockout were delivered to HSPCs by electroporation. Percentages of C to T conversion in samples analysed by Sanger sequencing are reported across different conditions (A). n = 2-6 HSPCs donors. 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 B2M knockout are achieved in samples analysed by flow cytometry (C). n = 2 HSPCs donors.

4 Highly efficient base editing achieved in the most primitive compartment

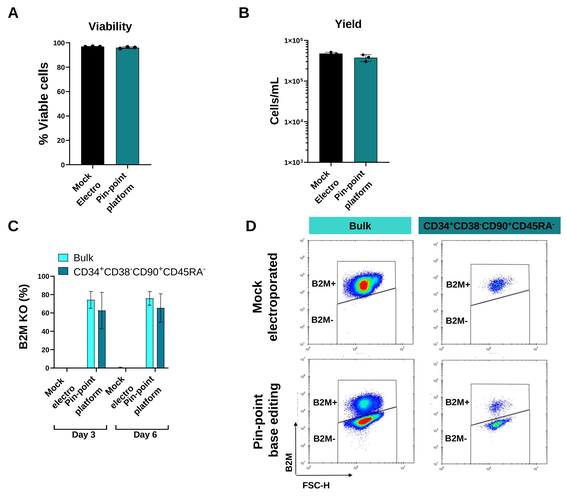

Figure 3
HSPCs were electroporated with Pin-point mRNAs and an aptameric sgRNA targeting B2M and cultured in StemSpan SFEM II media supplemented with SR1 and UM729. Cells were immunophenotyped by flow cytometry at day 3 and 6 post electroporation. High viability and cell recovery were observed at day 3 after delivery of the Pin-point base editing reagents (A, B). High percentage of B2M negative cells is observed in the HSPCs bulk population and in the stem compartment (CD34+ CD38- CD90+ CD45RA-) (C). n = 3 HSPCs donor. Representative flow cytometry plots from cells mock electroporated or edited with the Pin-point platform analysed at day 6 are reported (D). Gating strategy is as follow: (bulk) single cells> live cells > B2M - ; (CD34+ CD38- CD90+ CD45RA-) single cells> live cells > CD34 + CD38 - > CD90 + CD45RA - > B2M -.

5 Efficient base editing achieved at therapeutically relevant sites

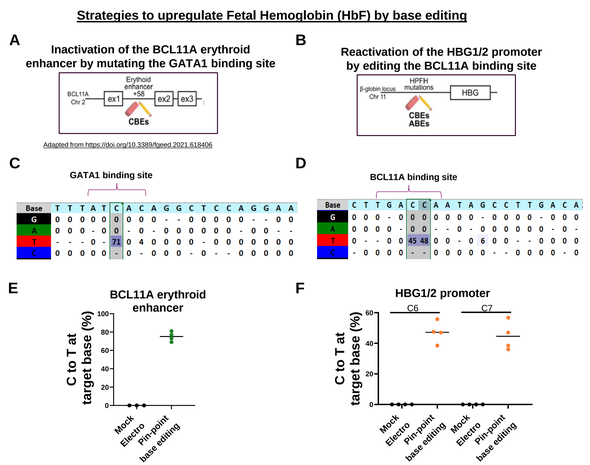

Figure 4
Reactivation of fetal γ-globin (HBG1/2) gene expression has therapeutic effects in patients with b-hemoglobinopathies, including b-thalassemia and sickle cell disease. HBG1/2 expression can be reactivated by two main base editing strategies: the mutation of the erythroid enhancer of BCL11A, a transcriptional repressor of HBG1/2 (A), or the 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) and of the BCL11A binding site in the HBG1/2 promoter (D). Editing rates ranging from 60-80% were achieved at the BCL11A erythroid enhancer (E) and from 40-50% at the HBG1/2 promoter (F). n = 4 HSPCs donors

6 Therapeutic levels of HbF achieved with Pin-point base editing

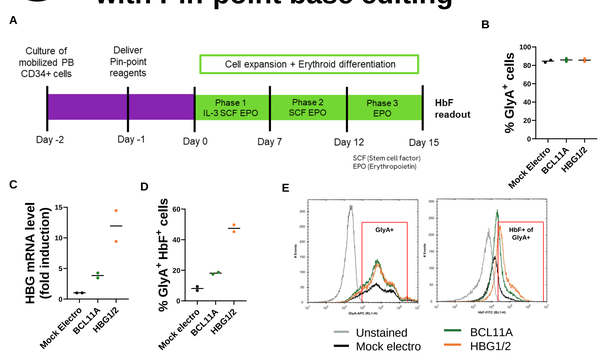

Figure 5
Cells edited with the Pin-point base editing platform and mock electroporated cells were subjected to *in vitro* erythroid differentiation (A). Cells differentiated into the erythroid lineage as assessed by flow cytometry staining for the erythroid marker GlyA (B). Editing at both the BCL11A erythroid enhancer or the HBG1/2 promoter upregulated the expression levels of HBG1/2 genes as measured by qPCR at day 15 of differentiation (C). The increased expression of HBG1/2 resulted in upregulation in HbF levels as measured by flow cytometry (D). Mutating the BCL11A binding motif located at the HBG1/2 promoters produced the highest HbF upregulation. Representative flow cytometry histograms of GlyA+ cells and GlyA+HbF+ cells are reported in (E). n = 2 HSPCs donors

7 Efficient multi gene editing achieved in other therapeutic cell types

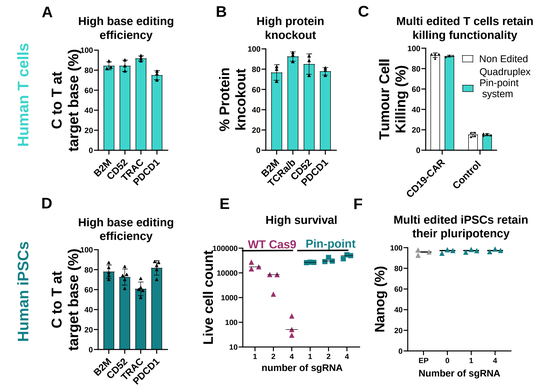

Figure 6
A) Four genes are edited simultaneously in T cells after delivery of Pin-point mRNAs and synthetic aptameric sgRNAs. Percentages of C to T conversion are reported. B) Protein knockout of 4 targets analysed by flow cytometry. C) In vitro tumour cell killing assay performed with either empty (control) or CD19-CAR expressing lentiviral vector transduced cells and mock electroporated (non edited) or T cells edited with the Pin-point platform (Quadruplex Pin-point system). n = 3 T cell donors for A-B; n = 2 T cell donors for C. D) Four genes are edited simultaneously in iPSCs after delivery of Pin-point mRNAs and synthetic aptameric sgRNAs. Percentages of C to T conversion are reported. E) Viability of iPSCs edited with single or multiple sgRNAs using the Pin-point system or Cas9 analysed by flow cytometry 48 hrs post electroporation. Viability was normalized to a no sgRNA control. C) Expression of the pluripotency marker Nanog in cells edited with the Pin-point base editing system analysed by flow cytometry 4 days post electroporation. Data shown in D, E, F are from two independent experiments with 2 iPSC lines.

8 Summary

- We have optimised reagent design and delivery conditions for the Pin-point base editing platform to achieve high levels of editing efficiency with high editing purity and low incidence of indels formation in HSPCs.
- Cell viability and yield are retained post editing.
- The highly efficient base editing is retained in the self-renewal and stem population.
- The Pin-point platform allows therapeutic editing to induce upregulated HbF levels.
- High levels of base editing are also achieved in other therapeutically relevant cell types such as T cells and iPSCs where we demonstrated efficient simultaneous editing at multiple sites with retained viability and functionality.

References

Collantes et al. The CRISPR Journal. Feb 2021.58–68
Porreca et al. bioRxiv 2023.06.20.545315

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