Pin-point™ Base Editing System: A Versatile Editing Platform Driving Cell Therapies

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Introduction

Gene editing technologies have successfully been used to improve the next generation of cell and gene therapies. However, standard gene editing platforms such as CRISPR-Cas9 trigger concerning cell toxicity and off-target effects due to the formation of DNA double-strand breaks (DSBs) that could hinder wider clinical applications. Base editing offers an ability to correct disease-causing mutations or knock out genes in a multiplexed manner, without introduction of DSBs. The first cohort of base editing therapeutics entered clinical trials only a few years after the development of the technology.

Horizon's modular Pin-point base editing system can use a nickase Cas9 with an aptameric guide RNA to recruit a deaminase to the site of interest, facilitating highly efficient and precise nucleotide conversion. We optimized design and delivery conditions of chemically modified synthetic guide RNAs and enzymatic mRNA to apply multiplex editing with our Pin-point base editing system to the development of engineered CAR-T cells. We target a set of therapeutically relevant loci for the development of allogeneic CAR-T cells achieving high knockout efficiency and editing purity at all sites simultaneously with a safer editing profile and enhanced cell viability compared to traditional nuclease systems. Our technology also enables robust simultaneous targeted knock-in and multiplex knockout without the requirement of additional sequence-targeting components. The ability to perform complex genome editing in multiple cell types (such as T cells, iPSCs, HSCs) safely, efficiently, and precisely opens the door to the application of the Pin-point system in a range of advanced cell therapies.

Horizon's Pin-point base editing system

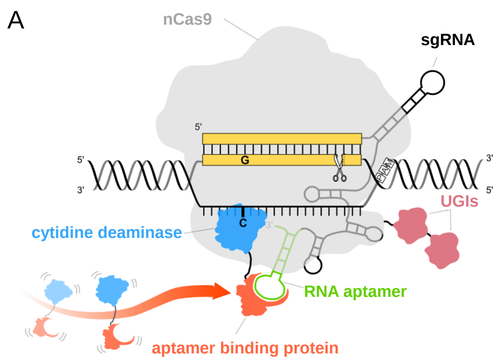
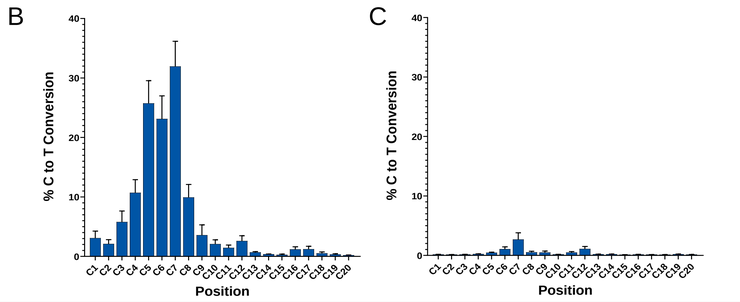


Figure 1: Aptamer-mediated base editing with Horizon's Pin-point system
A) Schematic of one possible configurations of the Pin-point base editing system.
B) Recruitment of APOBEC1 via an aptamer encoded in the sgRNA leads to efficient C to T conversion within a window centred on nucleotides 5-7 of the target sequence.
C) Negligible base editing occurs in the absence of an aptamer. Data are average base editing activity in a HEK293 cell line across a series of genomic loci targeted by the Pin-point system.



Multiplex gene editing in T cells

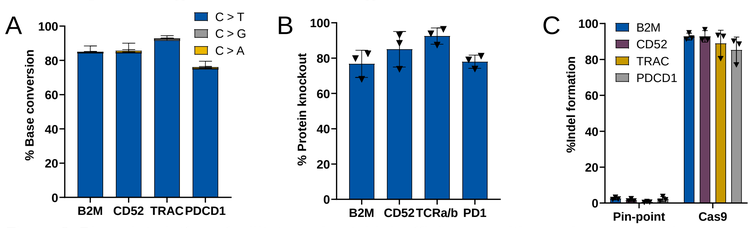


Figure 2: Pin-point platform facilitates robust, high efficiency multi-gene knockout without generating insertions or deletions (indels)
A) Distribution of edited outcomes at targeted cytosines B) Protein knockout of 4 targets analyzed by flow cytometry. C) Indel formation at target loci following Pin-point base editing and Cas9 gene editing analyzed by amplicon sequencing. *n* = 3 T cell donors.
T cells were activated before delivery of Pin-Point mRNA and synthetic sgRNAs targeting B2M, CD52, PDCD1 and TRAC by electroporation. Electroporated cells were either cultured unstimulated, or stimulated with PMA and Ionomycin 5 days post-electroporation for assessment of PD1 knockout. Analysis was performed 7 days post-electroporation.

Safety profile of multiplex edited T cells

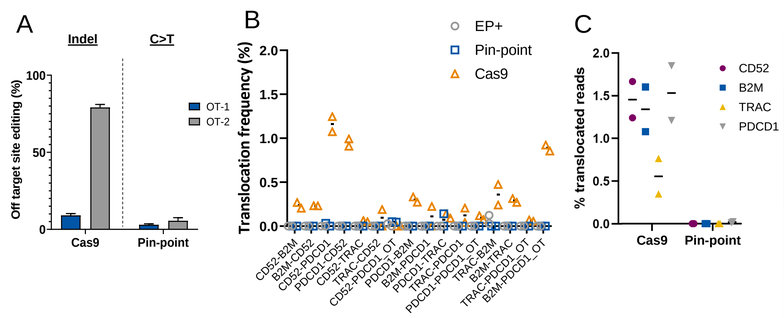


Figure 3: Minimal unintended editing outcomes following multi-gene knockout with the Pin-point system
A) Off-target editing at two in silico predicted sites following base editing with the Pin-point system and Cas9 gene editing analyzed by amplicon sequencing. B) Frequency of translocations between 4 target sites and an off-target site analyzed by digital droplet PCR. C) Frequency of translocations at each of 4 target sites analyzed by target enrichment sequencing. *n* = 3 T cell donors for A; *n* = 2 T cell donors for B, C.

Chimeric Antigen Receptor (CAR)-T cell engineering

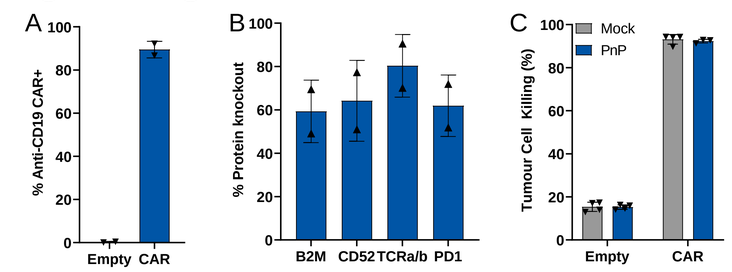


Figure 4: Multi-gene edited CAR-T cells using the Pin-point system retain therapeutic function
A) Percentage of CAR positive T cells in the transduced population. B) Protein knockout of 4 targets in CAR transduced T Cells analyzed by flow cytometry. C) In vitro tumor cell killing assay performed with mock electroporated or T cells edited with the Pin-point system and transduced with either empty or CAR expressing lentiviral vectors. *n* = 2 T cell donors for A-C.
T cells were activated, transduced with lentiviral particles containing either no insert "Empty", or anti-CD19 CAR, and selected with puromycin. Pin-Point mRNA and synthetic sgRNAs targeting B2M, CD52, PDCD1 and TRAC were delivered by electroporation. Electroporated cells were either cultured unstimulated, or stimulated with PMA and Ionomycin 5 days post-electroporation for assessment of PD1 knockout. Analysis was performed 7 days post-electroporation.

One-step simultaneous knock-in and multiplex knock-out in T cells

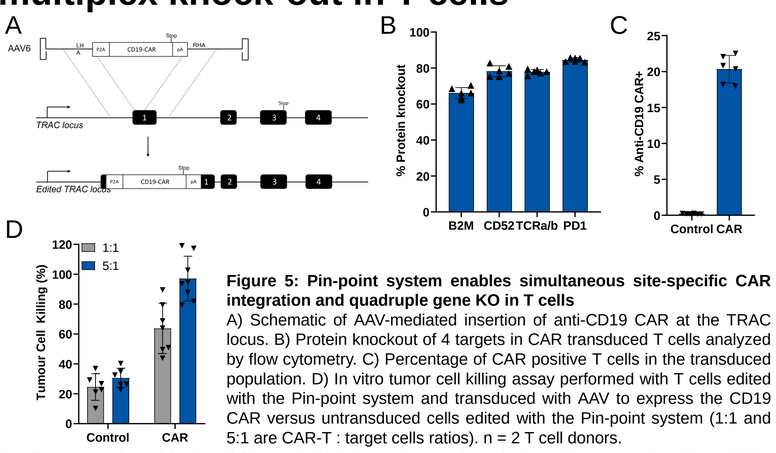


Figure 5: Pin-point system enables simultaneous site-specific CAR integration and quadruple gene KO in T cells
A) Schematic of AAV-mediated insertion of anti-CD19 CAR at the TRAC locus. B) Protein knockout of 4 targets in CAR transduced T cells analyzed by flow cytometry. C) Percentage of CAR positive T cells in the transduced population. D) In vitro tumor cell killing assay performed with T cells edited with the Pin-point system and transduced with AAV to express the CD19 CAR versus untransduced cells edited with the Pin-point system (1:1 and 5:1 are CAR-T : target cells ratios). n = 2 T cell donors.
T cells were activated before delivery of Pin-Point mRNA and synthetic sgRNAs targeting B2M, CD52, PD1 and TRAC by electroporation. This was followed by delivery of AAV particles loaded with the HDR donor DNA for the CD19-CAR by transduction. Flow cytometry analysis was performed 5 days post-electroporation. The killing assay was performed by co-culturing the CAR-T cells with antigen positive cancer cells and measuring the percentage of killed target cells.

iPSCs edited with the Pin-point system retain pluripotency

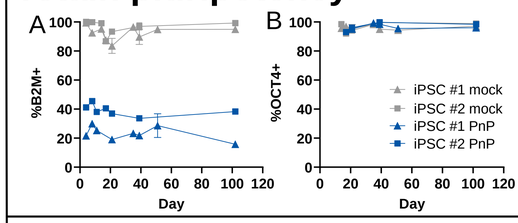


Figure 6: Stable expression of B2M (A) and the pluripotency marker OCT4 (B) over prolonged culture analyzed by flow cytometry after electroporation of two iPSCs lines with Pin-point mRNA and synthetic sgRNA targeting B2M.

Multiplex base editing in iPSCs

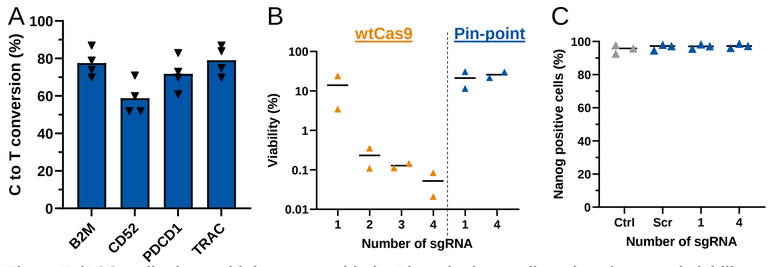


Figure 7: iPSCs edited at multiple targets with the Pin-point base editor show improved viability
A) Base editing at targeted cytosines analyzed by amplicon sequencing 7 days post electroporation.
B) Viability of iPSCs edited with single or multiple sgRNAs using Pin-point or Cas9 analyzed by flow cytometry 48hrs post electroporation. Viability was normalized to a no sgRNA control. C) Expression of the pluripotency marker Nanog analyzed by flow cytometry 4 days post electroporation.
Data shown in B,C are from two independent experiments with 2 iPSC lines.

One-step simultaneous knock-in and multiplex knock-out in iPSC

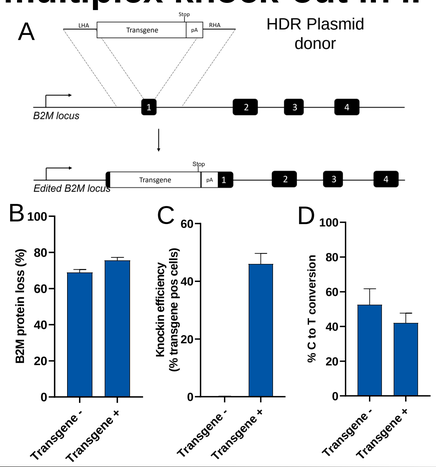


Figure 8: Pin-point system enables simultaneous site specific transgene integration and multiple gene KO in iPSCs
A) Schematic of plasmid-mediated insertion of a transgene (promoter-less GFP) at the B2M locus. B) B2M knockout analyzed by flow cytometry. C) Percentage of GFP positive iPSCs in the transduced population analyzed by flow cytometry. D) Percentage of C to T editing at the CIITA locus (CIITA knockout).

iPSCs were treated with Rho kinase inhibitor prior to dissociation to single cells and electroporated with Pin-point mRNA, synthetic sgRNAs (targeting B2M and CIITA) and with or without the HDR plasmid donor (Transgene + ; Transgene -).

Conclusions

- We applied multiplex base editing with the Pin-point system for the development of engineered CAR-T cells and hypoimmunogenic iPSCs.
- Base editing with the Pin-point system achieved greater than 70% knockout efficiency and high purity at therapeutically relevant target sites (B2M, CD52, TRAC and PDCD1) in T cells and iPSCs.
- Multiplex gene editing with the Pin-point platform substantially reduces guide-dependent off target editing and chromosomal translocations compared to Cas9-mediated knockout.
- CAR-T cells retain their cytotoxic activity in vitro following multiplex gene editing with the Pin-point system.
- The Pin-point platform enables simultaneous site-specific transgene knock-in and multi-gene knockout in T cells and iPSCs.
- iPSCs edited with the Pin-point platform retain pluripotency.
- We observed greatly improved viability of iPSCs following multi-gene editing with the Pin-point system compared to Cas9.

References

Collantes et al. The CRISPR Journal. Feb 2021.58–68

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