Prime Editing in Hematopoietic Diseases and Beyond: Efficacy and Safety

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Summary

- **Prime Editing precisely corrects** a predominant mutation that causes Chronic Granulomatous Disease (CGD)
- **Prime Editing restores protein expression and NADPH oxidase function** in myeloid progeny produced from Prime Edited CGD patient $CD34^{+}$ cells
- **We have developed a comprehensive toolset to identify potential off-target events** including new unbiased genome-wide tools
- **No unintended or off-target edits** were detected in multiple editing programs

Prime Editing to correct pathogenic mutation in *NCF1* that causes Chronic Granulomatous Disease (CGD)

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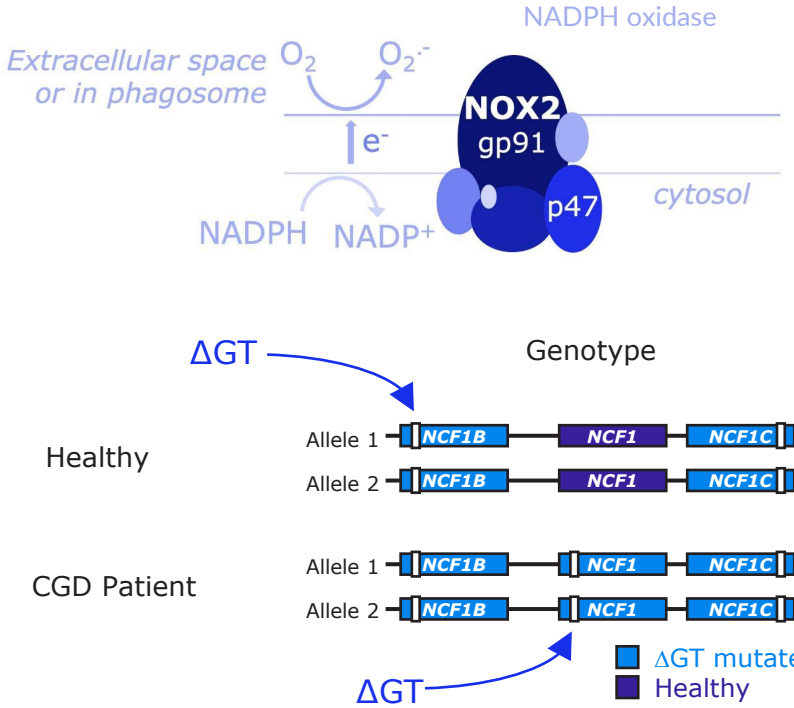
p47^{phox} Chronic Granulomatous Disease

- ***Description:***
  - Rare disease causing recurrent, debilitating infections & premature death
- ***Human genetics and biology:***
  - Autosomal recessive form caused by mutations in the *NCF1* gene, which encodes p47Phox, a component of NADPH oxidase
  - **c.75_76delGT mutation** (or **ΔGT**) is a 2-nt deletion found in **~80% of mutant *NCF1*** in patients with p47^{phox}-CGD
- ***Unmet need:***
  - No disease modifying approved therapies
- ***Prime Medicine's approach:***
  - Autologous hematopoietic stem cell transplant (HSCT) after *ex vivo* correction of the ΔGT mutation



NCF1B and *NCF1C* are pseudogenes of *NCF1*

Correction of ΔGT in *NCF1* in CD34⁺ cells to restore p47^{phox} protein expression & function in myeloid progeny

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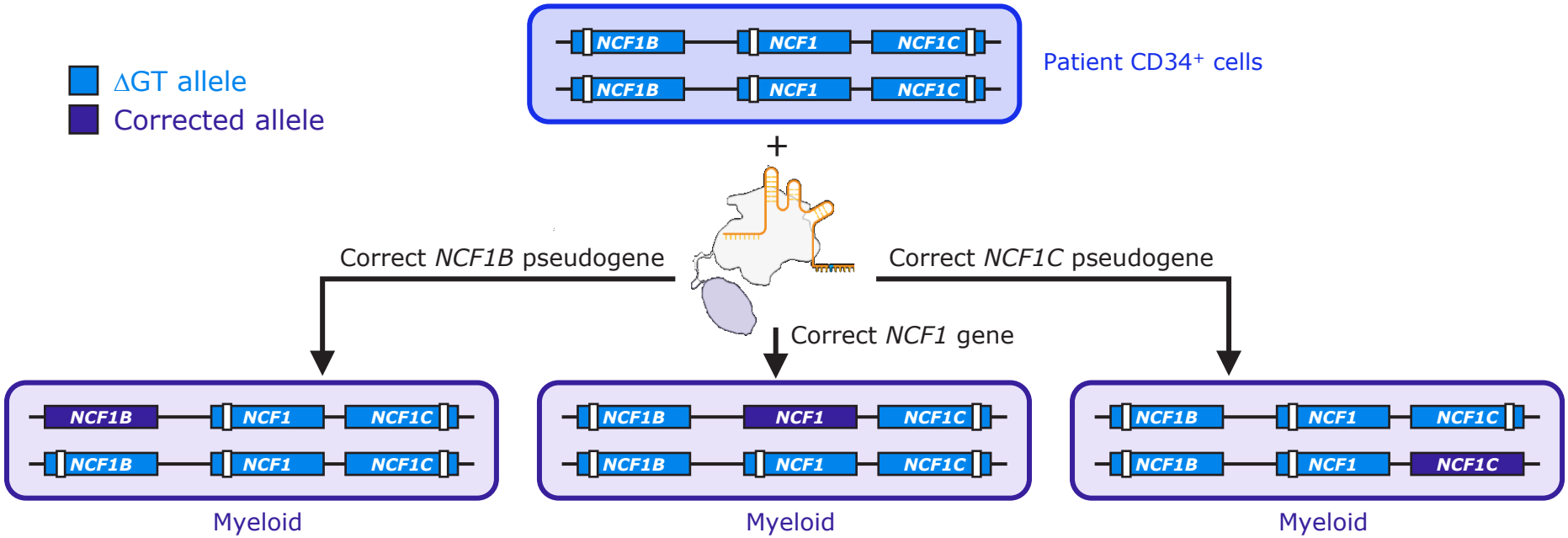
Correction of gene or pseudogene in CGD patient cells restores p47^{phox} expression



↑ p47^{phox} expression restores NADPH oxidase activity

Merling, et al. Gene-edited pseudogene resurrection corrects p47^{phox}-deficient chronic granulomatous disease. *Blood Adv*. **1**, 270-278 (2017).

Prime-Edited **patient** CD34+ cells generate myeloid cells that produce p47phox protein and oxidase activity

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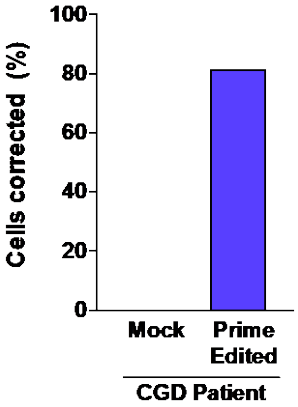
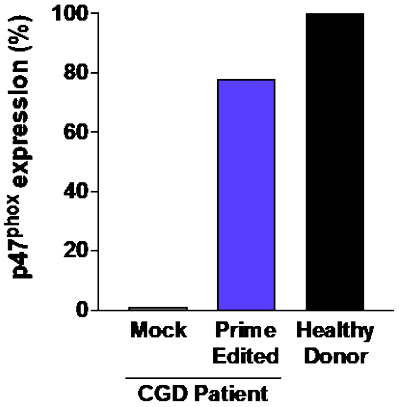
Myeloid progeny of Prime-Edited CD34+ cells from patient donor show functional p47phox expression

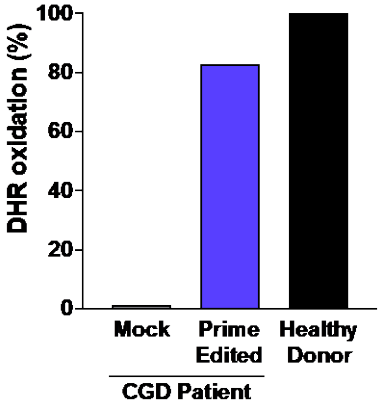
Post-editing patient CD34+ cell clonal analysis[1]



p47phox expression[2] restored in CGD myeloid cells[3]



PE restores oxidase activity in myeloid cell progeny[4]



Gene corrected → p47phox expression restored → Function recovered

Prime Editing results in ~80% of the p47phox levels in healthy donor myeloid cells, and restores oxidase activity in myeloid cells

[1]234 clones analyzed; [2]Normalized to healthy donor controls; [3]Myeloid cells produced from CD34+ cells were analyzed by flow cytometry for detection of myeloid markers including CD13 (percentage of cells expressing CD13 is depicted); [4] Oxidization of dihydrorhodamine (DHR) to fluorescent rhodamine by functional myeloid cells. Used a diagnostic for CGD patients (Jirapongsananuruk et al, J Allergy Clin Immunol, 2003). Performed in collaboration with Dr. Suk See DeRavin and Dr. Harry Malech.

CD34⁺ long-term hematopoietic stem cells self-renew & give rise to multiple cell lineages to support lifelong blood production

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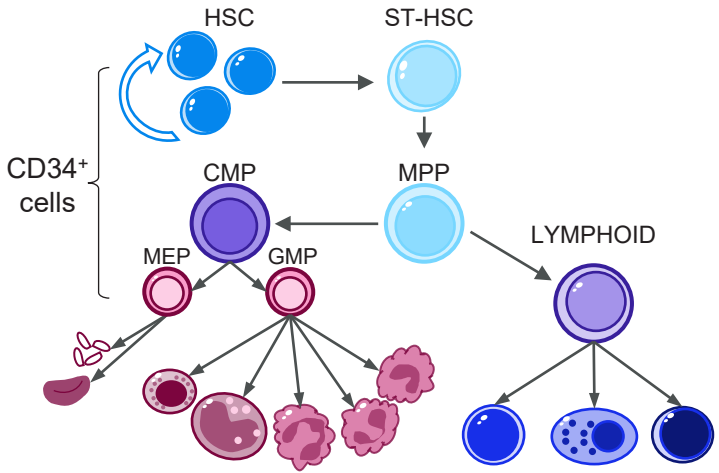
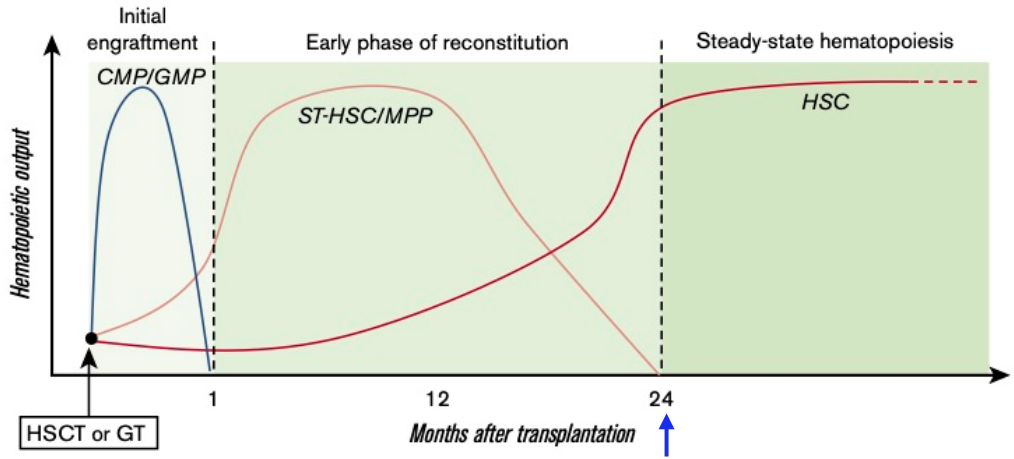
Given the heterogeneity within the CD34⁺ cell population, to evaluate human LT-HSC function, CD34⁺ cells are engrafted in immunodeficient mice until LT-HSCs repopulate the bone marrow and produce blood

Simplified schematic of blood cell development from hematopoietic stem cells



Mature terminally differentiated blood products

Human hematopoiesis after transplantation



Comparable timepoint in mouse is ~16 weeks

LT-HSC: long-term hematopoietic stem cell. ST-HSC: short-term repopulating hematopoietic stem cell. CMP: common myeloid progenitor. GMP: granulocyte macrophage progenitor. MEP: megakaryocyte erythroid progenitor. GT: gene therapy. HSCT: hematopoietic stem cell transplantation. Left Image concept adapted from Seita & Weissman, Wiley Interdiscip Rev Syst Biol Med. 2010. Right Image adapted from Scala & Aiuti Blood Advances 2019. Current gold standard for HSC function is in vivo engraftment into immunodeficient mice with human engraftment in the bone marrow as measured by the presence of hCD45+ cells 16 weeks post-transplant—Dever, et al Nature 2018

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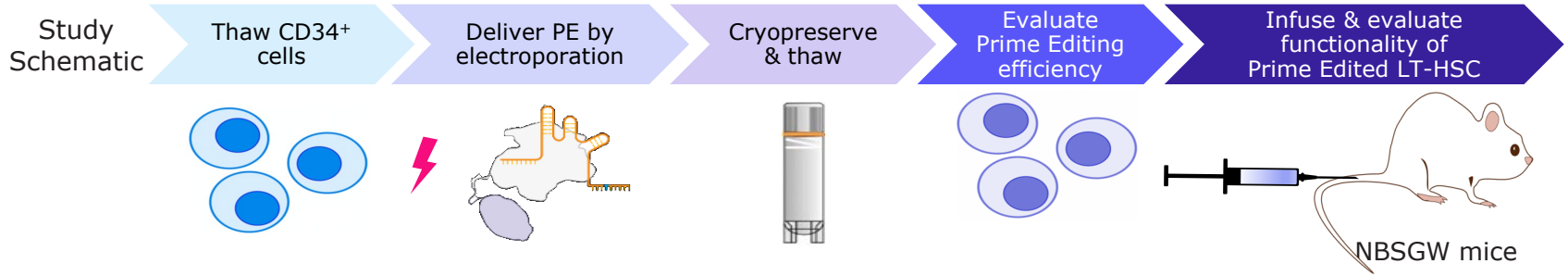
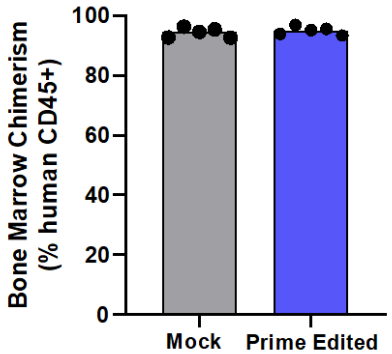
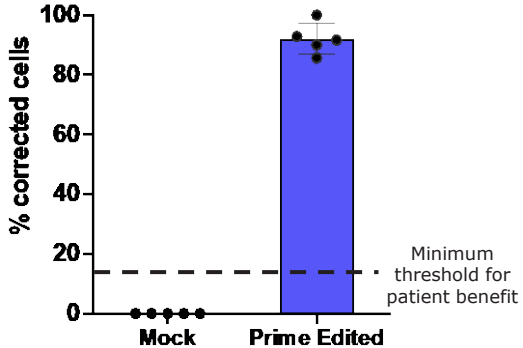
Successful Prime Editing long-term engrafted HSCs

16-week engraftment study with Donor 1 CD34⁺ cells



in vivo analysis shows
>95% human cell engraftment and **>92% editing in LT-HSCs*** (Donor 1)





NBSGW: NOD.Cg-*Kit*^{W41J} *Tyr* $^{+}$ *Prkdc*^{scid} *Il2rg*^{tm1Wjl}/ThomJ highly immunodeficient mice that support human CD34⁺ cell engraftment without irradiation. HSC: hematopoietic stem cell.
"Long-term" engraftment is characterization of engrafted CD34⁺ cells and progeny at >/= 16 weeks post cell infusion. No significant difference between Mock and PE groups. Statistical analyses by two-way ANOVA.

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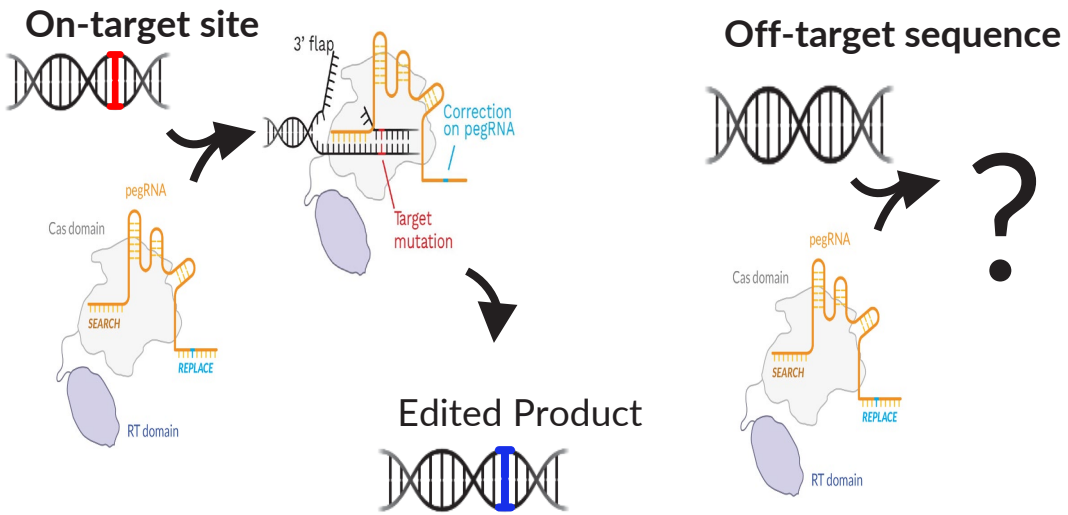
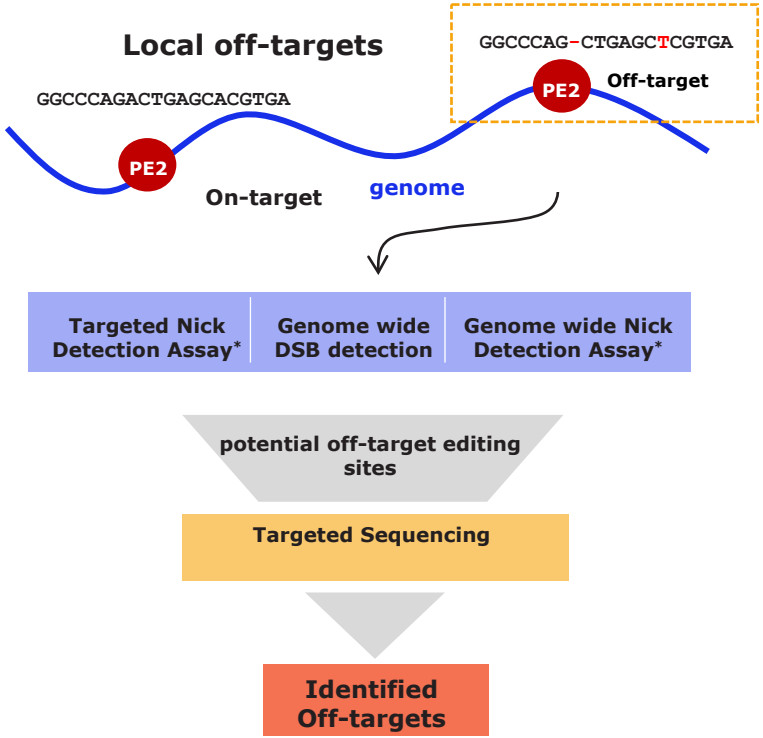
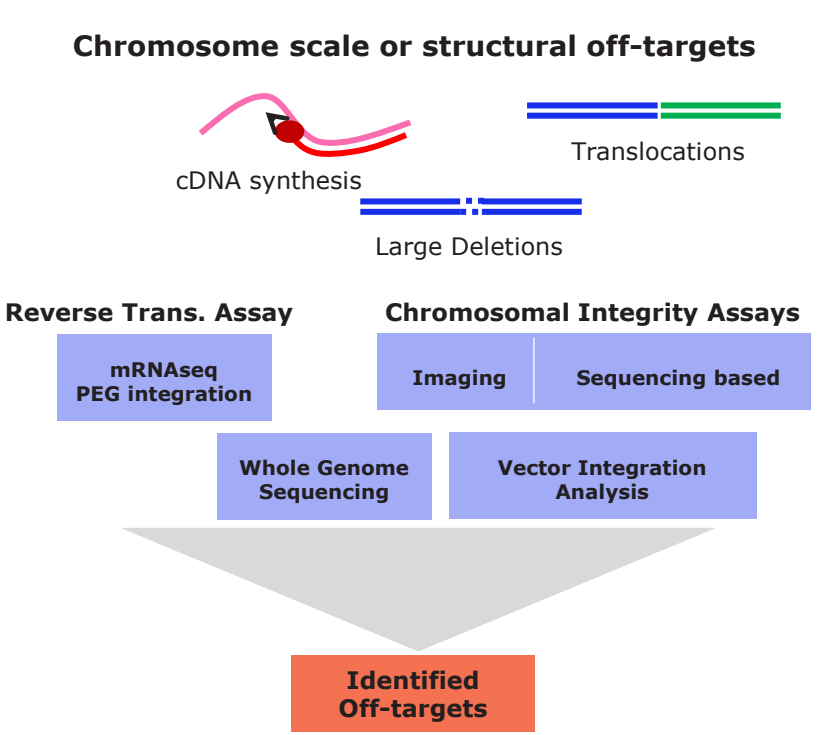
Safety: Prime editing and genotoxicity

- **Prime Editing generates single strand breaks in DNA driven by spacer interactions and uses reverse transcription (requiring two additional homology checks) to introduce a precise correction of a mutant sequence**
- **The mechanism of Prime Editing suggests there will generally be minimal risks of genotoxic events**
- **We have developed a suite of tools to evaluate potential off-target risks**



Safety: Prime's comprehensive suite of IND-ready assays for off-target discovery*





*Proprietary assay developed by Prime

New tools developed to identify potential Prime Editor Off-targets

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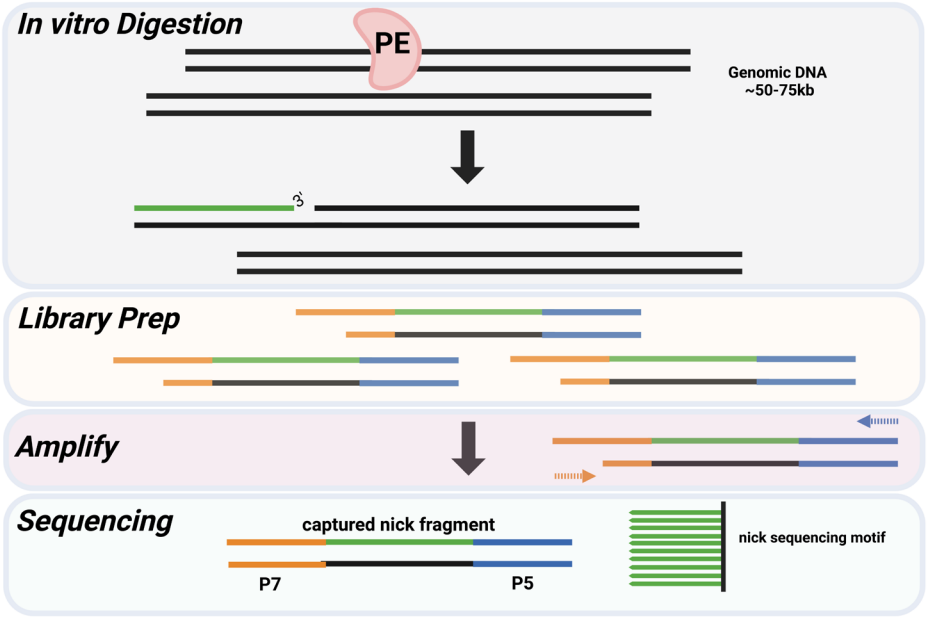
- **Prime Editing generates single strand breaks in DNA, most current off-target detection tools require double strand breaks.**
- **We are developing multiple single strand break specific techniques to identify potential off-target sites and better characterize the editing process**
- **PEG-seq is one approach that detects single strand breaks induced by gene editors genome wide and can identify potential off-targets of Prime Editors.**

3**P**rime **E**nd li**G**ation Sequencing (PEG-seq)



3Prime End liGation Sequencing (PEG-seq) is a novel method for identifying potential off-targets

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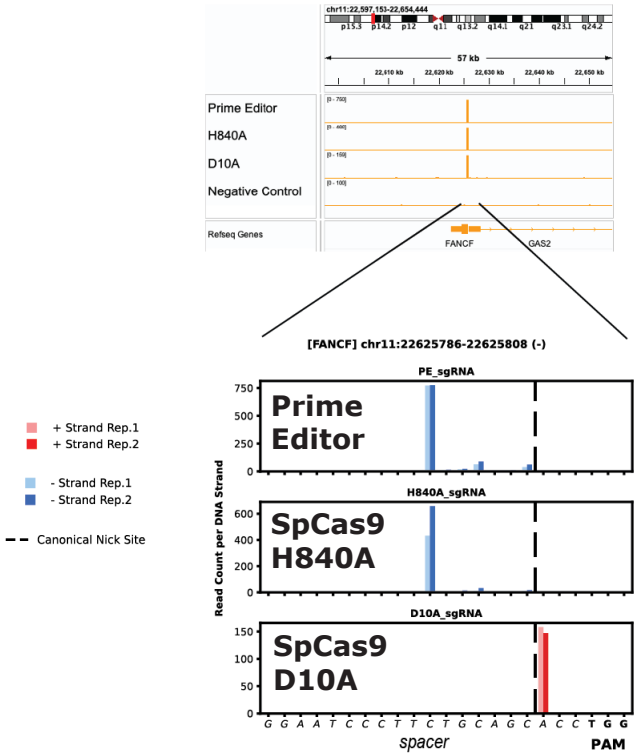
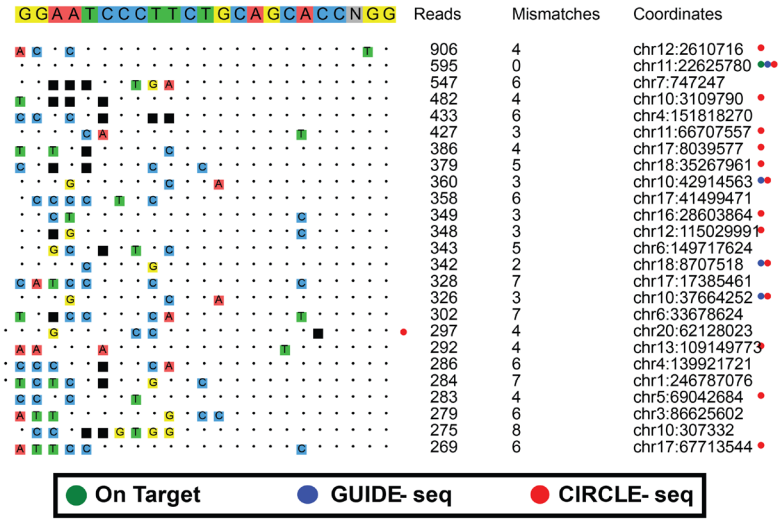
On-Target Signal



Potential off-target sites identified for FANCF spacer



Potential Prime Editor off-target sites overlap with sites identified using double strand break methods, but include new sites

Guide-seq data from Tsai et al., 2015; Circle-seq data from Tsai et al., 2017

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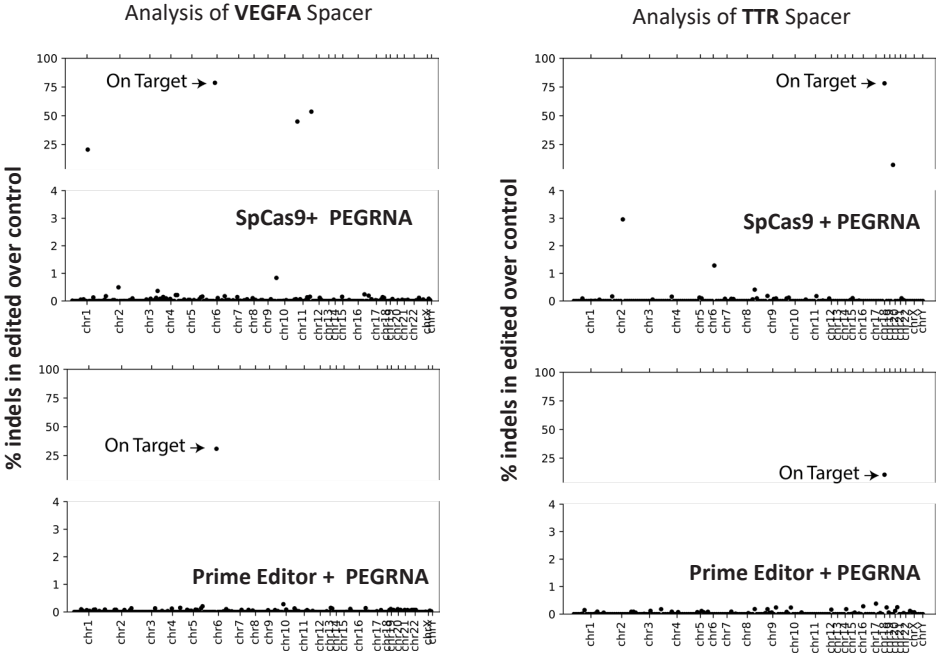
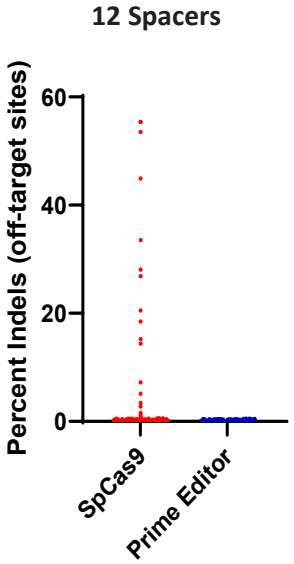
General Evaluations of Safety of Prime Editing

Evaluation of potential off-target sites in Prime Edited or SpCas9 edited cells by deep sequencing





- **In silico and biochemical tools can be used to define potential off-target sites for Prime Editors**
- **Direct comparisons of SpCas9 to Prime Editor reveal substantially fewer off-targets for Prime Editors.**

Editing in HEK293T cells by delivery of editor mRNA and PEGRNA

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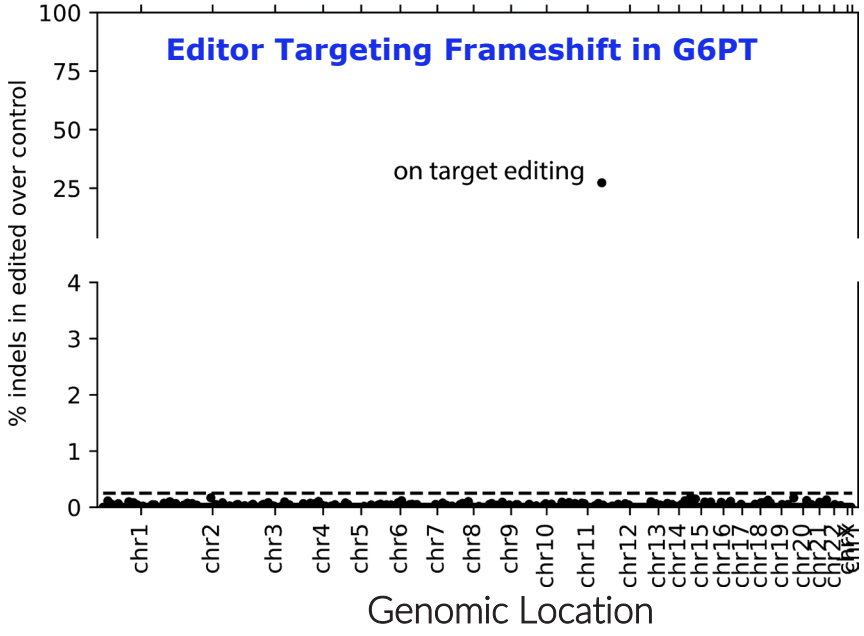
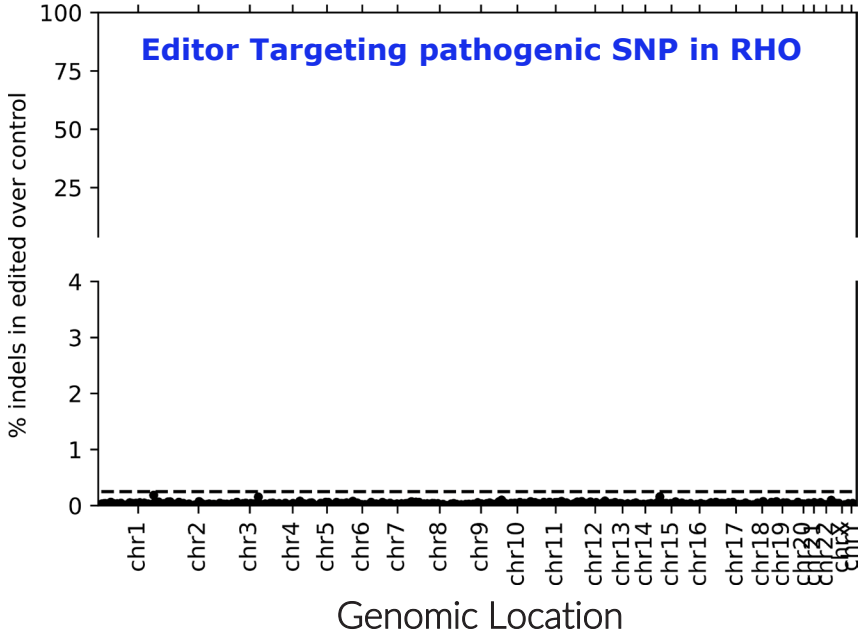
Safety evaluation of lead editors for our GSD1 and RHO Programs: preliminary off-target analyses do not identify off-target editing

Evaluating off-targets in Prime Edited patient cells (G6PT) or Human Retinal explants (Rho)





Targeted Analysis of potential off-target sites using deep sequencing in (left) patient iPSCs, (right) human retinal explants
GSD1 = Glycogen storage Disease type I; G6PT = Glucose 6 phosphate transporter; RHO = Rhodopsin mediated Retinitis Pigmentosa

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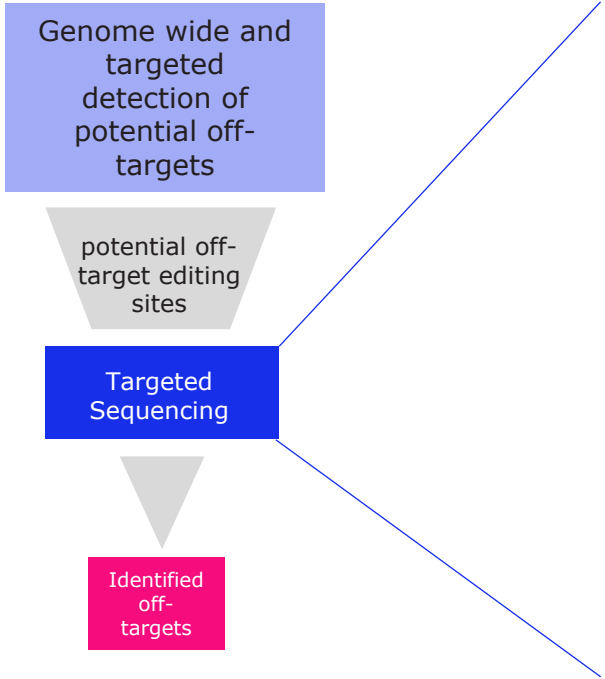
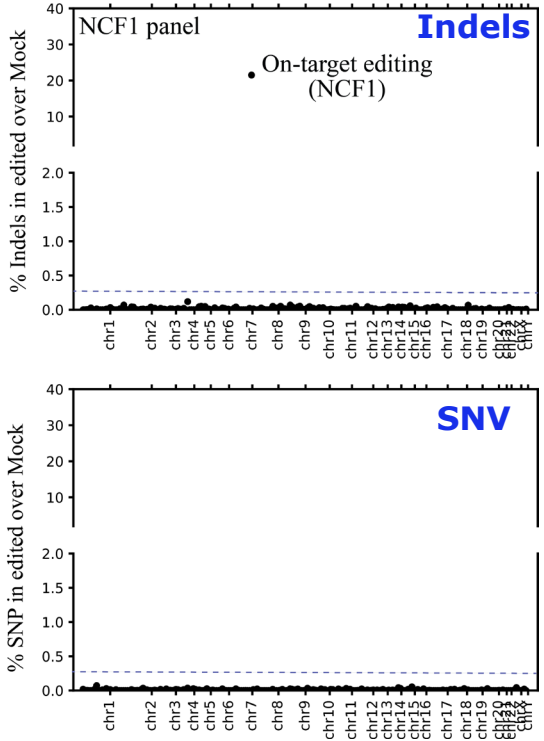
Safety evaluation of NCF1 editor for Prime's CGD program: preliminary off-target analyses do not identify off-target editing using IND ready assays

No off-target editing detected in long-term engrafted hematopoietic stem cells (LT-HSC)





0.25% threshold

Targeted Analysis of potential off-target sites using deep sequencing in Prime Edited human CD34⁺ cells

SNV = single nucleotide variant; CGD – chronic granulomatous disease

Safety evaluation of NCF1 editor: No large deletions or translocations detected in Prime Edited LT-HSCs*

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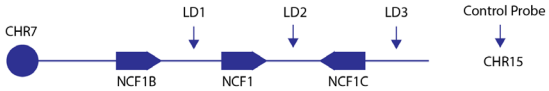
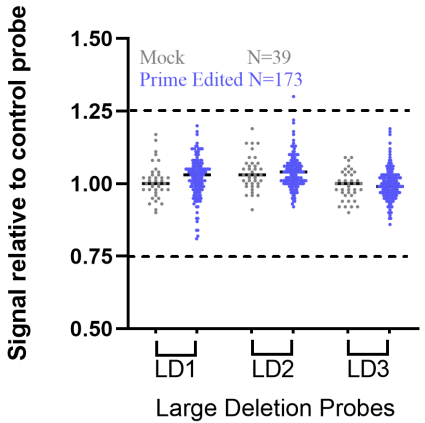
Digital PCR Large Deletion Assay

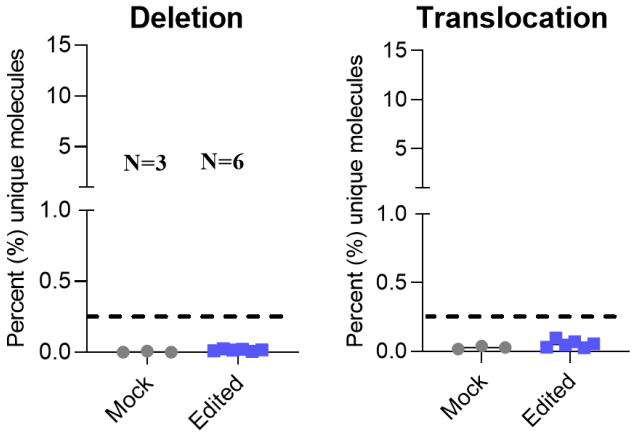


One-sided PCR Chromosomal alterations assay

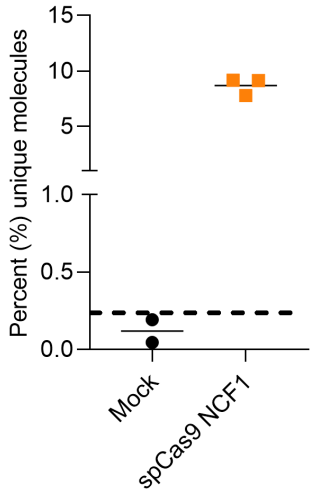
No large deletions in pre-infusion CD34⁺ cell clones



No large deletions or translocations in bone marrow engrafted LT-HSC*



Translocation Positive Control: Cas9 nuclease edited cells



*LT-HSC: long-term hematopoietic stem cell. Data from analysis of total human material from mouse bone marrow harvested 16 weeks after engraftment
dPCR: CD34⁺ population was sorted and expanded in colony forming media, individual colonies were picked and presence of the indicated chromosomal segments measured, N=number of colonies measured
One-Sided PCR: total material was amplified using a one-sided pcr protocol to identify genomic sequence changes adjacent to the edit site. Positive control sample was generated by transfecting HEK293T with sgRNA against NCF1 and SpCAS9 mRNA.

Summary

- **Prime Editing precisely and efficiently corrects** a predominant mutation that causes Chronic Granulomatous Disease (CGD)
- **Prime Edited HSCs maintain functionality *in vivo*** (long-term engraftment, multilineage blood cell production, and biodistribution of blood cell progeny)
- **We have developed of a suite of assays to evaluate Prime Editor safety risks** including the novel strand and base specific off-target method PEG-seq
- **No unintended (off-target) edits** have been detected from editors for our CGD program and our G6PT liver and Rho eye programs in preliminary data

THANK YOU!

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