Development of PM359, a Prime Edited CD34+ cell drug product for the treatment of p47phox Chronic Granulomatous Disease

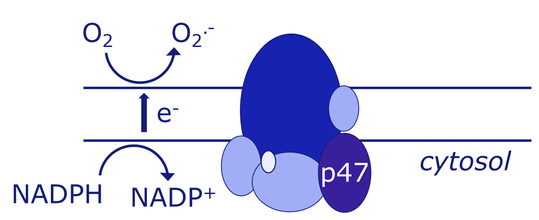
Jack Heath[1], Jacob Stewart-Ornstein[1], Ahmad Arabiyat[1], Christa Osuna[1], Justin Tedeschi[1], Allen Ng[1], Maria Collier[1], Marina Lilieholm[1], Julia Kushakji[1], Alan Wilhelm[1], Katie Green[1], Hari Subramanian[1], Sarah Trusiak[1], Katya Kosheleva[1], Mathew Irby[1], Rowshon Alam[1], Joseph Elich[1], Terence Ta[1], Suk See DeRavin[2], Harry Malech[2], Barrett Nehilla[1], Hetal Patel[1], Brian Beard[1], Ann Lee[1], Jeremy Duffield[1], Jennifer Gori[1]
[1]Prime Medicine, Inc. 60 First Street, Cambridge, MA, USA; [2]National Institutes of Allergy & Infectious Diseases, Bethesda, MD, USA

Background

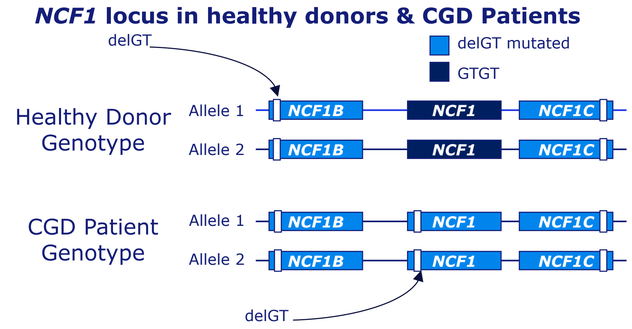
P47 phagocytic oxidase Chronic Granulomatous Disease (p47phox CGD, hereafter CGD) is an inherited immunodeficiency caused by mutations in *NCF1* which encodes the p47 protein, a subunit of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. When neutrophils encounter pathogens, NADPH oxidase produces superoxide that kills pathogens. CGD patients lack functional NADPH oxidase, which leads to recurrent infections and inflammation. Currently, allogeneic hematopoietic stem cell transplantation (HSCT) is the only available potential cure, but some patients may not be eligible.

The *NCF1* gene is flanked by 2 nonfunctional pseudogenes that are nearly identical to *NCF1* but contain a 2-nucleotide deletion in exon 2 (delGT). In CGD patients, delGT is present in both copies of the *NCF1* gene. Toward the development of a Prime Edited (PE) CD34+ cell therapy for CGD (PM359 hereafter), nonclinical pharmacology studies were conducted in which the optimized Prime Editor was designed to precisely correct the delGT mutation in *NCF1* in CGD CD34+ cells to restore neutrophil NADPH oxidase activity.

NADPH Oxidase in Phagocytic Myeloid Cells

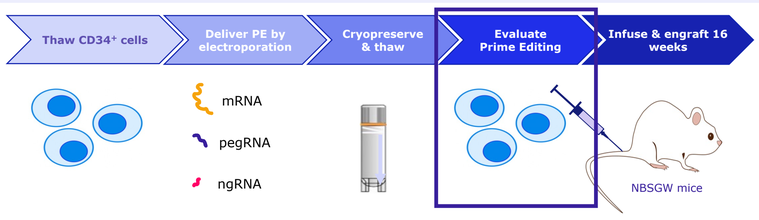


***NCF1* locus in healthy donors & CGD Patients**

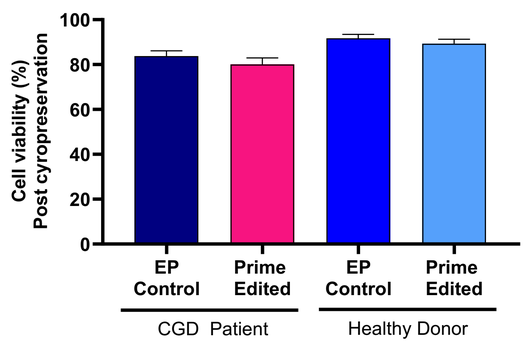


Results

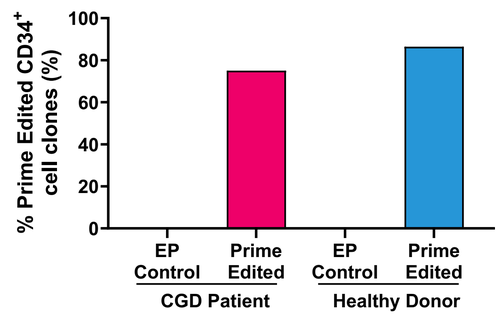
1 Pre-infusion assessment of Prime Edited CGD patient CD34+ cells

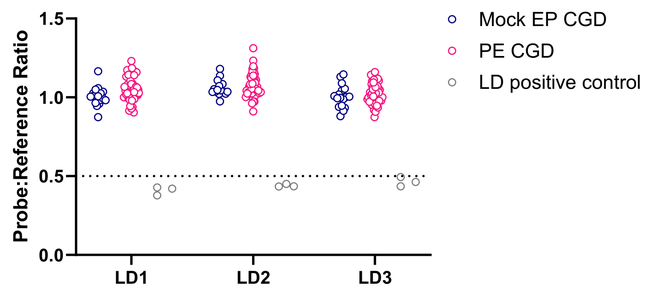


Prime Edited CGD patient CD34+ cells maintain viability



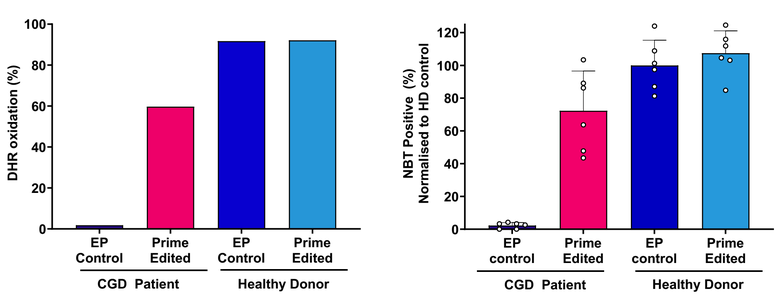
>70% of Prime Edited CGD Patient CD34+ cell clones carry ≥ 1 Prime Edit with no detectable large deletions



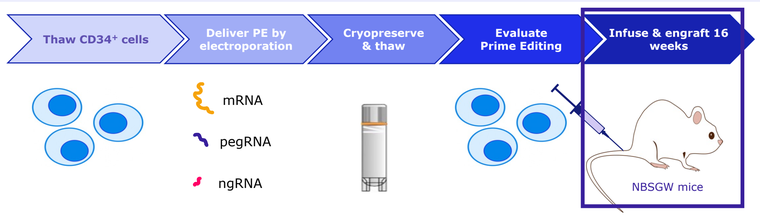


Prime Editing corrects the CGD causative mutation in patient CD34+ cells to restore NADPH oxidase activity

≧60% of myeloid cells produced from PE CGD CD34+ cells have restored NADPH oxidase activity as determined in DHR (left) and NBT (right) assays

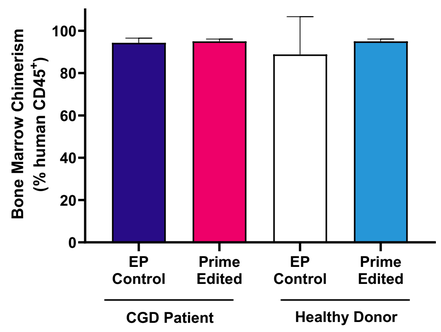


2 Week 16 in vivo engraftment of Prime Edited CGD patient CD34+ cells

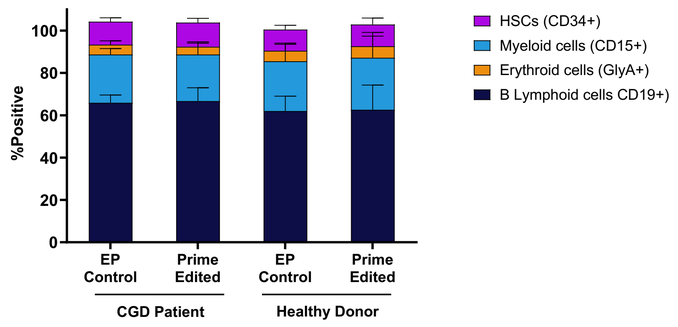


Engrafted Prime Edited CGD patient CD34+ cells maintain hematopoietic stem cell functionality in vivo

Mock EP vs. PE CGD cells:
>90% human CD45+ bone marrow chimerism

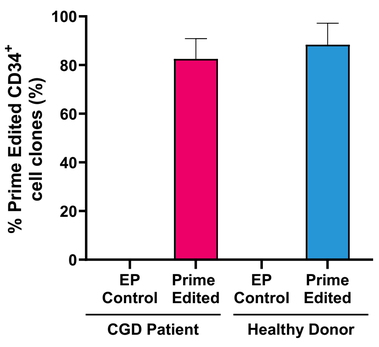


Mock EP vs. PE CGD cells:
No significant difference observed in blood reconstitution

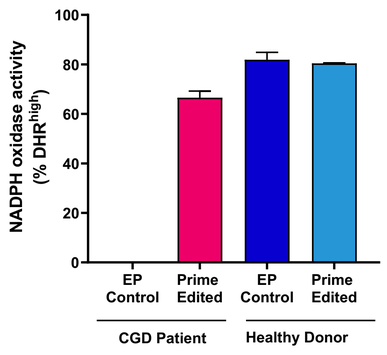


Restoration of NADPH oxidase activity in neutrophils produced by engrafted Prime Edited CGD patient CD34+ cells

>80% PE CGD Patient HSC clones in bone marrow have ≧1 Prime Edited allele



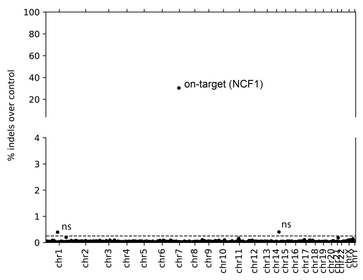
Restored oxidase activity in CD15+ neutrophils generated in vivo

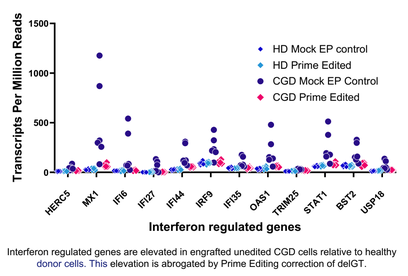


Off-target & transcriptional profiling of bone marrow engrafted PE vs. Mock EP control CD34+ cells

No off-target editing detected in genome of bone marrow engrafted Prime Edited CGD CD34+ cells.

Interferon regulated genes are elevated in engrafted unedited CGD cells relative to healthy donor cells. This elevation is abrogated by PE correction of delGT.



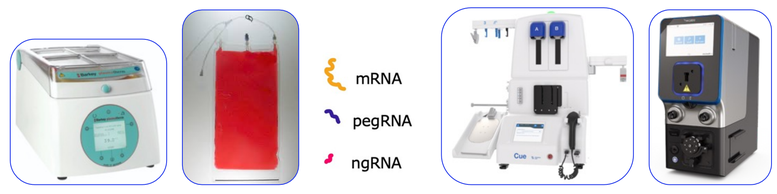


Interferon regulated genes are elevated in engrafted unedited CGD cells relative to healthy donor cells. This elevation is abrogated by Prime Editing correction of delGT.

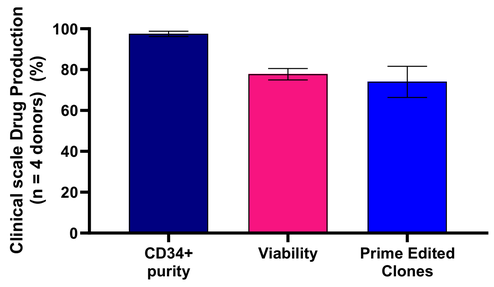
3 Clinical Scale-up of Process for Drug Product Manufacture

CD34+ cell enrichment process

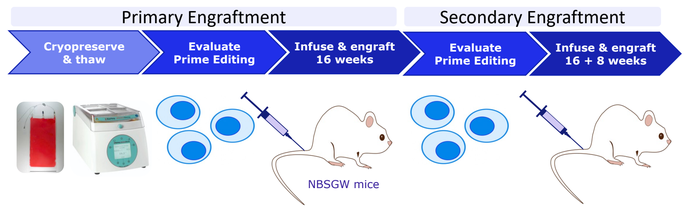
Large scale delivery of Prime Editors in closed-loop system



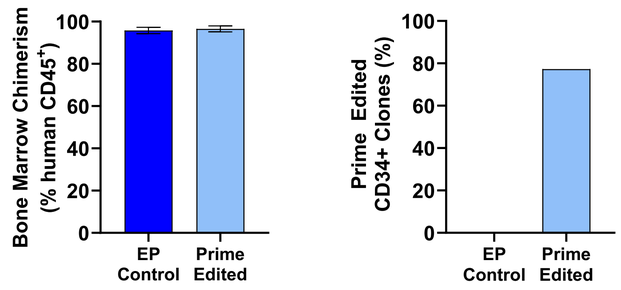
High purity, viability & efficient reproducible editing in Prime Edited CD34+ cells produced at clinical scale



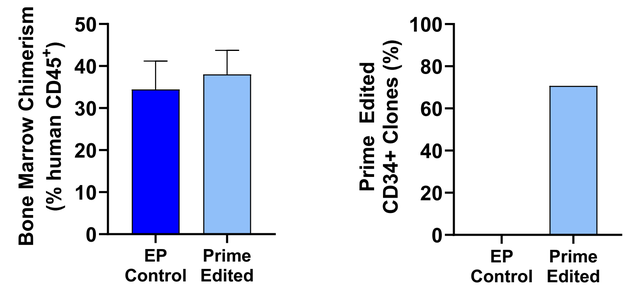
Prime Edited CD34+ cells produced at clinical scale repopulate the bone marrow of 1˚ and 2˚ recipient mice



Primary engraftment



Secondary engraftment



Conclusions

- Prime Editing precisely corrected >75% CGD patient CD34+ cells in preclinical studies
- >80% of bone marrow engrafted PE CGD patient CD34+ cells contained carried a corrected allele in nonclinical model
- NADPH oxidase activity restored in bone marrow neutrophils in mice engrafted with PE CGD CD34+ cells
- Bone marrow engrafted PE CGD patient cells in mice show reduced expression of interferon regulated genes compared to unedited CGD patient cells
- No unintended or off-target edits were detected in engrafted PE CGD CD34+ cells in nonclinical studies
- Clinical scale process developed that supports reproducible, efficient manufacturing of Prime Edited CD34+ cells with high purity, viability, Prime Editing efficiency, and cell potency
- **Prime Medicine has Received FDA Clearance of Investigational New Drug (IND) Application for PM359 for the Treatment of Chronic Granulomatous Disease (CGD)**

Abbreviations. CGD Chronic Granulomatous Disease; NADPH: Nicotinamide Adenine Dinucleotide Phosphate; PE: Prime Edited; EP: Electroporation Only; LD: Large Deletion, DHR: Dihydrorhodamine; NBT: Nitroblue Tetrazolium.