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Prime Editing Efficiently and Precisely Corrects Causative Mutation in Chronic Granulomatous Disease, Restoring Myeloid Function: Toward Development of a Prime Edited Autologous Hematopoietic Stem Cell Therapy

Jack M Heath[1], *Jacob Stuart Orenstein, PhD*[1], *Justin G Tedeschi*[1], *Allen Ng*[1], *Maria D Collier, PhD*[1], *Julia Kushakji*[1], *Alan J Wilhelm*[1], *Andrew Taylor*[1], *David P Waterman, PhD*[1], *Suk See De Ravin, MDPhD*[2], *Harry L Malech, MD*[3], *Andrew V Anzalone, MD PhD*[1], *Jeremy S Duffield, MD PhD*[1], *Jennifer L Gori, PhD*[1]

[1] Prime Medicine, Inc., Cambridge, MA
[2] NIAID, Bethesda, MD
[3] National Institute of Allergy and Infectious Diseases, LHD, NIAID, NIH, Bethesda, MD

Chronic granulomatous disease (CGD) is an inherited primary immunodeficiency characterized by a lack of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity in phagocytic myeloid cells such as neutrophils. NADPH oxidase produces reactive oxygen species (ROS) that directly kill bacteria and fungi to control infection. CGD patients lack this ability to control infection, which results in chronic and severe infections, inflammation, autoimmunity, poor quality of life, and reduced lifespan. Current standard care for CGD is antimicrobial prophylaxis and aggressive management of infection. For patients with a suitable human leukocyte antigen (HLA) matched donor, an allogeneic hematopoietic stem cell (HSC) transplant (allo-HSCT) offers a curative approach. However, many patients do not have access to HLA matched donors and allo-HSCT also carries a risk of GvHD and graft rejection. P47phox-deficient CGD, the most common autosomal recessive CGD, is caused by mutations in the *NCF1* gene, which encodes the p47phox protein, a subunit of the NADPH oxidase complex. Approximately 80% of p47phox-deficient CGD patients carry the same homozygous mutation, a 2 nucleotide GT deletion (delGT) in exon 2 of *NCF1* which is correctable using Prime Editing. Transplantation of Prime Edited patient autologous CD34 $^{+}$ cells, in which the delGT mutation has been corrected with Prime Editing, may provide a curative treatment for p47phox-deficient CGD patients. To evaluate whether correction of the delGT *NCF1* mutation in CGD patient CD34 $^{+}$ cells restores p47phox protein expression and NADPH oxidase activity in myeloid progeny, Prime Editors were designed to correct this mutation precisely and efficiently. The Prime Editors were delivered to CD34 $^{+}$ cells from two p47phox-deficient CGD patients homozygous for delGT *NCF1*. In both studies, ≧75% of Prime Edited patient CD34 $^{+}$ cells carried at least 1 corrected allele as determined by ddPCR and DNA sequencing analyses. Myeloid progeny differentiated from Prime Edited patient CD34 $^{+}$ cells had restored p47phox protein expression and NADPH oxidase activity to ˜80% of normal healthy donors as determined by multiple ROS detection assays. To evaluate reproducibility, Prime Editors were delivered to CD34 $^{+}$ cells from 6 different healthy donors. On average, greater than 90% of CD34 $^{+}$ cells were Prime Edited across 6 donors in independent experiments. To confirm that Prime Edited CD34 $^{+}$ cells retain stem cell multipotency, engraftment, and hematopoietic reconstitution properties, Prime Edited and mock treated CD34 $^{+}$ cells from three different donors were transplanted into immunodeficient mice. Sixteen weeks after CD34 $^{+}$ cell transplantation, >87% of long-term hematopoietic stem cells (LT-HSC) that repopulated the bone marrow had at least 1 allele corrected. There were no significant differences between 'electroporation only' mock treated and Prime Editor electroporated human CD34 $^{+}$ cells with respect to long-term CD34 $^{+}$ cell engraftment, human CD45 $^{+}$ blood cell chimerism, hematopoietic multilineage blood cell reconstitution and biodistribution, or LT-HSC cell potency. Across these studies, >95% human CD45 $^{+}$ cell chimerism was achieved with Prime Edited CD34 $^{+}$ cells. Despite high on-target correction of the delGT mutation in *NCF1*, off-target editing, unintended editing, and chromosomal rearrangements (larger deletions or translocation) events were not detected using a robust suite of genome-wide assays. In summary, Prime Editing corrects a mutation that causes the most common autosomal recessive from of CGD; correction of this delGT mutation at *NCF1* restores NADPH oxidase activity and function in myeloid progeny of Prime Edited CGD CD34 $^{+}$ cells; and Prime Edited long-term HSCs retain multipotency and functionality in vivo. These results support the development of a Prime Edited CGD patient autologous CD34 $^{+}$ cell therapy as a potential curative approach for p47phox-deficient CGD patients.

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Medicine, Inc.: Current Employment. **Collier:** *Prime Medicine, Inc.:* Current Employment, Current equity holder in publicly-traded company. **Kushakji:** *Prime Medicine, Inc.:* Current Employment. **Wilhelm:** *Prime Medicine, Inc.:* Current Employment. **Taylor:** *Prime Medicine, Inc.:* Current Employment. **Waterman:** *Prime Medicine, Inc.:* Current Employment, Current equity holder in publicly-traded company. **Anzalone:** *Prime Medicine, Inc.:* Current Employment, Current equity holder in publicly-traded company. **Duffield:** *Prime Medicine, Inc.:* Current Employment, Current equity holder in publicly-traded company. **Gori:** *Prime Medicine, Inc.:* Current Employment, Current equity holder in publicly-traded company.

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