Seamless Gene Correction in the Human Cystic Fibrosis Transmembrane Conductance Regulator Locus by Vector Replacement and Vector Insertion Events

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††: This manuscript is in honor of our colleague, friend, and mentor Dieter C Gruenert.

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Seamless gene editing via replacement & Insertion

Keywords (At least two keywords)

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Abstract (cannot exceed 250 words)

Gene and cell therapies have potential to overcome inherited diseases by correcting the responsible genetic mutations, rather than treating the symptoms over a patient's lifetime. Due to current developments in gene editing and iPS technology, gene correction via Homologous recombination (HR) in patient-derived iPSCs and regenerative medicine are becoming a more realistic approach to develop personalized and mutation-specific therapeutic strategies. Cystic fibrosis (CF) is the most common inherited disease in the Caucasian population, caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene. Since CF causes significant multi-organ damage and with over 2,000 reported *CFTR* mutations, CF patients could be one prominent population benefiting from gene and cell therapies. When considering gene-editing techniques for clinical applications, seamless gene corrections of the responsible mutations would be the most desirable approach. The studies reported here describe the generation of iPSCs from a CF patient homozygous for the *W1282X*, Class I *CFTR* mutation, and the seamless correction of the *W1282X CFTR* mutation using CRISPR/Cas9 nickase (Cas9n). In addition to the expected HR vector replacement product, we also discovered another class of HR products resulting in vector insertions with a partial duplication of the *CFTR* exon23 sequence. We show here that removal of the PuroΔTK drug resistance cassette and generation of seamless gene corrected cell lines by two independent processes: by treatment with the PiggyBac (PB) transposase or by intrachromosomal homologous recombination between the tandemly duplicated *CFTR* gene sequences. (237 words)