Generation of knock-in primary human T cells using Cas9 ribonucleoproteins

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T-cell genome engineering holds great promise for cell-based therapies for cancer, HIV, primary immune deficiencies, and autoimmune diseases, but genetic manipulation of human T cells has been challenging. Improved tools are needed to efficiently "knock out" genes and "knock in" targeted genome modifications to modulate T-cell function and correct disease-associated mutations. CRISPR/Cas9 technology is facilitating genome engineering in many cell types, but in human T cells its efficiency has been limited and it has not yet proven useful for targeted nucleotide replacements. Here we report efficient genome engineering in human CD4+ T cells using Cas9:single-guide RNA ribonucleoproteins (Cas9 RNPs). Cas9 RNPs allowed ablation of CXCR4, a coreceptor for HIV entry. Cas9 RNP electroporation caused up to ~40% of cells to lose high-level cell-surface expression of CXCR4, and edited cells could be enriched by sorting based on low CXCR4 expression. Importantly, Cas9 RNPs paired with homology-directed repair template oligonucleotides generated a high frequency of targeted genome modifications in primary T cells. Targeted nucleotide replacement was achieved in CXCR4 and PD-1 (PDCD1), a regulator of T-cell exhaustion that is a validated target for tumor immunotherapy. Deep sequencing of a target site confirmed that Cas9 RNPs generated knock-in genome modifications with up to ~20% efficiency, which accounted for up to approximately one-third of total editing events. These results establish Cas9 RNP technology for diverse experimental and therapeutic genome engineering applications in primary human T cells. Read the Article [1]