1231 VIRUS-FREE, TARGETED INSERTION OF LONG TRANSGENES WITHIN PRIMARY NATURAL KILLER CELLS USING CRISPR-CAS9

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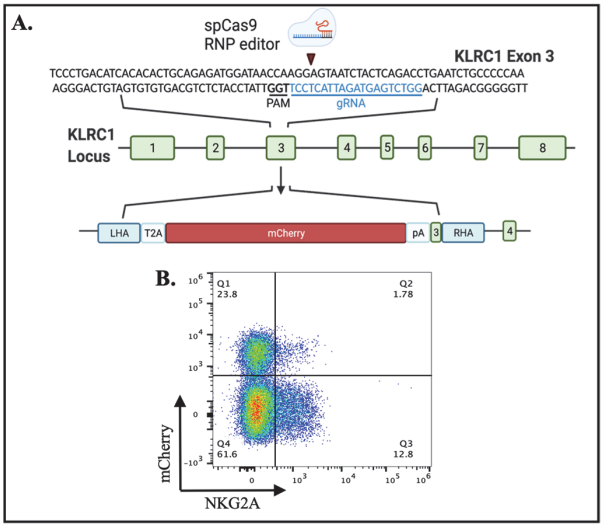
Background Natural killer (NK) cells are innate cytotoxic lymphocytes capable of killing virally infected cells and malignant tumors. Unlike T cells, NK cells are human leukocyte antigen (HLA)-agnostic and cause little to no Graft vs. Host Disease in allogenic transfusions, making them excellent candidates for off-the-shelf therapies. However, current techniques to insert a chimeric antigen receptor (CAR) gene into NK cells primarily employ viral vectors. Viral methods can give rise to complications such as insertional mutagenesis, which can lead to gene silencing or oncogene activation.[1] Here we have developed a CRISPR genome editing strategy to modify primary NK cells and report the targeted virus-free CAR integration within peripheral blood NK cells.

Methods In our strategy, the transgene (e.g., CAR) is encoded in a linear double stranded DNA (dsDNA) template and produced by polymerase chain reaction (PCR). The template includes homology to the intended target for insertion that is defined by nuclease mediated double strand break formation by a Cas9-single guide RNA (gRNA) ribonucleoprotein complex (figure 1A).[2]

Results We show efficient (>80%) CRISPR knock-out of the inhibitory NKG2A receptor encoded by the *KLRC1* gene within primary NK cells. Optimization of electroporation timing showed that delivery of 3 mg of donor DNA on day 4 of *ex vivo* expansion results in gene knock-in rates of up to 24% for the transgene (figure 1B). Lastly, we report that the use of K562-mbIL15-41BBL feeder cells improves expansion of CRISPR edited cells by approximately twenty-fold.

Conclusions These data provide a proof-of-principle for on-target integration of long transgenes, including CAR, without viral vectors within primary NK cells via CRISPR-Cas9 genome editing. Overall, this virus-free manufacturing strategy could enable the development of novel genetically-programmed NK immunotherapies.



Abstract 1231 Figure 1 Knock-in via CRISPR-Cas9 at the KLRC1 locus
(A) Schematic showing 2.5kb transgene encoding mCherry fluorescent protein and genome editing strategy. (B) Flow cytometry results showing 23.8% knock-in of a transgene into the KLRC1 locus in human primary NK cells 14 days after nucleofection of CRISPR reagents. RNP: ribonucleoprotein. NKG2A is encoded by the KLRC1 locus

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Ethics Approval The study was approved by the IRB at University of Wisconsin-Madison, approval number 2018-0103.