gene editing of natural killer (NK) cells. The disclosure provides for engineered NK cells that express one or more heterologous antigen receptors and that also have been gene edited to have disruption of one or more endogenous genes in the NK cells. In specific embodiments, the heterologous antigen receptors are engineered antigen receptors that have been produced by the hand of man, for example to be fusion proteins comprising components from different sources. In some embodiments, CAR-transduced NK cells are engineered to have disruption of expression of one or more endogenous genes in the NK cells, and in other embodiments NK cells having disruption of expression of one or more endogenous genes in the NK cells are transduced or transfected (*e.g.*, modified) to express one or more heterologous antigen receptors. Particular embodiments provide for large-scale CRISPR/Cas9-mediated engineering strategies of primary CAR-transduced NK cells.

[0005a] In one aspect, provided herein is an *in vitro* method of producing engineered NK cells, comprising the steps of: (a) expanding for a first time period NK cells with antigen presenting cells and an effective amount of interleukin (IL)-2 to produce expanded NK cells; one of (b1) and (c1), or (b2) and (c2): (b1) transducing or transfecting the expanded NK cells of (a) with one or more vectors each encoding one or more heterologous antigen receptors to produce modified NK cells; (c1) after a second time period, delivering to the modified NK cells an effective amount of Cas9 and one or more guide RNAs to disrupt expression of one or more genes in the NK cells, thereby producing gene edited modified NK cells; or (b2) delivering to the expanded NK cells of (a) an effective amount of Cas9 and one or more guide RNAs to disrupt expression of one or more genes in the NK cells, thereby producing gene edited NK cells; (c2) after a second time period, transducing or transfecting the gene edited NK cells with one or more vectors each encoding one or more heterologous antigen receptors to produce gene edited modified NK cells; and (d) expanding for a third time period the gene edited modified NK cells of (c1) or (c2) in the presence of an effective amount of antigen presenting cells and an effective amount of IL-2.

[0005b] In one aspect, provided herein is a population of NK cells produced by the method as described herein.

[0005c] In one aspect, provided herein is a composition comprising the population as described herein.

[0005d] In one aspect, provided herein is the NK cells produced by the method as described herein for use in therapy.

[0005e] In one aspect, provided herein is the use of NK cells produced by the method of as described herein in the manufacture of a medicament for treating an individual for a medical condition, wherein the medical condition is cancer, or wherein the medical condition is infectious disease and/or an immune-related disorder.

[0005f] In one aspect, provided herein is a kit comprising the NK cells produced by the method as described herein, and/or one or more reagents to produce the NK cells.

[0006] In certain embodiments, the process utilizes specific parameters that allow the process to be large-scale, including for engineering up to $1x10^9$ or more cells. In particular embodiments, the process utilizes successive steps including, in no particular order, optionally one or more expansion steps, optionally one or more depletion steps to remove cells positive for one or more markers, modification of the NK cells to have gene editing of one or more endogenous genes, and modification of the NK cells to express a heterologous antigen receptor. Any expansion step in any method encompassed herein may be optional. In specific cases, the process utilizes successive steps including one or multiple expansion steps, one or more depletion steps to remove cells positive for CD3, CD14, and/or CD19, modification of the cells to allow the NK cells to express one or more heterologous antigen receptors, and modification of the cells to lack expression, or have reduced expression, of one or more genes endogenous to the NK cells. In specific cases, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more endogenous genes are knocked out or knocked down using suitable mean, such as CRISPR and guide RNAs, for multiple genes. The process also encompasses specific durations of time for and/or between particular steps of the process.

[0007] The produced cells may be used for any purpose, including for the treatment of a medical condition such as cancer, infectious disease, and/or immune-related disorders. The produced cells may be stored, such as for an off-the-shelf purpose that upon their use may or may not be further modified, such as to customize the cells to an individual in need thereof. In some cases, no further modification is required, because the CAR expressed by the NK cells targets an