

lineage malignancies, adoptive T-cell approaches that utilize CARs which target CD19 have been developed (see, e.g., Jensen et al., *Biology of Blood and Marrow Transplantation*, 16: 1245-1256 (2010); Kochenderfer et al., *Blood*, 116(20): 4099-4102 (2010); Porter et al., *The New England Journal of Medicine*, 365(8): 725-733 (2011); Savoldo et al., *Journal of Clinical Investigation*, 121(5): 1822-1826 (2011), Cooper et al., *Blood*, 101(4): 1637-1644 (2003); Brentjens et al., *Nature Medicine*, 9(3): 279-286 (2003); Kalos et al., *Science Translational Medicine*, 3(95): 95ra73 (2011); Cheadle et al., *Journal of Immunology*, 184(4): 1885-1896 (2010); Brentjens et al., *Clinical Cancer Research*, 13(18 Pt 1): 5426-5435 (2007); Kochenderfer et al., *Blood*, 116(19): 3875-3886 (2010); Brentjens et al., *Blood*, 118(18): 4817-4828 (2011); and Kochenderfer et al., *Blood*, December 8, 2011 (epublication ahead of print (2012)). The B-cell antigen CD19 has been chosen as target for CARs because its expression is limited to normal and malignant B-cells (see, e.g., Nadler et al., *Journal of Immunology*, 131(1): 244-250 (1983)).

**[0005]** One disadvantage associated with the anti-CD19 CAR therapies reported to date is that they can induce significant toxicity associated with elevated levels of serum cytokines. The generation of human anti-mouse immune responses also is a potential risk associated with current anti-CD19 CARs, which contain murine sequences (see, e.g., Jensen et al., *supra*; Lamers et al., *Blood*, 117(1): 72-82 (2011); and Maus et al., *Cancer Immunol Res*, 2: 112-120 (2014)).

**[0006]** Thus, there remains a need for compositions that can be used in methods to treat B-cell malignancies which have reduced toxicity and immunogenicity in humans. This invention provides such compositions and methods.

## BRIEF SUMMARY

**[0006a]** In one aspect, the present disclosure refers to a chimeric antigen receptor (CAR) directed against CD19, which comprises an amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, or SEQ ID NO: 13. In yet another aspect, the present disclosure refers to a nucleic acid encoding the CAR of the present disclosure. In another aspect, there is provided a vector comprising the nucleic acid of the present disclosure. In another aspect, there is provided a T-cell or NK cell comprising the vector

of the present disclosure. In another aspect, the present disclosure refers to a T-cell or NK cell of the present disclosure for use in preventing or treating a B-cell malignancy in a subject. In yet another aspect, the present disclosure provides a method of destroying malignant B-cells *in vitro*, which method comprises contacting one or more of the T-cells or NK cells of the present disclosure with a population of malignant B-cells cells that express CD19, whereby the CAR binds to CD19 on the malignant B-cells and the malignant B-cells are destroyed. In another aspect of the disclosure, there is provided a method of making a cell expressing a chimeric antigen receptor (CAR), the method comprising introducing a vector encoding the CAR of any one of the present disclosure into a T-cell or NK cell.

**[0007]** The disclosure describes an isolated or purified chimeric antigen receptor (CAR) directed against CD19, which comprises an amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, or SEQ ID NO: 13.

**[0008]** In addition, also disclosed are isolated or purified nucleic acid sequences encoding the foregoing CARs, vectors comprising such nucleic acid sequences, isolated T-cells comprising such vectors, and methods of destroying malignant B-cells by contacting such isolated T-cells with a population of malignant CD19-expressing B-cells cells *in vivo* or *ex vivo*.

## CLAIMS:

1. A chimeric antigen receptor (CAR) directed against CD19, which comprises an amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, or SEQ ID NO: 13.
2. The CAR of claim 1, wherein the CAR comprises an amino acid sequence of SEQ ID NO: 10.
3. A nucleic acid encoding the CAR of claim 1 or 2.
4. A vector comprising the nucleic acid of claim 3.
5. A T-cell or NK cell comprising the vector of claim 4.
6. A T-cell or NK cell of claim 5 for use in preventing or treating a B-cell malignancy in a subject.
7. A method of destroying malignant B-cells *in vitro*, which method comprises contacting one or more of the T-cells or NK cells of claim 5 with a population of malignant B-cells that express CD19, whereby the CAR binds to CD19 on the malignant B-cells and the malignant B-cells are destroyed.
8. The method of claim 7, wherein the malignant B-cells are lymphoma cells.
9. The method of claim 7, wherein the malignant B-cells are leukemia cells.
10. A method of making a cell expressing a chimeric antigen receptor (CAR), the method comprising introducing a vector encoding the CAR of claim 1 or 2 into a T-cell or NK cell.