

CLAIMS

What is claimed is:

1. A CRISPR guide molecule, comprising:

a targeting region capable of binding a target nucleic acid sequence and

an activating region comprising the RNA sequence

UAAUUUCUACUCUUGUAGAU (SEQ ID NO: 6) including at least one

deoxyribonucleotide in place of a ribonucleotide, wherein the activating region is

capable of forming a nucleoprotein complex with a Cas12 protein.
2. The CRISPR guide molecule of claim 1, wherein one or more of positions 1, 3, 7, 10, 12, 14, 15, 16, 17, 18, and 19 in the activating region comprise a deoxyribonucleotide base.
3. The CRISPR guide molecule of claim 1, wherein the activating region comprises sequence selected from SEQ ID NOs.: 459-465.
4. The CRISPR guide molecule of claim 1, comprising one or more phosphorothioate chemical modifications.
5. The CRISPR guide molecule of claim 4, comprising a sequence selected from SEQ ID Nos: 512-517.
6. The CRISPR guide molecule of claim 1, wherein the targeting region targets the B2M gene and comprises the RNA sequence AGUGGGGGUGAAUUCAGUGU (SEQ ID NO: 211), wherein one or more of positions 1, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, and 20 in the targeting region comprise a deoxyribonucleotide base.

7. The CRISPR guide molecule of claim 6 comprising a sequence selected from SEQ ID NOs: 212-231, 275-294, 296-315, 331-334, and 346-350.
8. The CRISPR guide molecule of claim 6 comprising the sequence of SEQ ID NO: 416.
9. The CRISPR guide molecule of claim 1, wherein the targeting region targets the TRAC gene and comprises the RNA sequence GAGUCUCUCAGCUGGUACAC (SEQ ID NO: 232), wherein one or more of positions 1, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, and 20 in the targeting region comprise a deoxyribonucleotide base.
10. The CRISPR guide molecule of claim 9 comprising a sequence selected from SEQ ID NOs: 233-252, 317-329, 331-334, 491-492, and 508.
11. The CRISPR guide molecule of claim 9 comprising the sequence of SEQ ID NO: 415.
12. The CRISPR guide molecule of claim 1, wherein the targeting region targets the CISH gene and comprises the RNA sequence capable of hybridizing to a human genomic sequence selected from SEQ ID NOs: 156-165, wherein one or more of positions 1, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, and 20 in the targeting region comprise a deoxyribonucleotide base.
13. The CRISPR guide molecule of claim 1, wherein the targeting region targets the PDCD1 gene and comprises the RNA sequence capable of hybridizing to a human genomic sequence selected from SEQ ID NOs: 134-155, wherein one or more of

positions 1, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, and 20 in the targeting region comprise a deoxyribonucleotide base.

14. The CRISPR guide molecule of claim 1, wherein the targeting region targets the CBLB gene and comprises the RNA sequence capable of hybridizing to a human genomic sequence selected from SEQ ID NOs: 166-189, wherein one or more of positions 1, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, and 20 in the targeting region comprise a deoxyribonucleotide base.
15. A CRISPR nucleic acid/protein composition, comprising the CRISPR guide molecule of claim 1 and a Cas12 protein.
16. The CRISPR nucleic acid/protein composition of claim 15, wherein the Cas12 protein is a Cas12a protein comprising at the C-terminus, a linker and a nuclear localization signal (NLS)-containing sequence having at least 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 479-490.
17. The CRISPR nucleic acid/protein composition of claim 16, wherein the Cas12a protein comprises an amino acid sequence of SEQ ID NOs: 1.
18. An *in vitro* method for producing a chimeric antigen receptor (CAR)-expressing cell, said method comprising:
 - a) contacting a first target nucleic acid comprising a TRAC sequence in a cell with a first nucleoprotein complex of claim 15, wherein the first nucleoprotein complex is capable of cleaving the first target nucleic acid sequence;

- b) contacting a second target nucleic acid sequence comprising a B2M sequence in the same cell with a second nucleoprotein complex of claim 15, wherein the second nucleoprotein complex is capable of cleaving the second target nucleic acid sequence;
 - c) providing a first donor polynucleotide encoding a CAR comprising an scFv, a transmembrane domain, a co-stimulatory domain, and an activating domain, wherein the CAR is capable of being inserted into the cleavage site of the first target nucleic acid sequence;
 - d) providing a second donor polynucleotide encoding a B2M-HLA-E fusion construct comprising a B2M secretion signal, a HLA-G peptide signal sequence, a first linker sequence, a B2M sequence, a second linker sequence, and a HLA-E sequence, wherein the B2M-HLA-E fusion construct is capable of being inserted into the cleavage site of the second target nucleic acid sequence;
 - e) cleaving the first target nucleic acid sequence and inserting at least a portion of the first donor polynucleotide into the cleavage site; and
 - f) cleaving the second target nucleic acid sequence and inserting at least a portion of the second donor polynucleotide into the cleavage site.
19. The method of claim 18, wherein the second donor polynucleotide comprises the SEQ ID NO: 414.
20. The method of claim 18, wherein the CAR comprises a transmembrane domain derived from a CD8, a 4-1BB co-stimulatory domain or a CD28 co-stimulatory domain, and a CD3 ζ activating domain.

21. A CAR-expressing cell produced by the method of claim 18, wherein the cell is selected from a lymphocyte, a CAR-T cell, a TCR cell, a TCR-engineered CAR-T cell, a TIL, a CAR TIL, a dendritic cell, a CAR-DC, a macrophage, a CAR-M, an iPSC, a cell differentiated from an iPSC cell, an NK cell, or a CAR-NK cell.
22. Use of the CAR-expressing cell of claim 21 in the preparation of a medicament for use in a method of adoptive cell therapy.