

transfected cell, thus eliminating the concern that the introduced genetic material will disrupt the normal functioning of an essential gene, or cause a mutation that results in deleterious or oncogenic effects, and extraneous promoter sequences are not required for effective translation of the encoded protein, again avoiding possible deleterious side effects. In addition, it is not necessary for mRNA to enter the nucleus to perform its function, while DNA must overcome this major barrier.

[0004] Circular RNA is useful in the design and production of stable forms of RNA. The circularization of an RNA molecule provides an advantage to the study of RNA structure and function, especially in the case of molecules that are prone to folding in an inactive conformation (Wang and Ruffner, 1998). Circular RNA can also be particularly interesting and useful for *in vivo* applications, especially in the research area of RNA-based control of gene expression and therapeutics, including protein replacement therapy and vaccination.

[0005] Prior to this invention, there were three main techniques for making circularized RNA *in vitro*: the splint-mediated method, the permuted intron-exon method, and the RNA ligase-mediated method. However, the existing methodologies are limited by the size of RNA that can be circularized, thus limiting their therapeutic application.

SUMMARY

[0006] In one aspect, the present disclosure refers to a circular RNA polynucleotide comprising, in the following order, a post splicing 3' group I intron fragment, a first Internal Ribosome Entry Site (IRES), a first expression sequence, a second expression sequence, and a post splicing 5' group I intron fragment, wherein the first expression sequence encodes a CD19 or BCMA antigen binding molecule and/or the second expression sequence encodes a CD19 or BCMA antigen binding molecule.

[0006a] In another aspect, the present disclosure refers to a use of the circular RNA polynucleotide as described herein or the pharmaceutical composition as described herein in the manufacture of a medicament for treating cancer and/or an autoimmune disorder.

[0006b] In another aspect, the present disclosure refers to a vector for making a circular RNA polynucleotide, comprising, in the following order, a 5' duplex forming region, a 3' Group I intron fragment, an Internal Ribosome Entry Site (IRES), a first expression sequence, a second expression sequence, a 5' Group I intron fragment, and a 3' duplex forming region, comprising: a. a polynucleotide sequence encoding a cleavage site between the first expression sequence and the second expression sequence; and/or b. a first

spacer between the 5' duplex forming region and the 3' group I intron fragment, and a second spacer between the 5' group I intron fragment and the 3' duplex forming region wherein the first expression sequence encodes a CD19 or BCMA antigen binding molecule and/or the second expression sequence encodes a CD19 or BCMA antigen binding molecule.

[0006c] In another aspect, the present disclosure refers to a eukaryotic cell comprising a circular RNA polynucleotide as described herein, optionally wherein the cell is a human cell, an immune cell, and/or a T cell.

[0006d] In another aspect, the present disclosure refers to a pharmaceutical composition comprising at least one circular RNA polynucleotide of the disclosure, a nanoparticle, wherein the at least one circular RNA polynucleotide is encapsulated by the nanoparticle.

[0006e] Circular RNA, along with related compositions and methods are described herein. In some examples, the inventive circular RNA comprises post splicing group I intron fragments, spacers, an IRES, optional duplex forming regions, and more than one expression sequence. In some examples, the inventive circular RNA comprises duplex forming regions. In some examples, the expression sequences are separated by one or more polynucleotide sequences encoding a cleavage site. In some examples, a cleavage site is a self-cleaving peptide. In some examples, the self-cleaving peptide is a 2A self-cleaving peptide. In some examples, the first and second expression sequences are separated by ribosomal skipping element. In some examples, each expression sequence encodes a therapeutic protein. In some examples, the first expression sequence encodes a cytokine or a functional fragment thereof. In some examples, the first expression sequence encodes a transcription factor. In some examples, the first expression encodes an immune checkpoint inhibitor. In some examples, the first expression sequence encodes for a chimeric antigen receptor. In some examples, the first expression sequence encodes a first T-cell receptor (TCR) chain, and the second expression encodes a second TCR chain. In some examples, circular RNA of the disclosure has improved expression, functional

WHAT IS CLAIMED IS:

1. A circular RNA polynucleotide comprising, in the following order, a post splicing 3' group I intron fragment, a first Internal Ribosome Entry Site (IRES), a first expression sequence, a second expression sequence, and a post splicing 5' group I intron fragment, wherein the first expression sequence encodes a CD19 or BCMA antigen binding molecule and/or the second expression sequence encodes a CD19 or BCMA antigen binding molecule.
2. The circular RNA polynucleotide of claim 1, further comprising a cleavage site between the first expression sequence and the second expression sequence.
3. The circular RNA polynucleotide of claim 2, wherein the cleavage site is a self-cleaving spacer or a 2A self-cleaving peptide.
4. The circular polynucleotide of claim 1, comprising a second IRES between the first expression sequence and the second expression sequence.
5. The circular RNA polynucleotide of claim 4, wherein the first IRES and/or the second IRES consists of or comprises a sequence according to any of SEQ ID NO: 1-72.
6. The circular RNA of any one of claims 1-5, wherein the CD19 antigen-binding molecule and/or the BCMA antigen binding molecule is an antibody or antigen-binding fragment thereof or a chimeric antigen receptor (CAR).
7. The circular RNA polynucleotide of claim 6, wherein the antibody or antigen-binding fragment thereof is selected from a scFv, single domain antibody, and camelized antibody.
8. The circular RNA polynucleotide of any one of claims 1-7, wherein:
 - (a) the first expression sequence encodes a CD19 antigen binding molecule, and the second expression sequence encodes a therapeutic protein;
 - (b) the first expression sequence encodes a therapeutic protein and the second expression sequence encodes a CD19 antigen-binding molecule;
 - (c) the first expression sequence encodes a BCMA antigen binding molecule, and the second expression sequence encodes a therapeutic protein; or
 - (d) the first expression sequence encodes a therapeutic protein, and the second expression sequence encodes a BCMA antigen binding molecule.
9. The circular RNA polynucleotide of claim 8, wherein the expression sequence encoding the therapeutic protein encodes:
 - (a) a T cell receptor (TCR);

- (b) a chimeric antigen receptor (CAR);
 - (c) a PD1 or PDL1 antagonist;
 - (d) a cytokine;
 - (e) a chemokine;
 - (f) a tumor antigen;
 - (g) an immunosuppressive enzyme;
 - (h) a transcription factor;
 - (i) BCL-XL;
 - (j) a chaperone protein;
 - (k) a signaling protein; or
 - (l) an enzyme capable of converting 5-fluorocytosine (5-FC) into 5-fluorouracil (5-FU).
10. The circular RNA polypeptide of claim 9, wherein:
- a. The tumour antigen is a neoantigen;
 - b. the cytokine is IFN γ , IL-2, IL-7, IL-12, IL-15, IL-18, IL-10, TGF β , or IL-35;
 - c. the chemokine is selected from a CC chemokine, CXC chemokine, C chemokine, CX3C chemokine, CCL1, CCL2, CCL3, CCL4, CCL5, CCL6, CCL7, CCL8, CCL9/CCL10, CCL11, CCL12, CCL13, CCL14, CCL15, CCL16, CCL17, CCL18, CCL19, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28, CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCL17, XCL1, XCL2, and CX3CL1;
 - d. the transcription factor is selected from FOXP3, STAT5B, HELIOS, Tbet, GATA3, RORgt, and cd25;
 - e. the chaperone protein or complex is selected from Skp, Spy, FkpA, SurA, Hsp60, Hsp70, GroEL, GroES, Hsp90, HtpG, Hsp100, ClpA, ClpX, ClpP, and Hsp104; and/or
 - f. the expression sequence capable of converting 5-FC into 5-FU is cytosine deaminase.
11. The circular RNA polynucleotide of claim 1, comprising in the following order, a 5' duplex forming region, a post splicing 3' group I intron fragment, a first Internal Ribosome Entry

Site (IRES), a first expression sequence, a second expression sequence, a post splicing 5' group I intron fragment, and a 3' duplex forming region.

12. The circular RNA polynucleotide of claim 11, comprising a first spacer between the 5' duplex forming region and the post splicing 3' group I intron fragment, and a second spacer between the post splicing 5' group I intron fragment and the 3' duplex forming region.
13. The circular RNA polynucleotide of claim 12, wherein the first and second spacers each has a length of 10 to 60 nucleotides or 9 to 19 nucleotides.
14. The circular RNA polynucleotide of claim 12 or 13, wherein the first and second duplex forming regions each have a length of 30 nucleotides.
15. The circular RNA polynucleotide of any one of claims 1-14, wherein the IRES has a sequence of an IRES from Taura syndrome virus, Triatoma virus, Theiler's encephalomyelitis virus, Simian Virus 40, Solenopsis invicta virus 1, Rhopalosiphum padi virus, Reticuloendotheliosis virus, Human poliovirus 1, Plautia stali intestine virus, Kashmir bee virus, Human rhinovirus 2, Homalodisca coagulata virus- 1, Human Immunodeficiency Virus type 1, Homalodisca coagulata virus- 1, Himetobi P virus, Hepatitis C virus, Hepatitis A virus, Hepatitis GB virus , Foot and mouth disease virus, Human enterovirus 71, Equine rhinitis virus, Ectropis obliqua picorna-like virus, Encephalomyocarditis virus, Drosophila C Virus, Human coxsackievirus B3, Crucifer tobamovirus, Cricket paralysis virus, Bovine viral diarrhea virus 1, Black Queen Cell Virus, Aphid lethal paralysis virus, Avian encephalomyelitis virus, Acute bee paralysis virus, Hibiscus chlorotic ringspot virus, Classical swine fever virus, Human FGF2, Human SFTPA1, Human AML1/RUNX1, Drosophila antennapedia, Human AQP4, Human AT1R, Human BAG-1, Human BCL2, Human BiP, Human c-IAP1, Human c-myc, Human eIF4G, Mouse NDST4L, Human LEF1, Mouse HIF1 alpha, Human n.myc, Mouse Gtx, Human p27kipl, Human PDGF2/c-sis, Human p53, Human Pim-1, Mouse Rbm3, Drosophila reaper, Canine Scamper, Drosophila Ubx, Human UNR, Mouse UtrA, Human VEGF-A, Human XIAP, Drosophila hairless, S. cerevisiae TFIID, S. cerevisiae YAP1, tobacco etch virus, turnip crinkle virus, EMCV-A, EMCV-B, EMCV-Bf, EMCV-Cf, EMCV pEC9, Picobirnavirus, HCV QC64, Human Cosavirus E/D, Human Cosavirus F, Human Cosavirus JMY, Rhinovirus NAT001, HRV14, HRV89, HRVC-02, HRV-A21, Salivirus A SH1, Salivirus FHB, Salivirus NG-J1, Human Parechovirus 1, Crohivirus B, Yc-3,

Rosavirus M-7, Shanbavirus A, Pasivirus A, Pasivirus A 2, Echovirus E14, Human Parechovirus 5, Aichi Virus, Hepatitis A Virus HA16, Phopivirus, CVA10, Enterovirus C, Enterovirus D, Enterovirus J, Human Pegivirus 2, GBV-C GT110, GBV-C K1737, GBV-C Iowa, Pegivirus A 1220, Pasivirus A 3, Sapelovirus, Rosavirus B, Bakunsa Virus, Tremovirus A, Swine Pasivirus 1, PLV-CHN, Pasivirus A, Sicinivirus, Hepacivirus K, Hepacivirus A, BVDV1, Border Disease Virus, BVDV2, CSFV-PK15C, SF573 Dicistrovirus, Hubei Picorna-like Virus, CRPV, Salivirus A BN5, Salivirus A BN2, Salivirus A 02394, Salivirus A GUT, Salivirus A CH, Salivirus A SZ1, Salivirus FHB, CVB3, CVB1, Echovirus 7, CVB5, EVA71, CVA3, CVA12, EV24 or an aptamer to eIF4G.

16. The circular RNA polynucleotide of any one of claims 1-15, consisting of natural nucleotides.
17. The circular RNA polynucleotide of any one of claims 1-16, wherein the expression sequence is codon optimized.
18. The circular RNA polynucleotide of any one of claims 1-17, optimized to lack:
 - a. at least one microRNA binding site present in an equivalent pre-optimized polynucleotide;
 - b. at least one endonuclease susceptible site present in an equivalent pre-optimized polynucleotide; and/or
 - c. at least one RNA-editing susceptible site present in an equivalent pre-optimized polynucleotide.
19. The circular RNA polynucleotide of any one of claims 1-18, wherein the circular RNA polynucleotide is from 100 nucleotides to 15 kilobases in length.
20. The circular RNA polynucleotide of any one of claims 1-19, having an *in vivo* duration of therapeutic effect in humans of at least 20 hours and/or a functional half-life of at least 20 hours.
21. The circular RNA polynucleotide of any one of claims 1-20, having:
 - a. a duration of therapeutic effect in a human cell greater than or equal to that of an equivalent linear RNA polynucleotide comprising the same expression sequence;
 - b. a functional half-life in a human cell greater than or equal to that of an equivalent linear RNA polynucleotide comprising the same expression sequence;

- c. an *in vivo* duration of therapeutic effect in humans greater than that of an equivalent linear RNA polynucleotide having the same expression sequence; and/or
 - d. an *in vivo* functional half-life in humans greater than that of an equivalent linear RNA polynucleotide having the same expression sequence.
- 22. A pharmaceutical composition comprising at least one circular RNA polynucleotide of any one of claims 1-21, a nanoparticle, wherein the at least one circular RNA polynucleotide is encapsulated by the nanoparticle.
- 23. The pharmaceutical composition of claim 22, wherein the nanoparticle is a lipid nanoparticle, a core-shell nanoparticle, a biodegradable nanoparticle, a biodegradable lipid nanoparticle, a polymer nanoparticle, or a biodegradable polymer nanoparticle.
- 24. The pharmaceutical composition of claim 22 or 23, comprising a targeting moiety, wherein the targeting moiety mediates receptor-mediated endocytosis or direct fusion into selected cells of a selected cell population or tissue in the absence of cell isolation or purification, and wherein the targeting moiety is operably connected to the nanoparticle.
- 25. The pharmaceutical composition of any one of claims 22-24, wherein the targeting moiety is a scFv, nanobody, peptide, minibody, polynucleotide aptamer, heavy chain variable region, light chain variable region or fragment thereof.
- 26. The pharmaceutical composition of any one of claims 22-25, wherein
 - a. less than 1%, by weight, of the polynucleotides in the pharmaceutical composition are double stranded RNA, DNA splints, or triphosphorylated RNA; and/or
 - b. less than 1%, by weight, of the polynucleotides and proteins in the pharmaceutical composition are double stranded RNA, DNA splints, triphosphorylated RNA, phosphatase proteins, protein ligases, and capping enzymes.
- 27. The pharmaceutical composition of any one of claims 22-26, wherein the nanoparticle comprises:
 - a. one or more cationic lipids, ionizable lipids, or poly β -amino esters;
 - b. one or more non-cationic lipids;
 - c. one or more PEG-modified lipids, polyglutamic acid lipids, or hyaluronic acid lipids;
 - d. cholesterol; and/or
 - e. arachidonic acid or oleic acid.

28. The pharmaceutical composition of any one of claims 22-27, wherein the nanoparticle comprises more than one circular RNA polynucleotide.
29. Use of the circular RNA polynucleotide of any one of claims 1-21 or the pharmaceutical composition of any one of claims 22-28 in the manufacture of a medicament for treating cancer and/or an autoimmune disorder.
30. The use of claim 29, wherein the cancer and/or autoimmune disorder is:
 - a. a cancer selected from the group consisting of acute lymphocytic leukemia; acute myeloid leukemia (AML); alveolar rhabdomyosarcoma; B cell malignancies; bladder cancer (*e.g.*, bladder carcinoma); bone cancer; brain cancer (*e.g.*, medulloblastoma); breast cancer; cancer of the anus, anal canal, or anorectum; cancer of the eye; cancer of the intrahepatic bile duct; cancer of the joints; cancer of the neck; gallbladder cancer; cancer of the pleura; cancer of the nose, nasal cavity, or middle ear; cancer of the oral cavity; cancer of the vulva; chronic lymphocytic leukemia; chronic myeloid cancer; colon cancer; esophageal cancer, cervical cancer; fibrosarcoma; gastrointestinal carcinoid tumor; head and neck cancer (*e.g.*, head and neck squamous cell carcinoma); Hodgkin lymphoma; hypopharynx cancer; kidney cancer; larynx cancer; leukemia; liquid tumors; liver cancer; lung cancer (*e.g.*, non-small cell lung carcinoma and lung adenocarcinoma); lymphoma; mesothelioma; mastocytoma; melanoma; multiple myeloma; nasopharynx cancer; non-Hodgkin lymphoma; B-chronic lymphocytic leukemia; hairy cell leukemia; acute lymphocytic leukemia (ALL); Burkitt's lymphoma; ovarian cancer; pancreatic cancer; cancer of the peritoneum; cancer of the omentum; mesentery cancer; pharynx cancer; prostate cancer; rectal cancer; renal cancer; skin cancer; small intestine cancer; soft tissue cancer; solid tumors; synovial sarcoma; gastric cancer; testicular cancer; thyroid cancer; and ureter cancer; and/or
 - b. an autoimmune disorder selected from scleroderma, Grave's disease, Crohn's disease, Sjorgen's disease, multiple sclerosis, Hashimoto's disease, psoriasis, myasthenia gravis, autoimmune polyendocrinopathy syndromes, Type I diabetes mellitus (T1DM), autoimmune gastritis, autoimmune uveoretinitis, polymyositis,

colitis, thyroiditis, and the generalized autoimmune diseases typified by human Lupus.

31. A vector for making a circular RNA polynucleotide, comprising, in the following order, a 5' duplex forming region, a 3' Group I intron fragment, an Internal Ribosome Entry Site (IRES), a first expression sequence, a second expression sequence, a 5' Group I intron fragment, and a 3' duplex forming region, comprising:
 - a. a polynucleotide sequence encoding a cleavage site between the first expression sequence and the second expression sequence; and/or
 - b. a first spacer between the 5' duplex forming region and the 3' group I intron fragment, and a second spacer between the 5' group I intron fragment and the 3' duplex forming regionwherein the first expression sequence encodes a CD19 or BCMA antigen binding molecule and/or the second expression sequence encodes a CD19 or BCMA antigen binding molecule.
32. A eukaryotic cell comprising a circular RNA polynucleotide according to any of claims 1-21, wherein the cell is a human cell, an immune cell, and/or a T cell.