proliferative potential; and b) TIL growth is dependent on metabolic state and phenotypic state of TILs, i.e. expression of costimulatory receptors. Bystander TILs take up valuable tumor real-estate after adoptive transfer and create a “traffic jam” getting to tumor. Allen, *et al.*, *Science*, **2022**, 378, eaba1624.

[0005] Thus, there remains a need for improved TIL therapies for the treatment of cancers. Disclosed herein are gene-edited TILs with an inducible and membrane-bound IL-12, which showed superior cytotoxic function. The present invention further provides an improved expansion process for preferentially producing TS-TILs over bystander TILs.

SUMMARY

[0005a] In one aspect, the present disclosure relates to a nucleic acid molecule comprising a nucleotide sequence encoding i) a tethered IL-12 (TeIL-12), wherein the TeIL-12 is under the control of a nuclear factor of activated T-cells (NFAT)-responsive promoter; and ii) a tethered IL-15 (TeIL-15), wherein the TeIL-15 is under the control of a constitutive promoter.

[0005b] In another aspect, the present disclosure relates to a recombinant expression vector comprising the nucleic acid molecule as disclosed herein.

[0005c] In another aspect, the present disclosure relates to a lentiviral or retroviral expression system comprising the recombinant expression vector as disclosed herein and one or more helper plasmids encoding an Env protein, a Gag protein, a Pol protein, and/or a Rev protein.

[0005d] In another aspect, the present disclosure relates to a method of making a recombinant lentiviral particle, comprising: a) culturing a population of packaging cells in a cell culture medium; b) contacting the population of packaging cells with the lentiviral expression system as disclosed herein; and c) harvesting the supernatant of the cell culture medium, wherein the supernatant comprises the recombinant lentiviral particle.

[0005e] In another aspect, the present disclosure relates to a gene-edited tumor infiltrating lymphocyte (TIL) expressing the nucleic acid molecule as disclosed herein.

[0005f] In another aspect, the present disclosure relates to a method of making a population of gene-edited tumor-infiltrating lymphocytes (TILs), comprising: a) performing a first expansion by culturing a population of TILs in a first cell culture medium; b) performing a second expansion by culturing the population of TILs in a second cell culture medium; and

c) at any time, transducing the population of TILs with the recombinant expression vector as disclosed herein to produce the population of gene-edited TILs, wherein the population of gene-edited TILs expresses a TeIL-12 and a TeIL-15.

[0005g] In another aspect, the present disclosure relates to a pharmaceutical composition comprising the gene-edited TILs as disclosed herein.

[0005h] In another aspect, the present disclosure relates to use of a composition in the manufacture of a medicament for treating a cancer in a patient in need thereof, wherein the composition comprises: i) a nucleic acid molecule as disclosed herein; ii) a recombinant expression vector as disclosed herein; iii) a lentiviral expression system as disclosed herein; iv) a gene-edited tumor infiltrating lymphocyte (TIL) as disclosed herein; or vii) a pharmaceutical composition as disclosed herein.

[0005i] In another aspect, the present disclosure relates to a composition for use in therapy, wherein the composition comprises: i) a nucleic acid molecule as disclosed herein; ii) a recombinant expression vector as disclosed herein; iii) a lentiviral expression system as disclosed herein; iv) a gene-edited tumor infiltrating lymphocyte (TIL) as disclosed herein; or vii) a pharmaceutical composition as disclosed herein.

[0006] Some embodiments of the present disclosure provide a nucleic acid molecule comprising a nucleotide sequence encoding a tethered IL-12 (TeIL-12), wherein the TeIL-12 is under the control of a nuclear factor of activated T-cells (NFAT)-responsive promoter.

[0007] Some embodiments of the present disclosure provide a nucleic acid molecule comprising a nucleotide sequence encoding a tethered IL-12 (TeIL-12), wherein the TeIL-12 is under the control of a nuclear factor of activated T-cells (NFAT)-responsive promoter, and wherein the TeIL-12 comprises an amino acid sequence set forth in SEQ ID NO:62.

[0008] Some embodiments of the present disclosure provide a nucleic acid molecule comprising a nucleotide sequence encoding a tethered IL-12 (TeIL-12), wherein the TeIL-12 is under the control of a nuclear factor of activated T-cells (NFAT)-responsive promoter, and a nucleotide sequence encoding a tethered IL-15 (TeIL-15), wherein the TeIL-15 is under the control of an EF1α promoter.

[0009] Some embodiments of the present disclosure provide a nucleic acid molecule comprising a nucleotide sequence encoding a tethered IL-12 (TeIL-12), wherein the TeIL-12 is under the control of a nuclear factor of activated T-cells (NFAT)-responsive promoter, and a nucleotide sequence encoding a tethered IL-15 (TeIL-15), wherein the TeIL-15 is under the control of an EF1α promoter, and wherein the TeIL-15 has the amino acid sequence of SEQ ID NO:73.

[0010] In some embodiments, the TeIL-12 comprises a membrane anchor, and a human IL-12 p40 subunit fused to a human IL-12 p35 subunit. In some embodiments, the human IL-12 p35 subunit has the amino acid sequence of SEQ ID NO:60 and the human IL-12 p40 subunit has the amino acid sequence of SEQ ID NO:61. In some embodiments, the TeIL-12

period of about 7-11 days. In some embodiments, the second expansion is performed over a period of about 10 days. Some embodiments of the present disclosure provide a method of treating a cancer in a patient in need thereof comprising:

a. resecting a tumor sample from the patient;

b. digesting the tumor sample to obtain a tumor digest;

c. producing a population of TILs enriched for tumor-reactive TILs from the tumor digest using the methods disclosed herein; and

d. administering the therapeutic population of gene-edited TILs to the patient.

[0065] In some embodiments, the cancer is selected from the group consisting of melanoma (including mucosal melanoma, uveal melanoma, cutaneous melanoma, choroidal melanoma, ciliary body melanoma, or iris melanoma), ovarian cancer, cervical cancer, endometrial cancer, non-small-cell lung cancer (NSCLC), lung cancer, bladder cancer, breast cancer, triple negative breast cancer, cancer caused by human papilloma virus, head and neck cancer (including head and neck squamous cell carcinoma (HNSCC)), renal cancer, and renal cell carcinoma. In some embodiments, the method further comprises the step of treating the patient with a non-myeloablative lymphodepletion regimen prior to administering the TILs to the patient. In some embodiments, the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m^2/day for two days followed by administration of fludarabine at a dose of 25 mg/m^2/day for three days. In some embodiments, the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m^2/day and fludarabine at a dose of 25 mg/m^2/day for two days followed by administration of fludarabine at a dose of 25 mg/m^2/day for three days. In some embodiments, the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m^2/day and fludarabine at a dose of 25 mg/m^2/day for two days followed by administration of fludarabine at a dose of 25 mg/m^2/day for one day. In some embodiments, the cyclophosphamide is administered with mesna. In some embodiments, the method further comprises a step of treating the patient with an IL-2 regimen starting on the day after the administration of TILs to the patient. In some embodiments, the method further comprises a step of treating the patient with an IL-2 regimen starting on the same day as administration of TILs to the patient. In some embodiments, the IL-2 regimen is a high-dose IL-2 regimen comprising 600,000 or 720,000 IU/kg of aldesleukin, or a biosimilar or variant thereof,

[00293] Non-limiting examples of immune checkpoint genes that may be silenced or inhibited by the shRNA include PD-1, CTLA-4, LAG-3, HAVCR2 (TIM-3), CISH, TGFβ, PKA, CBL-B, PPP2CA, PPP2CB, PTPN6, PTPN22, PDCD1, BTLA, CD160, TIGIT, TET2, BAFF (BR3), CD96, CRTAM, LAIR1, SIGLEC7, SIGLEC9, CD244, TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7, FADD, FAS, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1, IL10RA, IL10RB, HMOX2, IL6R, IL6ST, EIF2AK4, CSK, PAG1, SIT1, FOXP3, PRDM1, BATF, GUCY1A2, GUCY1A3, GUCY1B2, GUCY1B3, TOX, SOCS1, ANKRD11, and BCOR. For example, immune checkpoint genes that may be silenced or inhibited the shRNA may be selected from the group comprising PD-1, CTLA-4, LAG-3, TIM-3, Cish, CBL-B, TIGIT, TET2, TGFβ, and PKA. BAFF (BR3) is described in Bloom, *et al., J. Immunother.,* **2018,** in press. According to another example, immune checkpoint genes that may be silenced or inhibited the shRNA may be selected from the group comprising PD-1, LAG-3, TIM-3, CTLA-4, TIGIT, TET2, CISH, TGFβR2, PRA, CBLB, BAFF (BR3), and combinations thereof. Exemplary PD-1 shRNA sequences are provided in the table below.

TABLE 26 – PD-1 shRNA sequences

Construct	DNA sequence
PD-1 shRNA #1	GGATTTCCAGTGGCGAGAGAACTCGAGTTCTCTCGCCACTGGAAATCC (SEQ ID NO:141)
PD-1 shRNA #2	CATTGTCTTTCCTAGCGGAATCTCGAGATTCCGCTAGGAAAGACAATG (SEQ ID NO:142)
PD-1 shRNA #3	CAAGGCGCAGATCAAAGAGAGCTCGAGCTCTCTTTGATCTGCGCCTTG (SEQ ID NO:143)
PD-1 shRNA #4	AAGGCGCAGATCAAAGAGAGCCTCGAGGCTCTCTTTGATCTGCGCCTT (SEQ ID NO:144)
PD-1 shRNA #5	ATCGGAGAGCTTCGTGCTAAACTCGAGTTTAGCACGAAGCTCTCCGAT (SEQ ID NO:145)
PD-1 shRNA #6	AGTATGCCACCATTGTCTTTCCTCGAGGAAAGACAATGGTGGCATACT (SEQ ID NO:146)
PD-1 shRNA #7	GGATTTCCAGTGGCGAGAGAACTCGAGTTCTCTCGCCACTGGAAATCC (SEQ ID NO:147)

[00294] In some embodiments of the invention, the nucleic acid molecule disclosed herein further comprises long terminal repeat (LTR) sequences, for example, a 5' LTR sequence and

[00341] Further provided herein is a recombinant lentiviral particle comprising the recombinant lentiviral RNA molecule disclosed herein. In some embodiments, the recombinant lentiviral particle is produced by the packaging cell line disclosed herein.

[00342] In some embodiments, the recombinant lentiviral particle comprises the recombinant lentiviral vector disclosed herein. In some embodiments, the recombinant lentiviral vector comprises the recombinant lentiviral RNA molecule disclosed herein. In some embodiments, the recombinant lentiviral particle further comprises a capsid enclosing the recombinant RNA viral genome. In some embodiments, the recombinant lentiviral particle further comprises an Env protein selected from the group consisting of Ba-EVTR, VSV-G, and RD114. In some embodiments, the recombinant lentiviral particle further comprises a reverse transcriptase.

VI. Method Of Making Gene-Edited TILs

[00343] In some embodiments of the present invention directed to methods for expanding TIL populations, the methods comprise one or more steps of gene-editing at least a portion of the TILs in order to enhance their therapeutic effect. As used herein, "gene-editing," "gene editing," and "genome editing" refer to a type of genetic modification in which DNA is permanently modified in the genome of a cell, *e.g.*, DNA is inserted, deleted, modified or replaced within the cell's genome. In some embodiments, gene-editing causes the expression of a DNA sequence to be silenced (sometimes referred to as a gene knockout) or inhibited/reduced (sometimes referred to as a gene knockdown). In other embodiments, gene-editing causes the expression of a DNA sequence to be enhanced (*e.g.*, by causing over-expression). In accordance with embodiments of the present invention, gene-editing technology is used to enhance the effectiveness of a therapeutic population of TILs.

[00344] In some embodiments of the present invention, provided herein is a method of making a population of gene-edited TILs, comprising: a) performing a first expansion by culturing a population of TILs in a first cell culture medium; b) performing a second expansion by culturing the population of TILs in a second cell culture medium; and c) at any time, transducing the population of TILs with the recombinant lentiviral particle disclosed herein to produce the population of gene-edited TILs, wherein the population of gene-edited TILs expresses one or more cytokines, such as IL-12, IL-2, IL-6, IL-7, IL-9, IL-15, IL-18, IL-

from the group consisting of glycine, L- histidine, L-isoleucine, L-methionine, L-phenylalanine, L-proline, L- hydroxyproline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, thiamine, reduced glutathione, L-ascorbic acid-2-phosphate, iron saturated transferrin, insulin, and compounds containing the trace element moieties Ag^{+}, Al^{3+}, Ba^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Ge^{4+}, Se^{4+}, Br, T, Mn^{2+}, P, Si^{4+}, V^{5+}, Mo^{6+}, Ni^{2+}, Rb^{+}, Sn^{2+} and Zr^{4+}. In some embodiments, the basal cell media is selected from the group consisting of Dulbecco's Modified Eagle's Medium (DMEM), Minimal Essential Medium (MEM), Basal Medium Eagle (BME), RPMI 1640, F-10, F-12, Minimal Essential Medium (αMEM), Glasgow's Minimal Essential Medium (G-MEM), RPMI growth medium, and Iscove's Modified Dulbecco's Medium.

[0066] In some embodiments, the concentration of glycine in the defined medium is in the range of from about 5-200 mg/L, the concentration of L- histidine is about 5-250 mg/L, the concentration of L-isoleucine is about 5-300 mg/L, the concentration of L-methionine is about 5-200 mg/L, the concentration of L-phenylalanine is about 5-400 mg/L, the concentration of L-proline is about 1-1000 mg/L, the concentration of L- hydroxyproline is about 1-45 mg/L, the concentration of L-serine is about 1-250 mg/L, the concentration of L-threonine is about 10-500 mg/L, the concentration of L-tryptophan is about 2-110 mg/L, the concentration of L-tyrosine is about 3-175 mg/L, the concentration of L-valine is about 5-500 mg/L, the concentration of thiamine is about 1-20 mg/L, the concentration of reduced glutathione is about 1-20 mg/L, the concentration of L-ascorbic acid-2-phosphate is about 1-200 mg/L, the concentration of iron saturated transferrin is about 1-50 mg/L, the concentration of insulin is about 1-100 mg/L, the concentration of sodium selenite is about 0.000001-0.0001 mg/L, and the concentration of albumin (*e.g.*, AlbuMAX® I) is about 5000-50,000 mg/L.

[0067] In some embodiments, the non-trace element moiety ingredients in the defined medium are present in the concentration ranges listed in the column under the heading “Concentration Range in 1X Medium” in Table 27below. In other embodiments, the non-trace element moiety ingredients in the defined medium are present in the final concentrations listed in the column under the heading “A Preferred Embodiment of the 1X Medium” in Table 27. In other embodiments, the defined medium is a basal cell medium comprising a serum free supplement. In some of these embodiments, the serum free supplement comprises non-trace moiety ingredients of the type and in the concentrations

listed in the column under the heading “A Preferred Embodiment in Supplement” in Table 27 below.

TABLE 27: Concentrations of Non-Trace Element Moiety Ingredients

Ingredient	A preferred embodiment in supplement (mg/L) (About)	Concentration range in 1X medium (mg/L) (About)	A preferred embodiment in 1X medium (mg/L) (About)
Glycine	150	5-200	53
L-Histidine	940	5-250	183
L-Isoleucine	3400	5-300	615
L-Methionine	90	5-200	44
L-Phenylalanine	1800	5-400	336
L-Proline	4000	1-1000	600
L-Hydroxyproline	100	1-45	15
L-Serine	800	1-250	162
L-Threonine	2200	10-500	425
L-Tryptophan	440	2-110	82
L-Tyrosine	77	3-175	84
L-Valine	2400	5-500	454
Thiamine	33	1-20	9
Reduced Glutathione	10	1-20	1.5
Ascorbic Acid-2-PO_4 (Mg Salt)	330	1-200	50
Transferrin (iron saturated)	55	1-50	8
Insulin	100	1-100	10
Sodium Selenite	0.07	0.000001-0.0001	0.00001
AlbuMAX®I	83,000	5000-50,000	12,500

[0068] In some embodiments, the osmolarity of the defined medium is between about 260 and 350 mOsmol. In some embodiments, the osmolarity is between about 280 and 310 mOsmol. In some embodiments, the defined medium is supplemented with up to about 3.7 g/L, or about 2.2 g/L sodium bicarbonate. The defined medium can be further supplemented with L-glutamine (final concentration of about 2 mM), one or more antibiotics, non-essential amino acids (NEAA; final concentration of about 100 μM), 2-mercaptoethanol (final concentration of about 100 μM).

[0069] In some embodiments, the defined media described in Smith, *et al.*, *Clin Transl Immunology*, 4(1) 2015 (doi: 10.1038/cti.2014.31) are useful in the present invention. Briefly, RPMI or CTS™ OpTmizer™ was used as the basal cell medium, and supplemented with either 0, 2%, 5%, or 10% CTS™ Immune Cell Serum Replacement.

"Concentration Range in 1X Medium" in Table 28 below. In other embodiments, the non-trace element moiety ingredients in the defined medium are present in the final concentrations listed in the column under the heading "A Preferred Embodiment of the 1X Medium" in Table 28. In other embodiments, the defined medium is a basal cell medium comprising a serum free supplement. In some of these embodiments, the serum free supplement comprises non-trace moiety ingredients of the type and in the concentrations listed in the column under the heading "A Preferred Embodiment in Supplement" in Table 28 below.

TABLE 28: Concentrations of Non-Trace Element Moiety Ingredients

Ingredient	A preferred embodiment in supplement (mg/L) (About)	Concentration range in 1X medium (mg/L) (About)	A preferred embodiment in 1X medium (mg/L) (About)
Glycine	150	5-200	53
L-Histidine	940	5-250	183
L-Isoleucine	3400	5-300	615
L-Methionine	90	5-200	44
L-Phenylalanine	1800	5-400	336
L-Proline	4000	1-1000	600
L-Hydroxyproline	100	1-45	15
L-Serine	800	1-250	162
L-Threonine	2200	10-500	425
L-Tryptophan	440	2-110	82
L-Tyrosine	77	3-175	84
L-Valine	2400	5-500	454
Thiamine	33	1-20	9
Reduced Glutathione	10	1-20	1.5
Ascorbic Acid-2-PO_4 (Mg Salt)	330	1-200	50
Transferrin (iron saturated)	55	1-50	8
Insulin	100	1-100	10
Sodium Selenite	0.07	0.000001-0.0001	0.00001
AlbuMAX®I	83,000	5000-50,000	12,500

[00104] In some embodiments, the osmolarity of the defined medium is between about 260 and 350 mOsmol. In some embodiments, the osmolarity is between about 280 and 310 mOsmol. In some embodiments, the defined medium is supplemented with up to about 3.7 g/L, or about 2.2 g/L sodium bicarbonate. The defined medium can be further supplemented with L-glutamine (final concentration of about 2 mM), one or more antibiotics, non-essential

Method/Compound	Mechanism of Action	Target Process/Pathway	Potential Use in TILs	Considerations/Challenges
Nicotinamide Riboside (NR)	Precursor to NAD+; converted to NMN and then to NAD+	NAD+ salvage pathway	Enhance TIL metabolism and function	Dosage optimization to avoid side effects
Nicotinamide Mononucleotide (NMN)	Direct precursor to NAD+; converted to NAD+ by NMNAT enzymes	NAD+ salvage pathway	Improve TIL anti-tumor activity	Stability and cellular uptake considerations
Niacin (Vitamin B3)	Converted to NAD+ via the Preiss-Handler pathway	NAD+ biosynthesis pathway	Support TIL energy generation and DNA repair	Flushing reaction at high doses
NAMPT Inhibitors	Increase NAD+ levels by inhibiting its degradation	NAD+ salvage pathway	Potentially extend TIL lifespan	Risk of systemic NAD+ depletion
PARP Inhibitors	Prevent NAD+ depletion by inhibiting PARP enzymes	DNA repair pathway	Preserve NAD+ for metabolic functions in TILs	Balance between inhibiting PARP and cancer therapy

Method/Compound	Mechanism of Action	Target Process/Pathway	Potential Use in TILs	Considerations/Challenges
Sirtuin Activators	Increase NAD+ utilization efficiency	Sirtuin-mediated deacetylation	Enhance TIL mitochondrial function	Long-term effects on TILs are not fully understood
CD38 Inhibitors	Reduce NAD+ degradation	ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase pathway	Increase NAD+ bioavailability for TILs	May affect calcium signaling
Ketogenic Diet	May elevate NAD+ levels via increased fatty acid oxidation	Metabolic shift to ketone body usage	Could indirectly support TILs in vivo	Feasibility in patients and impact on immune cells
Caloric Restriction	Can upregulate NAD+ biosynthesis pathways	General metabolic downregulation	Potentially increase TIL efficacy	Patient compliance and nutritional concerns
Exercise	Upregulates NAD+ biosynthesis and salvage pathways	Enhanced muscle contraction and energy demand	Indirect systemic effects on immune function	Direct impact on TILs is unclear
NAD+ Infusions	Direct supplementation of NAD+	Bypasses NAD+ biosynthesis pathways	Immediate replenishment of NAD+ in TILs	Risk of imbalances and homeostasis disruption

Method/Compound	Mechanism of Action	Target Process/Pathway	Potential Use in TILs	Considerations/Challenges
Gene Therapy	Overexpression of NAD+ biosynthesis enzymes	Targeted enhancement of NAD+ production	Sustained increase in TIL NAD+ levels	Vector design, delivery, and integration risks

[00379] The evolutionary conserved Wnt/β-catenin pathway is critically involved in pluripotency and differentiation of embryonic stem cells via maintenance of DNA methylation patterns promoting epigenetic stability necessary for correct cell fate decisions during states of high cell division (Clevers, H. and R. Nusse, *Cell*, **2012**. 149(6): p. 1192-205). In humans, rapid recall of memory T cells upon re-exposure to antigen suggests the requirement of a stable epigenetic program. Most TIL-based ACT protocols for solid tumors are generated from terminally differentiated effector memory T cells resulting from repetitive rounds of TCR ligation during the ex vivo phase (Hinrichs, C.S. and S.A. Rosenberg, *Immunol Rev*, **2014**. 257(1): p. 56-71). However, the critical involvement of DNA methylation in shaping expansion, memory state status and thus functionality of antigen-specific TILs undergoing TCR ligation-dependent expansion ex vivo is not known (Abdelsamed, H.A., et al., *J Exp Med*, **2017**. 214(6): p. 1593-1606).

[00380] There is evidence to show that pharmacological activation of the Wnt/β-catenin signaling reprograms tumor-specific CD8+ TILs in tumor-bearing mice by increasing expression of Tcf1 (encoded by Tcf7) (Gattinoni, L., et al., *Nat Med*, **2009**. 15(7): p. 808-13). In human melanoma, CD8+TCF7+ uncovers a subset of TILs with a memory precursor-like cell state with bystander cytotoxic function and is associated with positive outcome to immune checkpoint blockade (Li, H., et al., *Cell*, **2019**. 176(4): p. 775-789 e18; Sade-Feldman, M., et al., *Cell*, **2018**. 175(4): p. 998-1013 e20). Downregulation of the components of the Wnt pathway has been reported in PD1+ CD8+ memory TILs in vivo and a further downregulation upon *in vitro expansion* in all T cell populations (Lipp, J.J., et al., *Oncoimmunology*, **2022**. 11(1): p. 2019466), whereas compensating the lack of Wnt signaling through pharmacological inhibition of GSK3β improves the effector function of in vitro expanded populations. As Wnt signaling is known to arrest the development of effector

consisting of Dulbecco's Modified Eagle's Medium (DMEM), Minimal Essential Medium (MEM), Basal Medium Eagle (BME), RPMI 1640, F-10, F-12, Minimal Essential Medium (αMEM), Glasgow's Minimal Essential Medium (G-MEM), RPMI growth medium, and Iscove's Modified Dulbecco's Medium.

[00196] In some embodiments, the concentration of glycine in the defined medium is in the range of from about 5-200 mg/L, the concentration of L- histidine is about 5-250 mg/L, the concentration of L-isoleucine is about 5-300 mg/L, the concentration of L-methionine is about 5-200 mg/L, the concentration of L-phenylalanine is about 5-400 mg/L, the concentration of L-proline is about 1-1000 mg/L, the concentration of L- hydroxyproline is about 1-45 mg/L, the concentration of L-serine is about 1-250 mg/L, the concentration of L-threonine is about 10-500 mg/L, the concentration of L-tryptophan is about 2-110 mg/L, the concentration of L-tyrosine is about 3-175 mg/L, the concentration of L-valine is about 5-500 mg/L, the concentration of thiamine is about 1-20 mg/L, the concentration of reduced glutathione is about 1-20 mg/L, the concentration of L-ascorbic acid-2-phosphate is about 1-200 mg/L, the concentration of iron saturated transferrin is about 1-50 mg/L, the concentration of insulin is about 1-100 mg/L, the concentration of sodium selenite is about 0.000001-0.0001 mg/L, and the concentration of albumin (e.g., AlbuMAX® I) is about 5000-50,000 mg/L.

[00197] In some embodiments, the non-trace element moiety ingredients in the defined medium are present in the concentration ranges listed in the column under the heading “Concentration Range in 1X Medium” in Table 29 below. In other embodiments, the non-trace element moiety ingredients in the defined medium are present in the final concentrations listed in the column under the heading “A Preferred Embodiment of the 1X Medium” in Table 29. In other embodiments, the defined medium is a basal cell medium comprising a serum free supplement. In some of these embodiments, the serum free supplement comprises non-trace moiety ingredients of the type and in the concentrations listed in the column under the heading “A Preferred Embodiment in Supplement” in Table 27 above.

[00198] In some embodiments, the osmolarity of the defined medium is between about 260 and 350 mOsmol. In some embodiments, the osmolarity is between about 280 and 310 mOsmol. In some embodiments, the defined medium is supplemented with up to about 3.7 g/L, or about 2.2 g/L sodium bicarbonate. The defined medium can be further supplemented with L-glutamine (final concentration of about 2 mM), one or more antibiotics, non-essential

[00238] In some embodiments, the non-trace element moiety ingredients in the defined medium are present in the concentration ranges listed in the column under the heading "Concentration Range in 1X Medium" in Table 28 below. In other embodiments, the non-trace element moiety ingredients in the defined medium are present in the final concentrations listed in the column under the heading "A Preferred Embodiment of the 1X Medium" in Table 28. In other embodiments, the defined medium is a basal cell medium comprising a serum free supplement. In some of these embodiments, the serum free supplement comprises non-trace moiety ingredients of the type and in the concentrations listed in the column under the heading "A Preferred Embodiment in Supplement" in Table 28 above.

[00239] In some embodiments, the osmolarity of the defined medium is between about 260 and 350 mOsmol. In some embodiments, the osmolarity is between about 280 and 310 mOsmol. In some embodiments, the defined medium is supplemented with up to about 3.7 g/L, or about 2.2 g/L sodium bicarbonate. The defined medium can be further supplemented with L-glutamine (final concentration of about 2 mM), one or more antibiotics, non-essential amino acids (NEAA; final concentration of about 100 μM), 2-mercaptoethanol (final concentration of about 100 μM).

[00240] In some embodiments, the defined media described in Smith, *et al.*, *Clin Transl Immunology*, 4(1) 2015 (doi: 10.1038/cti.2014.31) are useful in the present invention. Briefly, RPMI or CTS™ OpTmizer™ was used as the basal cell medium, and supplemented with either 0, 2%, 5%, or 10% CTS™ Immune Cell Serum Replacement.

[00241] In some embodiments, the cell medium in the first and/or second gas permeable container is unfiltered. The use of unfiltered cell medium may simplify the procedures necessary to expand the number of cells. In some embodiments, the cell medium in the first and/or second gas permeable container lacks beta-mercaptoethanol (BME or βME; also known as 2-mercaptoethanol, CAS 60-24-2).

[00242] In some embodiments, the second expansion is performed in a closed system bioreactor. In some embodiments, a closed system is employed for the TIL expansion, as described herein. In some embodiments, a single bioreactor is employed. In some embodiments, the single bioreactor employed is for example a G-REX -10 or a G-REX -100. In some embodiments, the closed system bioreactor is a single bioreactor.

Method/Compound	Mechanism of Action	Target Process/Pathway	Potential Use in TILs	Considerations/Challenges
Nicotinamide Riboside (NR)	Precursor to NAD+; converted to NMN and then to NAD+	NAD+ salvage pathway	Enhance TIL metabolism and function	Dosage optimization to avoid side effects
Nicotinamide Mononucleotide (NMN)	Direct precursor to NAD+; converted to NAD+ by NMNAT enzymes	NAD+ salvage pathway	Improve TIL anti-tumor activity	Stability and cellular uptake considerations
Niacin (Vitamin B3)	Converted to NAD+ via the Preiss-Handler pathway	NAD+ biosynthesis pathway	Support TIL energy generation and DNA repair	Flushing reaction at high doses
NAMPT Inhibitors	Increase NAD+ levels by inhibiting its degradation	NAD+ salvage pathway	Potentially extend TIL lifespan	Risk of systemic NAD+ depletion
PARP Inhibitors	Prevent NAD+ depletion by inhibiting PARP enzymes	DNA repair pathway	Preserve NAD+ for metabolic functions in TILs	Balance between inhibiting PARP and cancer therapy

Method/Compound	Mechanism of Action	Target Process/Pathway	Potential Use in TILs	Considerations/Challenges
Sirtuin Activators	Increase NAD+ utilization efficiency	Sirtuin-mediated deacetylation	Enhance TIL mitochondrial function	Long-term effects on TILs are not fully understood
CD38 Inhibitors	Reduce NAD+ degradation	ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase pathway	Increase NAD+ bioavailability for TILs	May affect calcium signaling
Ketogenic Diet	May elevate NAD+ levels via increased fatty acid oxidation	Metabolic shift to ketone body usage	Could indirectly support TILs in vivo	Feasibility in patients and impact on immune cells
Caloric Restriction	Can upregulate NAD+ biosynthesis pathways	General metabolic downregulation	Potentially increase TIL efficacy	Patient compliance and nutritional concerns
Exercise	Upregulates NAD+ biosynthesis and salvage pathways	Enhanced muscle contraction and energy demand	Indirect systemic effects on immune function	Direct impact on TILs is unclear
NAD+ Infusions	Direct supplementation of NAD+	Bypasses NAD+ biosynthesis pathways	Immediate replenishment of NAD+ in TILs	Risk of imbalances and homeostasis disruption

Method/Compound	Mechanism of Action	Target Process/Pathway	Potential Use in TILs	Considerations/Challenges
Gene Therapy	Overexpression of NAD+ biosynthesis enzymes	Targeted enhancement of NAD+ production	Sustained increase in TIL NAD+ levels	Vector design, delivery, and integration risks

[00408] The evolutionary conserved Wnt/β-catenin pathway is critically involved in pluripotency and differentiation of embryonic stem cells via maintenance of DNA methylation patterns promoting epigenetic stability necessary for correct cell fate decisions during states of high cell division (Clevers, H. and R. Nusse, *Cell*, **2012**. 149(6): p. 1192-205). In humans, rapid recall of memory T cells upon re-exposure to antigen suggests the requirement of a stable epigenetic program. Most TIL-based ACT protocols for solid tumors are generated from terminally differentiated effector memory T cells resulting from repetitive rounds of TCR ligation during the ex vivo phase (Hinrichs, C.S. and S.A. Rosenberg, *Immunol Rev*, **2014**. 257(1): p. 56-71). However, the critical involvement of DNA methylation in shaping expansion, memory state status and thus functionality of antigen-specific TILs undergoing TCR ligation-dependent expansion ex vivo is not known (Abdelsamed, H.A., et al., *J Exp Med*, **2017**. 214(6): p. 1593-1606).

[00409] There is evidence to show that pharmacological activation of the Wnt/β-catenin signaling reprograms tumor-specific CD8+ TILs in tumor-bearing mice by increasing expression of Tcf1 (encoded by Tcf7) (Gattinoni, L., et al., *Nat Med*, **2009**. 15(7): p. 808-13). In human melanoma, CD8+TCF7+ uncovers a subset of TILs with a memory precursor-like cell state with bystander cytotoxic function and is associated with positive outcome to immune checkpoint blockade (Li, H., et al., *Cell*, **2019**. 176(4): p. 775-789 e18; Sade-Feldman, M., et al., *Cell*, **2018**. 175(4): p. 998-1013 e20). Downregulation of the components of the Wnt pathway has been reported in PD1+ CD8+ memory TILs in vivo and a further downregulation upon *in vitro expansion* in all T cell populations (Lipp, J.J., et al., *Oncoimmunology*, **2022**. 11(1): p. 2019466), whereas compensating the lack of Wnt signaling through pharmacological inhibition of GSK3β improves the effector function of in vitro expanded populations. As Wnt signaling is known to arrest the development of effector

threonine is about 10-500 mg/L, the concentration of L-tryptophan is about 2-110 mg/L, the concentration of L-tyrosine is about 3-175 mg/L, the concentration of L-valine is about 5-500 mg/L, the concentration of thiamine is about 1-20 mg/L, the concentration of reduced glutathione is about 1-20 mg/L, the concentration of L-ascorbic acid-2-phosphate is about 1-200 mg/L, the concentration of iron saturated transferrin is about 1-50 mg/L, the concentration of insulin is about 1-100 mg/L, the concentration of sodium selenite is about 0.000001-0.0001 mg/L, and the concentration of albumin (e.g., AlbuMAX® I) is about 5000-50,000 mg/L.

[00331] In some embodiments, the non-trace element moiety ingredients in the defined medium are present in the concentration ranges listed in the column under the heading "Concentration Range in 1X Medium" in Table 29 below. In other embodiments, the non-trace element moiety ingredients in the defined medium are present in the final concentrations listed in the column under the heading "A Preferred Embodiment of the 1X Medium" in Table 29. In other embodiments, the defined medium is a basal cell medium comprising a serum free supplement. In some of these embodiments, the serum free supplement comprises non-trace moiety ingredients of the type and in the concentrations listed in the column under the heading "A Preferred Embodiment in Supplement" in Table 29 below.

TABLE 29: Concentrations of Non-Trace Element Moiety Ingredients

Ingredient	A preferred embodiment in supplement (mg/L) (About)	Concentration range in 1X medium (mg/L) (About)	A preferred embodiment in 1X medium (mg/L) (About)
Glycine	150	5-200	53
L-Histidine	940	5-250	183
L-Isoleucine	3400	5-300	615
L-Methionine	90	5-200	44
L-Phenylalanine	1800	5-400	336
L-Proline	4000	1-1000	600
L-Hydroxyproline	100	1-45	15
L-Serine	800	1-250	162
L-Threonine	2200	10-500	425
L-Tryptophan	440	2-110	82
L-Tyrosine	77	3-175	84
L-Valine	2400	5-500	454
Thiamine	33	1-20	9
Reduced Glutathione	10	1-20	1.5
Ascorbic Acid-2-PO_4 (Mg Salt)	330	1-200	50

mg/L, the concentration of thiamine is about 1-20 mg/L, the concentration of reduced glutathione is about 1-20 mg/L, the concentration of L-ascorbic acid-2-phosphate is about 1-200 mg/L, the concentration of iron saturated transferrin is about 1-50 mg/L, the concentration of insulin is about 1-100 mg/L, the concentration of sodium selenite is about 0.000001-0.0001 mg/L, and the concentration of albumin (*e.g.*, AlbuMAX® I) is about 5000-50,000 mg/L.

[00388] In some embodiments, the non-trace element moiety ingredients in the defined medium are present in the concentration ranges listed in the column under the heading "Concentration Range in 1X Medium" in Table 29. In other embodiments, the non-trace element moiety ingredients in the defined medium are present in the final concentrations listed in the column under the heading "A Preferred Embodiment of the 1X Medium" in Table 29. In other embodiments, the defined medium is a basal cell medium comprising a serum free supplement. In some of these embodiments, the serum free supplement comprises non-trace moiety ingredients of the type and in the concentrations listed in the column under the heading "A Preferred Embodiment in Supplement" in Table 29.

[00389] In some embodiments, the osmolarity of the defined medium is between about 260 and 350 mOsmol. In some embodiments, the osmolarity is between about 280 and 310 mOsmol. In some embodiments, the defined medium is supplemented with up to about 3.7 g/L, or about 2.2 g/L sodium bicarbonate. The defined medium can be further supplemented with L-glutamine (final concentration of about 2 mM), one or more antibiotics, non-essential amino acids (NEAA; final concentration of about 100 μM), 2-mercaptoethanol (final concentration of about 100 μM).

[00390] In some embodiments, the defined media described in Smith, *et al.*, *Clin Transl Immunology*, 4(1) 2015 (doi: 10.1038/cti.2014.31) are useful in the present invention. Briefly, RPMI or CTS™ OpTmizer™ was used as the basal cell medium, and supplemented with either 0, 2%, 5%, or 10% CTS™ Immune Cell Serum Replacement.

[00391] In some embodiments, the cell medium in the first and/or second gas permeable container is unfiltered. The use of unfiltered cell medium may simplify the procedures necessary to expand the number of cells. In some embodiments, the cell medium in the first and/or second gas permeable container lacks beta-mercaptoethanol (BME or βME; also known as 2-mercaptoethanol, CAS 60-24-2).

[00588] After lentiviral transduction, TILs will be expanded for 11 days in a REP process and surface expression of membrane-bound cytokine receptors will then be assessed by flow cytometry. Harvested REP cells will be assessed for CD8+ T cells. Previous reports show that IL-15 and IL-21 act as a potent stimulator, which preferentially stimulates the proliferation and survival of CD8 T cells.

[00589] Phenotype of mIL-15/mIL-21 expressing REP TIL

[00590] To characterize the immune regulatory effect of mIL-15 and mIL-21 on REP cells, phenotypic analysis will be performed with freshly thawed REP cells in both gated CD8+CD3+T cells and CD4+CD3+ T cell subset. Expression of the following will be assessed: CD27, PD1, TIM3, CD62L, TOX, T-bet, CD38, CXCR3, Eomes, and CD25.

EXAMPLE 6: PRODUCTION AND CHARACTERIZATION OF MODIFIED TILs THAT EXPRESSED TEHTERED IL-12 UNDER THE CONTROL OF AN NFAT PROMOTER AND REDUCED EXPRESSION OF PD-1

[00591] For this study, modified TILs with membrane anchored IL-12 immunomodulatory fusion proteins and one or more shRNAs for reducing endogenous PD-1 expression will be made. Figure 5 depicts an exemplary nucleic acid construct that allows for expression of the member anchored IL-12 (TeIL-12) and PD-1 shRNA. As shown in Figure 5, expression of the membrane anchored immunomodulatory IL-12 is controlled using an NFAT promoter that includes NFAT responsive elements linked to a minimal human IL-2 promoter, see, e.g., Table 23. The shRNA is under the control of a PolIII promoter (U6).

Virus preparation and T cell transduction

[00592] To make lentivirus, pLenti-vectors containing the tethered cytokine gene sequence (Table 23) and PD-1 shRNA (Table 26) and packaging helper vectors (BaEV-TR, Gag/Pol) will be co-transfected into 293T cells. Lentivirus supernatant will be collected from day 2-3 culture supernatants followed by ultracentrifuge (120,000 g) to concentrate lentivirus for TIL transduction. 1E5 pre-REP cells will be added into Retronectin pre-coated 48 well plates with concentrated lentivirus. Two days after gene transduction, pre-REP cells will be harvested for REP process according to the methods described herein or further phenotypic characterization and functional assays.

[00752] To evaluate the impact of media formulation on TIL function with respect to inhibiting tumor cell growth, pre-REP TILs transduced with a TCR construct targeting KRAS G12D were cultured with KRAS G12D-expressing HPAC tumor cells in conditions that simulate the solid TME. A modest benefit in controlling tumor growth was observed when antigen-specific TILs were expanded in optimized media formulations compared with media containing IL-2 only (IL-2/NAD+, 1.4 fold change; ITARG, 1.2 fold change; ITARG/NAD+ 1.7 fold change) (Fig. 79A). Removing IL-2 (30 IU/mL) from the simulated TME resulted in a loss of TIL reactivity irrespective of media condition used to expand antigen-specific TIL (IL-2/NAD+, 1.05 fold change; ITARG, 1.03 fold change; ITARG/NAD+ 1.2 fold change) (Fig. 79B).

[00753] Membrane-tethered IL12/IL15 antigen-specific TILs show enhanced cytotoxicity in vitro

[00754] Next, the impact of membrane-tethered cytokines on antigen-directed TIL expanded in optimized medias on tumor cell growth was evaluated upon coculture at low E:T ratios over time. Compared with all experimental groups, KRAS.mteth.IL15.IL21 TILs expanded in ITARG/NAD+ exhibited the most potent antitumor activity (5.0 fold change) (Fig. 80A). Moreover, enhanced activity of KRAS.mteth.IL15.IL21 TILs expanded in ITARG/NAD+ was maintained (5.2 fold change) in the absence of IL-2 (Fig. 80B). The level of mteth.IL15 on the surface of KRAS transduced TIL detected using flow cytometry serves as a control for transduction efficiency, as shown in Fig. 80C.

EXAMPLE 26: MEMBRANE-TETHERED IL-12/IL-15 EXPRESSING TILS SHOW ENHANCED PERSISTENCE IN VIVO

[00755] Materials and Methods

[00756] On Day -10, 1E6 PDX M1152 tumor cells were inoculated to 7 or 8 NOG mice for each experimental group #1-#7. On Day 0, 10E6 REP TILs were infused to each mouse in the treatment groups #2-#7 (see Table 25).

[00757] Table 25. PDX M1152 ACT experiment summary.

	Group ID	Engineered REP-TIL	REP process	# NOG mice
M1152 (ReREP-TIL) efficacy	1	M1152 tumor only		8
	2	+ ACT (Gen2 only)	Gen2	7

	3	+ ACT (NFAT-TeIL-12/tCD19)	Gen2	7
	4	+ACT(NFAT-TeIL-12/TeIL-15)	Gen2	7
	5	+ ACT (Gen2 only) +Proleukin	Gen2	7
	6	+ ACT (NFAT-TeIL-12/tCD19) +Proleukin	Gen2	7
	7	+ ACT (NFAT-TeIL-12/TeIL15) +Proleukin	Gen2	7

[00758] Nine doses of Proleukin (45,000IU) regimen were administered via ip injection for each mouse in treatment groups #5, #6 and #7; the administration schedule was:

Day 0: One dose Proleukin (45,000 IU) after ACT

Day 1: Two doses Proleukin (45,000 IU) - early morning and late afternoon

Day 2: Two doses Proleukin (45,000 IU) - early morning and late afternoon

Day 3: One dose Proleukin (45,000 IU)

Days 4-6: One dose Proleukin (45,000 IU) each day

[00759] Starting from Day 7, peripheral blood was collected from TIL-infused mice in EDTA-coated tubes as follows:

Day 7: Groups #2, #3, #4

Day 8: Groups #5, #6, #7

Day 14: Groups #2, #3, #4

Day 15: Groups #5, #6, #7

[00760] TILs persistence in PB were stained with CD2+CD3+ antibody and quantified by flow cytometry with absolute counting beads.

[00761] **Results**

[00762] TeIL-12/TeIL-15 expressing TILs show trends of increased persistence after infusion in the absence of Proleukin (Fig. 81).

* * *

WHAT IS CLAIMED IS:

1. A nucleic acid molecule comprising a nucleotide sequence encoding

i) a tethered IL-12 (TeIL-12), wherein the TeIL-12 is under the control of a nuclear factor of activated T-cells (NFAT)-responsive promoter; and

ii) a tethered IL-15 (TeIL-15), wherein the TeIL-15 is under the control of a constitutive promoter.

2. The nucleic acid molecule of claim 1, wherein

i) the TeIL-12 comprises a first membrane anchor, and a human IL-12 p40 subunit fused to a human IL-12 p35 subunit; and/or

ii) the TeIL-15 comprises a second membrane anchor and a human IL-15.

3. The nucleic acid molecule of claim 2, wherein

i) the human IL-12 p35 subunit comprises the amino acid sequence of SEQ ID NO:60 and the human IL-12 p40 subunit comprises the amino acid sequence of SEQ ID NO:61; and/or

ii) the human IL-15 comprises the amino acid sequence of SEQ ID NO:64.

4. The nucleic acid molecule of any one of claims 1-3, wherein

i) the TeIL-12 comprises an amino acid sequence set forth in SEQ ID NO:62; and/or

ii) the TeIL-15 comprises an amino acid sequence set forth in SEQ ID NO:73.

5. The nucleic acid molecule of any one of claims 1-4, wherein the constitutive promoter is selected from the group consisting of an EF1α promotor, a CMV promotor, a CAG promotor, an MND promotor, and an SSFV promoter.

6. The nucleic acid molecule of any one of claims 1-5, comprising the nucleic acid sequence set forth in any one of SEQ ID NOs:148-150.

7. The nucleic acid molecule of any one of claims 1-6, further comprising a nucleotide sequence encoding an shRNA.

8. The nucleic acid molecule of claim 7, wherein the shRNA inhibits the expression of an immune checkpoint gene selected from the group consisting of PD-1, CTLA-4, LAG-3, HAVCR2 (TIM-3), CISH, TGFβ, PKA, CBL-B, PPP2CA, PPP2CB, PTPN6, PTPN22, PDCD1, BTLA, CD160, TIGIT, TET2, CD96, CRTAM, LAIR1, SIGLEC7, SIGLEC9, CD244, TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7, FADD,

FAS, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1, IL10RA, IL10RB, HMOX2, IL6R, IL6ST, EIF2AK4, CSK, PAG1, SIT1, FOXP3, PRDM1, BATF, GUCY1A2, GUCY1A3, GUCY1B2, GUCY1B3, TOX, SOCS1, ANKRD11, and BCOR.

9. A recombinant expression vector comprising the nucleic acid molecule of any one of claims 1-8.

10. The recombinant expression vector of claim 9, wherein the vector is a recombinant lentiviral or retroviral vector.

11. A lentiviral or retroviral expression system comprising the recombinant expression vector of claim 10 and one or more helper plasmids encoding an Env protein, a Gag protein, a Pol protein, and/or a Rev protein.

12. The lentiviral or retroviral expression system of claim 11, wherein the Env protein comprises Ba-EVTR, VSV-G, and/or RD114 Env proteins.

13. A method of making a recombinant lentiviral particle, comprising:

a) culturing a population of packaging cells in a cell culture medium;

b) contacting the population of packaging cells with the lentiviral expression system of claim 11 or 12; and

c) harvesting the supernatant of the cell culture medium, wherein the supernatant comprises the recombinant lentiviral particle.

14. A gene-edited tumor infiltrating lymphocyte (TIL) expressing the nucleic acid molecule of any one of claims 1-8.

15. A method of making a population of gene-edited tumor-infiltrating lymphocytes (TILs), comprising:

a) performing a first expansion by culturing a population of TILs in a first cell culture medium;

b) performing a second expansion by culturing the population of TILs in a second cell culture medium; and

c) at any time, transducing the population of TILs with the recombinant expression vector of claim 9 or 10 to produce the population of gene-edited TILs,

wherein the population of gene-edited TILs expresses a TeIL-12 and a TeIL-15.

16. The method of claim 15, further comprising

i) activating the population of TILs for 1 day or 2 days before transducing the population of TILs with the recombinant lentiviral particle; and/or

ii) resting the population of TILs for 2 or 3 days after the transducing step.

17. A pharmaceutical composition comprising the gene-edited TILs of claim 14.

18. Use of a composition in the manufacture of a medicament for treating a cancer in a patient in need thereof, wherein the composition comprises:

i) a nucleic acid molecule of any one of claims 1-8;

ii) a recombinant expression vector of any one of claims 9-10;

iii) a lentiviral expression system of any one of claims 11-12;

iv) a gene-edited tumor infiltrating lymphocyte (TIL) of claim 14; or

vii) a pharmaceutical composition of claim 17.

19. The use of claim 18, wherein the cancer is selected from the group consisting of melanoma (including mucosal melanoma, uveal melanoma, cutaneous melanoma, choroidal melanoma, ciliary body melanoma, or iris melanoma), ovarian cancer, cervical cancer, endometrial cancer, non-small-cell lung cancer (NSCLC), lung cancer, bladder cancer, breast cancer, triple negative breast cancer, cancer caused by human papilloma virus, head and neck cancer (including head and neck squamous cell carcinoma (HNSCC)), renal cancer, and renal cell carcinoma.

20. The use of any one of claims 18-19, wherein the subject is to be further subjected to a non-myeloablative lymphodepletion regimen prior to the use of the composition, and/or an IL-2 regimen starting on the day after the use of TILs.

21. A composition for use in therapy, wherein the composition comprises:

i) a nucleic acid molecule of any one of claims 1-8;

ii) a recombinant expression vector of any one of claims 9-10;

iii) a lentiviral expression system of any one of claims 11-12;

iv) a gene-edited tumor infiltrating lymphocyte (TIL) of claim 14; or

vii) a pharmaceutical composition of claim 17.