NO: 16, CDRH2 as set forth in SEQ ID NO: 17, and CDRH3 as set forth in SEQ ID NO: 18; and a VL comprising or consisting of CDRL1 as set forth in SEQ ID NO: 19, CDRL2 as set forth in SEQ ID NO: 20, and CDRL3 as set forth in SEQ ID NO: 21. In some embodiments, the anti-mesothelin scFv antibody comprises a VH comprising a sequence having about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 22; and a VL comprising a sequence having about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 23.

[0050] In some embodiments, the VH and the VL of the anti-mesothelin scFv antibody are connected through a peptide linker. The peptide linker can include 3 or more amino acid residues, for example, from about 3 to about 30, from about 3 to about 25, from 3 to about 20, from about 4 to about 30, from about 4 to about 20, from about 4 to about 10, from about 5 to about 30, from about 5 to about 20, or from about 5 to about 10. The peptide linker can include 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or 30 amino acid residues.

[0051] The peptide linker can include a plurality of poly-alanines, poly-glycines, or a mixture of alanine and glycine residues. The peptide linker can include a $(Gly_4Ser)n$ linker, in which n is an integer from 1 to 30 (SEQ ID NO: 76), preferably 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30, further preferably 15, 16, 17, 18, 19 or 20. In some instances, the peptide linker comprises GILGSGGGGSGGGGSGGGGS (SEQ ID NO: 45) the peptide linker comprises GGGGSGGGGSGGGGS (SEQ ID NO: 30). In some instances, the peptide linker comprises GSGGGGSGGGGSGGGGS (SEQ ID NO: 41). In some instances, the peptide linker comprises SEQ ID NO: 45.

[0052] In some embodiments, the anti-mesothelin scFv antibody comprises a sequence comprising about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to
QVQLQQSGPGLVTPSQTLSLTCAISGDSVSSNSATWNWIRQSPSRGLEWLGRTY
YRSKWYNDYAVSVKSRMSINPDTSKNQFSLQLNSVTPEDTAVYYCARGMMTY
YYGMDVWGQGTTVTVSS***GILGSGGGGSGGGGSGGGGS***QPVLTQSSSLSASPGA
SASLTCTLRSGINVGPYRIYWYQQKPGSPPQYLLNYKSDSDKQQGSGVPSRFSG
SKDASANAGVLLISGLRSEDEADYYCMIWHSSAAVFGGGTQLTVLS (SEQ ID NO: 1), in which the linker is italicized. The anti-mesothelin scFv is also referred to herein as P4. In some embodiments, the anti-mesothelin scFv antibody comprises a sequence comprising about 85% sequence identity to SEQ ID NO: 1. In some

fusion protein (e.g., linked to the whole or a portion of IL-15Rα). IL-15 may be a membrane bounded protein or secreting protein.

A. mIL15/Ra

[0067] In some instances, the IL-15 comprises a membrane bound IL-15 fused to the IL-15 receptor α chain (IL-15Rα). The fusion IL-15 protein is referred to herein as mIL15/Ra. The IL-15Rα encompasses the entire wild-type IL-15Rα (without the signal peptide), but also encompasses a functional fragment thereof (e.g., an extracellular domain or a sushi domain of IL-15Rα), and variants of IL-15Rα, optionally comprising one or more substitutions, deletions, or additions.

[0068] In some instances, the mIL15/Ra fusion protein comprises an IL-15Rα polypeptide fused to the C-terminus of a membrane bound IL-15 polypeptide, either directly or indirectly via a linker. The peptide linker can include 1 or more amino acid residues, for example, from about 1 to about 30, from about 3 to about 20, from 3 to about 10, from about 4 to about 30, from about 4 to about 20, from about 4 to about 10, from about 5 to about 30, from about 5 to about 20, or from about 5 to about 10. The peptide linker can include 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or 30 amino acid residues.

[0069] The peptide linker can include a plurality of poly-alanines, poly-glycines, or a mixture of alanine and glycine residues. The peptide linker can include a $(Gly_4Ser)n$ linker, in which n is an integer from 1 to 30 (SEQ ID NO: 76), preferably 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30, further preferably 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30. In some instances, the peptide linker comprises SGGGSGGGGSGGGGSGGGGSGGGSLQ (SEQ ID NO: 32). In some instances, the peptide linker comprises SGGSGGGGSGGGSGGGGSLQ (SEQ ID NO: 33). In some instances, the peptide linker comprises GGGGSGGGGSGGGGS (SEQ ID NO: 30). In some instances, the peptide linker comprises GSGGGGSGGGGSGGGGS (SEQ ID NO: 41). In some instances, the peptide linker comprises SEQ ID NO: 45.

[0070] In some embodiments, an immune cell described herein expresses mIL15/Ra. In some embodiments, the mIL15/Ra comprises a sequence comprising about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESG DASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFI NTS*SGGGSGGGGSGGGGSGGGGSGGGSLQ*ITCPPPMSVEHADIWVKSYSLYSRE

mIL15/Ra polypeptide comprises about 97% sequence identity to SEQ ID NO: 28. In some cases, the IL2sp-mIL15/Ra polypeptide comprises about 98% sequence identity to SEQ ID NO: 28. In some cases, the IL2sp-mIL15/Ra polypeptide comprises about 99% sequence identity to SEQ ID NO: 28. In some cases, the IL2sp-mIL15/Ra polypeptide comprises SEQ ID NO: 28. In some cases, the IL2sp-mIL15/Ra polypeptide consists of SEQ ID NO: 28.

B. sushi15

[0073] In some instances, the IL-15 comprises an IL-15 receptor α chain (IL-15Rα) sushi domain fused to an IL-15. This fusion IL-15 protein is referred to herein as sushi15. The IL-15Rα sushi domain is fused to the C-terminus of the IL-15 polypeptide, either directly or indirectly via a linker.

[0074] In some case, the sushi domain of the IL-15Rα is fused to the IL-15 polypeptide indirectly via a linker. The peptide linker can include 1 or more amino acid residues, for example, from about 1 to about 30, from about 3 to about 20, from 3 to about 10, from about 4 to about 30, from about 4 to about 20, from about 4 to about 10, from about 5 to about 30, from about 5 to about 20, or from about 5 to about 10. The peptide linker can include 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or 30 amino acid residues.

[0075] The peptide linker can include a plurality of poly-alanines, poly-glycines, or a mixture of alanine and glycine residues. The peptide linker can include a $(Gly_4Ser)n$ linker, in which n is an integer from 1 to 30 (SEQ ID NO: 76), preferably 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30, further preferably 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30. In some instances, the peptide linker comprises SEQ ID NO: 32. In some instances, the peptide linker comprises SEQ ID NO: 33. In some instances, the peptide linker comprises SEQ ID NO: 30. In some instances, the peptide linker comprises SEQ ID NO: 41. In some instances, the peptide linker comprises SEQ ID NO: 45.

[0076] In some embodiments, an immune cell described herein expresses sushi15. In some embodiments, the sushi15 comprises a sequence comprising about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR*SGGGSGGGGSGGGGSGGGGSGGGSLQ*NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 10). In

C. mIL15/Ra-LSP

[0079] In some instances, the IL-15 comprises a membrane bound IL-15 fused to an IL-15 receptor α chain (IL-15Rα) , IL15 propeptide and long signal peptide of IL-15 (LSP) fusion protein. This fusion IL-15 protein is referred to herein as mIL15/Ra-LSP. The IL-15Rα polypeptide comprises a full-length IL-15Rα chain without the signal peptide. The IL-15Ra is fused to the C-terminus of the IL-15 polypeptide, either directly or indirectly via a linker. The IL15 propeptide is fused to the C-terminus of LSP, either directly or indirectly. IL15 propeptide is fused to the C-terminus of IL-15Rα polypeptide, either directly or indirectly. In some cases, the IL15 propeptide comprises GIHVFILGCFSAGLPKTEA (SEQ ID NO: 48). In some cases, the IL15 propetide consists of SEQ ID NO: 48. In some cases LSP comprises MRISKPHLRSISIQCYLCLLLNSHFLTEA (SEQ ID NO: 47). In some cases, the LSP consists of SEQ ID NO: 47.

[0080] In some case, the IL-15Ra is fused to the C-terminus of the IL-15 polypeptide indirectly via a linker. The peptide linker can include 1 or more amino acid residues, for example, from about 1 to about 30, from about 3 to about 20, from 3 to about 10, from about 4 to about 30, from about 4 to about 20, from about 4 to about 10, from about 5 to about 30, from about 5 to about 20, or from about 5 to about 10. The peptide linker can include 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or 30 amino acid residues.

[0081] The peptide linker can include a plurality of poly-serines, poly-glycines, or a mixture of serine and glycine residues. The peptide linker can include a $(Gly_4Ser)n$ linker, in which n is an integer from 1 to 30 (SEQ ID NO: 76), preferably 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30, further preferably 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30. In some instances, the peptide linker comprises SEQ ID NO: 32. In some instances, the peptide linker comprises SEQ ID NO: 33. In some instances, the peptide linker comprises SEQ ID NO: 30. In some instances, the peptide linker comprises SEQ ID NO: 41. In some instances, the peptide linker comprises SEQ ID NO: 45.

[0082] In some embodiments, an immune cell described herein expresses mIL15/Ra-LSP. In some embodiments, the mIL15/Ra-LSP comprises a sequence comprising about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to MRISKPHLRSISIQCYLCLLLNSHFLTEAGIHVFILGCFSAGLPKTEANWVNVISD

T2A. P2A is derived from porcine teschovirus-1 2A. E2A is derived from equine rhinitis A virus. F2A is derived from foot-and-mouth disease virus 18. T2A is derived from thosea asigna virus 2A. Exemplary sequences for 2A peptide members include:

[0108] P2A - ATNFSLLKQAGDVEENPGP (SEQ ID NO: 77);

[0109] E2A - QCTNYALLKLAGDVESNPGP (SEQ ID NO: 78);

[0110] F2A - VKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 79); and

[0111] T2A – EGRGSLLTCGDVEENPGP (SEQ ID NO: 80).

[0112] In some embodiments, a peptide linker is further added to the terminus of the 2A peptide, e.g., at the N-terminus. In some embodiments, the peptide linker comprises GSG. In some cases, the P2A comprises GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 5).

[0113] In some embodiments, the polynucleotide encoding the CAR, the polynucleotide encoding IL-15, and optionally the polynucleotide encoding CCL19 are each independently transcribed under a promoter comprising a polynucleotide encoding a 2A peptide. In some instances, the polynucleotide encoding IL-15 and the polynucleotide encoding CCL19 are each transcribed under a promoter comprising the same 2A peptide. In some instances, the polynucleotide encoding IL-15 and the polynucleotide encoding CCL19 are each transcribed under a promoter comprising two different 2A peptides. In some cases, the polynucleotide encoding the CAR, the polynucleotide encoding IL-15, and the polynucleotide encoding CCL19 are each independently transcribed under a promoter comprising different 2A peptides.

[0114] In some embodiments, the polynucleotide encoding the CAR, the polynucleotide encoding IL-15, and optionally the polynucleotide encoding CCL19 are each independently transcribed under a promoter comprising a polynucleotide encoding the P2A peptide. The P2A peptide can comprise ATNFSLLKQAGDVEENPGP (SEQ ID NO: 77). The P2A peptide can also comprise SEQ ID NO: 5.

[0115] In some embodiments, the polynucleotide encoding the CAR and the polynucleotide encoding IL-15 are arranged in the nucleic acid molecule from the 5’ terminus to the 3’ terminus as:

CTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCT
CAGGGCCAAGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCT
GTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAG
ATGCGGTCCAGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGT
GCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTT
CGCTTCTCGCTTCTGTTCGCGCGCTTCTGCTCCCCGAGCTCAATAAAAGAGC
CCACAACCCCTCACTCGGCGCGCCAGTCCTCCGATTGACTGAGTCGCCCGGG
TACCCGTGTATCCAATAAACCCTCTTGCAGTTGCATCCGACTTGTGGTCTCG
CTGTTCCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTCAGCGGGGGT
CTTTCA (SEQ ID NO: 81). Alternatively, tetracycline-responsive promoter which is induced by tetracycline, Mx1 promoter which is induced by interferon, or the like may be used. Use of the promoter which is induced by a particular substance in the expression vector of the present invention permits control of induction of IL-15 expression and optionally CCL19 expression according to the course of treatment of cancer, for example, when the immune cell containing the vector of the present invention is used as a pharmaceutical composition for use in the treatment of cancer.

[0182] Examples of the viral vector can include a retrovirus vector, a lentivirus vector, an adenovirus vector, and an adeno-associated virus vector and can preferably include a retrovirus vector, preferably a gamma retrovirus vector, more preferably a pMSGV vector (Tamada k et al., Clin Cancer Res 18: 6436-6445 (2002)), a pMSCV vector (manufactured by Takara Bio Inc.), or a pSFG vector. Use of a retrovirus vector permits long-term and stable expression of a transgene because the transgene is integrated in the genome of a host cell.

[0183] One or more assays can be used to confirm the containment of the expression vector of the present invention in the immune cell. Exemplary assays can include flow cytometry for screening the expression of CAR by the engineered immune cells, Northern blotting, Southern blotting, PCR such as RT-PCR, ELISA, or Western blotting. In some embodiments, the expression vector further comprises a marker gene (e.g., encoding a fluorescent protein such as green fluorescent protein (GFP), red fluorescent protein (RFP), or yellow fluorescent protein (YFP)) to detect the expression of the CAR, IL-15, and/or CCL19 by the immune cell.

[0302] ***1. anti-Mesothelin-CAR gene***

[0303] The nucleic acids comprising the gene encoding anti-Mesothelin-CAR were artificially synthesized. 11 patterns of artificial nucleic acids were synthesized for the transfection into iγδT cells or iγ9δ2T cells using the genes listed in Table 7.

Table 7

Abbreviation	order from N-terminal	gene
ssVH	1	lead sequence of immunoglobulin heavy chain
P4 scFv (P4)	2	variable region of anti-Mesothelin antibody (P4) heavy chain
	3	GILGSGGGGSGGGGSGGGGS linker (SEQ ID NO: 45)
	4	variable region of anti-Mesothelin antibody (P4) light chain
CD8hinge/TM	5	CD8-derived sequence (including transmembrane region)
CD28	6a (either)	intracellular domain region of CD28
41BB	6b (or)	intracellular domain region of 4-1BB
CD3z	7	intracellular domain region of CD3ζ
P2A	8	P2A
IL2sp or IL15Rasp	9 (with/without)	Signal peptide
mIL15/Ra, mIL15/Ra-LSP, sushi15 or sIL15-LSP	10	IL-15
P2A	11 (with/without)	P2A
endosp	12	Signal peptide
CCL19	13 (with/without)	CCL19

[0304] ***2. IL-15 genes***

[0305] The artificial nucleic acid comprised the gene encoding the each IL-15 version. The genes of IL-15 versions are shown in Table 8.

Table 8

Sequence List

Summary Table ("GILGSSG" is disclosed as SEQ ID NO: 82)

1	AA	P4 scFv (w/o signal peptide)	
2	AA	ssVH signal peptide (sp)	
3	AA	ssVHsp-P4 scFv	
4	AA	peptide linker (P4 scFv-CD8hinge)	
5	AA	P2A peptide with GSG linker	
6	AA	IL2sp	
7	AA	IL15Rasp	
8	AA	mIL15/Ra (w/o IL2sp)	
9	AA	mIL15/Ra-LSP	
10	AA	Sushi15 (w/o IL15Rasp)	
11	AA	sIL15-LSP	
PRP	AA	IL15 propeptide	
LSP	AA	IL15LSP	
12	AA	Endosp	
13	AA	CCL19	
14	AA	IL2sp-mIL15/Ra-P2A- endosp-CCL19	
15	AA	SEE BELOW	
16	AA	CDR1_VH	
17	AA	CDR2_VH	
18	AA	CDR3_VH	
19	AA	CDR1_VL	
20	AA	CDR2_VL	
21	AA	CDR3_VL	
22	AA	VH	
23	AA	VL	
24	AA	4-BB	
25	AA	CD3z(wt)	
26	AA	CD8 hinge	
27	AA	CD8 TM domain	
28	AA	IL2sp-mIL15/Ra	
29	AA	IL15/Rasp-sushi15	
30	AA	Peptide linker	15AAs
31	AA	IL15Rasp-sushi15/Ra-P2A- endosp-CCL19	
32	AA	Peptide linker (IL15-Ra/sushi-IL15)	26 AAs
33	AA	Peptide linker	20 AAs
34*	AA	ssVHsp-P4-BB-mIL15/Ra	DNA: SEQ ID NO: 37
53	AA	ssVHsp-P4-BB-mIL15/Ra-LSP	DNA: SEQ ID NO: 58
35*	AA	ssVHsp-P4-BB-sushi15	DNA: SEQ ID NO: 38