mRNA Tumor Vaccine Encoding Membrane-Bound IL-12 Cytokine Adjuvant and Use Thereof

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority to Chinese Application No. 202410245327.9, filed on March 4, 2024, the entire contents of which are incorporated herein by reference.

TECHNICAL FIELD

The present disclosure relates to the field of biomedicine, more particularly, to an mRNA tumor vaccine encoding a membrane-tethered IL-12 cytokine adjuvant and uses thereof.

BACKGROUND ART

Cancer poses a major threat to human health. Abnormal cancer cell proliferation or differentiation leads to functional abnormalities, uncontrolled cell growth, local tissue invasion, and metastasis. As a result of genetic mutations, cancer cells express tumor-specific neoantigens that can be recognized and eliminated by immune T lymphocytes, providing a basis for tumor immunotherapy.

Insufficiency of cytotoxic T lymphocytes (CTLs) targeting immunogenic tumors represents a major challenge in immunotherapy (Kalbasi and Ribas, 2020). Conventional vaccines, such as alum-adjuvanted protein vaccines, generally fail to elicit robust CTL responses against viral infections or tumors. More potent adjuvants can enhance CTL responses, but are often associated with severe toxicity (Awate et al., 2013; Coffman et al., 2010). Both antigen production and adjuvant selection are critical to the success of a vaccine. The mRNA vaccine platform developed in recent years provides a promising strategy for generating more potent antigens and effective adjuvants for prevention of viral infections and treatment of cancer (Barbier et al., 2022; Beck et al., 2021; Chaudhary et al., 2021). To increase antigen expression, most clinical mRNA vaccines use methylated bases to prevent innate immune sensing and reduce toxicity, although this approach may also limit the adjuvant effect of the mRNA (Andries et al., 2015; Kariko et al., 2005). Lipid nanoparticles (LNPs) used for mRNA delivery can function as built-in adjuvants inducing the production of proinflammatory cytokines such as IL-1β and IL-6, thereby promoting antigen-specific CD4+

T follicular helper (Tfh) cell and B-cell responses (Alameh et al., 2021; Tahtinen et al., 2022). However, cytokines such as IL-6 may inhibit the differentiation of naive T cells into effector cells, which are essential for antitumor immune responses (Huseni et al., 2023). Selecting an appropriate adjuvant for an mRNA cancer vaccine is therefore critical to ensuring both efficacy and safety. Creating a Th1-type immune microenvironment dominated by IL-12 and TNF-α is important for inducing tumor-specific cytotoxic T cells. However, IL-12 itself is highly toxic. Direct co-injection of an RNA vaccine with IL-12 as an adjuvant, or expression of soluble IL-12 from mRNA, causes severe treatment-related adverse effects. Accordingly, creating an immune microenvironment that favors cytotoxic T-cell differentiation for mRNA vaccine-mediated T-cell activation, while reducing adverse effects caused by immune processes, is essential for improving the efficacy of mRNA tumor vaccines.

Recent studies have shown that intramuscularly administered mRNA-LNPs primarily drain to the draining lymph nodes and spleen, where they are taken up by macrophages and dendritic cells, resulting in expression of protein (Kimberly, J.H. et al., Mol. Ther. Nucleic Acids, November 24, 2023, 35(1):102083). The draining lymph nodes and spleen are important organs where tumor vaccines trigger adaptive immune responses. Therefore, specifically delivering IL-12 to the draining lymph nodes and spleen, without releasing IL-12 to peripheral tissues, may enhance the immunization efficacy of tumor mRNA vaccines and also reduce peripheral toxicity caused by IL-12.

SUMMARY OF THE INVENTION

In view of the foregoing, an objective of the present disclosure is to provide an mRNA tumor vaccine encoding a membrane-tethered IL-12 cytokine adjuvant. According to the present disclosure, by RNA-based technologies, IL-12 and a tumor antigen are co-expressed on antigen-presenting cells in peripheral lymphoid tissues, and the membrane-tethered IL-12 serves as an adjuvant to improve the therapeutic efficacy of a conventional mRNA tumor vaccine. Compared with direct expression of soluble IL-12 protein, the cell membrane-tethered IL-12 is anchored to the surface of antigen-presenting cells in peripheral lymphoid tissues, thereby preventing release of IL-12 to peripheral tissues and the resulting peripheral toxicity, without compromising its adjuvant effect.

To achieve the foregoing objective, the present disclosure provides the following

technical solutions.

In one aspect, the present disclosure provides an mRNA tumor vaccine encoding a membrane-tethered IL-12 cytokine adjuvant, wherein the mRNA comprises three components, namely an IL-12-encoding sequence (IL12), a membrane-domain-encoding sequence (MD), and a tumor-antigen-encoding sequence (Ag).

In certain embodiments of the present disclosure, the Il-12-encoding sequence (IL12) and the membrane-domain-encoding sequence (MD) are located on the same mRNA, and the tumor-antigen-encoding sequence (Ag) is located on another mRNA; or the IL-12-encoding sequence (IL12), the membrane-domain-encoding sequence (MD), and the tumor-antigen-encoding sequence (Ag) are all on the same mRNA, arranged in the order of IL12-L1-MD-L2-Ag or Ag-L2-IL12-L1-MD; wherein L1 is a glycine/serine (Gly/Ser)-based polypeptide linker, and L2 is an IRES sequence, a glycine/serine polypeptide-based linker, or a 2A polypeptide linker, preferably, a 2A polypeptide linker.

In certain embodiments of the present disclosure, the tumor antigen comprises a mutation-derived tumor neoantigen, a virus-derived tumor-specific antigen, or a tumor-associated antigen that is highly expressed in tumors.

In certain embodiments of the present disclosure, the mutation-derived tumor neoantigen comprises KRAS G12C, KRAS G12D, EGFRvIII, or $BRAF^{V600E}$; the virus-derived tumor-specific antigen comprises HPV E6/E7, the endogenous retroviral antigen hERT, LMP1, or LMP2; and the tumor-associated antigen highly expressed in tumors comprises WT1, MAGE-A3, GP100, NY-ESO-1, HER2/Neu, Claudin18.2, Mesothelin, or MUC.

In certain embodiments of the present disclosure, the sequence encoding the IL-12 cytokine comprises ORFs encoding an IL-12B polypeptide and an IL-12A polypeptide, wherein the IL-12B and IL-12A are linked via a peptide linker; the linker comprises a Gly/Ser-based linker containing $(GnS)_m$, wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20, and m is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20; preferably, the linker is a $(G4S)_3$ linker.

In certain embodiments of the present disclosure, the membrane domain comprises a transmembrane domain, and preferably comprises a transmembrane domain and an intracellular domain. The transmembrane domain may comprise a CD80 transmembrane domain, a CD86 transmembrane domain, a CD4 transmembrane domain, a CD8 transmembrane domain, a VEGFR transmembrane domain, or a PDGF-RB transmembrane

domain, and preferably comprises a CD80 transmembrane domain. The intracellular domain may comprise a CD80 intracellular domain, a CD86 intracellular domain, a CD4 intracellular domain, a CD8 intracellular domain, a VEGFR intracellular domain, a PDGF-RB intracellular domain, or a truncated PDGF-RB intracellular domain. The transmembrane domain and the intracellular domain may be derived from the same gene or from different genes, and are preferably the combination of a CD80 transmembrane domain and a CD80 intracellular domain.

In certain embodiments of the present disclosure, the mRNA tumor vaccine further comprises a nucleic acid carrier loaded with the mRNA. The nucleic acid carrier may comprise a liposome, LPP, or LPX, preferably an LNP (lipid nanoparticle).

In another aspect, the present disclosure provides a pharmaceutical composition comprising the mRNA tumor vaccine according to the present disclosure and a second anticancer agent, wherein the second anticancer agent comprises an immune checkpoint inhibitor and/or a bispecific T-cell engager (BiTE). Preferably, the pharmaceutical composition further comprises a pharmaceutically acceptable excipient.

In a further aspect, the present disclosure provides use of the mRNA tumor vaccine or pharmaceutical composition according to the present disclosure in the manufacture of a medicament for the treatment or prevention of a tumor. In another aspect, the present disclosure provides use of the mRNA tumor vaccine or pharmaceutical composition according to the present disclosure for the treatment or prevention of a tumor. In yet another aspect, the present disclosure provides a method for treating or preventing a tumor in a subject, comprising administering the mRNA tumor vaccine or pharmaceutical composition according to the present disclosure to the subject.

In certain embodiments of the present disclosure, the tumor is selected from the group consisting of B-cell lymphoma, bronchial carcinoma, prostate cancer, bladder cancer, cancer of the brain or central nervous system, cancer of the peripheral nervous system, esophageal cancer, head and neck cancer, cervical cancer, uterine or endometrial cancer, oral cancer, laryngeal cancer, salivary gland cancer, thymic cancer, adrenal cancer, osteosarcoma, chondrosarcoma, liposarcoma, testicular cancer, malignant fibrous histiocytoma, colorectal cancer, melanoma, gastric cancer, pancreatic cancer, lung cancer, liver cancer, kidney cancer, cholangiocarcinoma, small-intestinal or appendiceal cancer, squamous cell carcinoma, breast

cancer, and ovarian cancer.

BENEFICIAL EFFECTS

According to the present disclosure, we incorporated a sequence encoding a cell membrane-tethered IL-12 (mtIL12) into an mRNA, which successfully improved the therapeutic efficacy of existing mRNA tumor vaccines. Compared with direct expression of soluble IL-12, cell membrane-tethered IL-12 restricts IL-12 to the surface of antigen-presenting cells in peripheral lymphoid tissues. This configuration preserves the adjuvant effect of IL-12 while greatly reducing its release into the peripheral blood, thereby substantially reducing systemic toxicity caused by IL-12. Incorporation of the membrane-tethered IL-12 sequence significantly improved the therapeutic efficacy of various types of mRNA tumor vaccines. We therefore believe that mRNA tumor vaccines based on the membrane-tethered IL-12 have the potential to become a next-generation therapeutic cancer vaccine providing an improved therapeutic outcome across a broad range of clinical tumor types.

BRIEF DESCRIPTION OF THE DRAWINGS

To provide a better understanding of the technical solutions of embodiments of the present application, the drawings used in the embodiments are described below.

Figure 1 shows that IL-12 can serve as an adjuvant to enhance the antitumor efficacy of an mRNA vaccine. (A-E) C57BL/6J mice (n = 4-6) were inoculated subcutaneously with 4×10^5 B16F10-OVA cells, and on days 8 and 12 after tumor inoculation, were administered 5 μg of OVA-encoding mRNA by intramuscular injection. Six hours after vaccination, 2.5 μg of IL2-Fc (C), 5 μg of IL15-Fc (D), or 0.25 μg of IL12-Fc protein (E), mixed with sodium alginate, was injected intramuscularly at the same site. (B) Peripheral blood was collected on day 9 after the first vaccination, and the number of OVA-specific T cells was determined by flow cytometry. (C-E) Tumor growth curves for each treatment. Data are presented as mean ± standard error (SEM) from two independent experiments. P values were determined by two-way analysis of variance followed by Bonferroni's multiple-comparison test (C-E). ** $P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

Figure 2 shows the construction of RNA tumor vaccines containing soluble IL12 or membrane-tethered IL12 as an adjuvant. (A) Schematic representation of structures of the

soluble sIL12-OVA and membrane-tethered mtIL12-OVA mRNA vaccines. (B) DC2.4 cells were transfected with CTRL mRNA (top panel), mtEGFP-OVA RNA (middle panel), or mtIL12-OVA mRNA (bottom panel). 24 hours after transfection, cells were collected and stained with antibodies against IL12p40 and the OVA peptide-MHC complex, and the expression of membrane-tethered IL12 or OVA-pMHC complexes was analyzed by flow cytometry. (C) DC2.4 cells were transfected with mtIL12-OVA mRNA or sIL12-OVA mRNA. 24 hours after transfection, cultures were collected to measure the concentration of secreted soluble IL12.

Figure 3 shows that an RNA tumor vaccine containing a membrane-tethered IL12 adjuvant had antitumor efficacy comparable to that of a vaccine containing a soluble IL12 adjuvant, but showed lower peripheral toxicity. (A, B) C57BL/6J mice (n = 6) were inoculated with 4×10^5 B16F10-OVA cells, and on days 8 and 12 after inoculation, were administered 2.5 μg of an mRNA-LNP vaccine encoding mtEGFP-OVA, mtIL12-OVA, or sIL12-OVA by intramuscular injection. Tumor growth and mouse survival (A) and changes in body weight (B) were recorded. (C) C57BL/6J mice (n = 4) were administered 2.5 μg of mRNA encoding mtIL12-OVA or sIL12-OVA by intramuscular injection. Serum samples were collected 24 and 48 hours after injection, and proinflammatory cytokine concentrations were measured.

Figure 4 shows that the mtIL12 mRNA vaccine specifically activated antigen-specific T cells without affecting bystander T cells or NK cells. (A) C57/B6J mice (n=4) were administered 2.5 μg of mRNA encoding mtIL12-OVA or sIL12-OVA by intramuscular injection. 24 hours after vaccination, the draining lymph nodes (dLNs) were dissected, and the cell subset expressing surface IL12p40 was identified by flow cytometry. (B) C57BL/6J mice (n = 4-5) were administered 5×10^5 OT1 T cells by adoptive transfer, and one day later were vaccinated with 5 μg of an mRNA vaccine encoding mtEGFP-OVA, mtIL12-OVA, or sIL12-OVA. 42 hours after immunization, 50 μL BFA was administered by intraperitoneal injection. 48 hours after immunization, the draining lymph nodes (dLN) were dissected and digested, and IFN-γ-positive cells were detected by flow cytometry. (C) C57BL/6J mice were administered 5 μg of mtIL12 mRNA or 5 μg of sIL12 mRNA by intramuscular injection. After 36 hours, peripheral blood was collected and blocked with a BFA solution for 6 hours, after which the proportion of IFNγ-positive cell subset was analyzed by flow cytometry.

Figure 5 shows that the mtIL12-OVA mRNA vaccine exhibited greater antitumor

efficacy than either OVA mRNA alone or mtIL12 mRNA alone. C57BL/6J mice (n=6) were inoculated with 4×10^5 B16F10-OVA cells. On days 8 and 12, the tumor-bearing mice were administered 2.5 μg of mRNA encoding mtEGFP-OVA, mtIL12-OVA, or mtIL12 by intramuscular injection, and tumor growth was recorded.

Figure 6 shows that the antitumor efficacy of the RNA tumor vaccine containing a membrane-tethered IL12 adjuvant depended on $CD8^+$ T cells. C57BL/6J mice (n=6) were inoculated with 4×10^5 B16F10-OVA cells, and on days 8 and 12 after inoculation, were treated with 2.5 μg mRNA encoding mtIL12-OVA by intramuscular injection. Anti-NK1.1 (200 μg), anti-CD8 (200 μg), anti-CD4 (200 μg), and anti-CSF1R (500 μg) antibodies were administered 1 day before the treatment began, and then twice weekly for the following 2 weeks. The tumor growth curve (A) and survival curve (B) were recorded. (C) C57BL/6J mice were administered 2.5 μg of mtEGFP-OVA mRNA or 2.5 μg of mtIL12-OVA mRNA by intramuscular injection on days 0 and 5. On day 8 after the first vaccination, OVA-specific T cells were measured in the draining lymph nodes, and on day 13 after the first vaccination, OVA-specific T cells were measured in the spleen.

Figure 7 shows that the mtIL12-based vaccine generated more potent effector T cells in an OVA antigen model. (A) B6 mice were immunized by intramuscular injection of 2.5 μg of mtEGFP-OVA or mtIL12-OVA RNA on days 0 and 7, and blood was collected on day 14 for flow cytometric analysis. (B) Flow cytometry showed that IL12-mediated immunization promoted the differentiation of OVA-specific T cells from a progenitor phenotype (Ly108+ CD69-) into an effector phenotype (Ly108- CD69-). (C) Peripheral blood cells were co-incubated with OT1 peptide (SIINFEKL) for 3 hours and then stained and analyzed by flow cytometry. OVA-specific T cells from mice immunized with the IL12-adjuvanted vaccine expressed higher levels of effector molecules GZMB and IFN-γ and the effector T-cell-specific transcription factor T-bet, but a lower level of the immunoinhibitory molecule PD-1. (D) Equal numbers of OVA-specific T cells were sorted from mice immunized with mtEGFP-OVA or mtIL12-OVA, and co-incubated with different concentrations of OT1 peptide (SIINFEKL).The IFN-γ concentration in the culture supernatant was measured after 4 hours.

Figure 8 shows that cis-delivery of IL12 and an antigen generated more antigen-specific T cells. (A) B6 mice were administered 2 μg of mtEGFP-OVA, 2 μg of mtEGFP-OVA+1 μg

mtIL12, or 1 μg of mtEGFP-OVA+1 μg mtIL12-OVA RNA-LNP by intravenous injection on days 0 and 7. Blood samples were collected on day 14 for flow cytometric analysis. (B) Flow cytometry showed that mice treated with the vaccine providing cis-delivery of IL12 and antigen (1 μg of mtEGFP-OVA + 1 μg of mtIL12-OVA) generated more antigen-specific T cells in the peripheral blood.

Figure 9 shows that the membrane-tethered IL12-adjuvanted mRNA vaccine overcame the resistance to immune checkpoint blockade. (A) C57BL/6J mice (n=6) were subcutaneously inoculated with 4×10^5 B16F10-OVA cells, and on days 11 and 15 after inoculation, were treated with 2.5 μg of mRNA encoding mtIL12-OVA. Anti-CTLA4 (200 μg) and anti-PD1 (200 μg) were administered on day 6 after the first vaccination, and twice weekly for 2 weeks. Tumor growth and mouse survival were recorded.

Figure 10 shows that the mtIL12-E7 vaccine inhibited the growth of HPV-associated TC-1 tumors. C57BL/6J mice (n=6) were inoculated with 4×10^5 TC-1 cells. On day 14, tumor-bearing mice were administered 1 μg of an mRNA vaccine encoding mtEGFP-E7, mtIL12-E7, or mtIL12 by intramuscular injection, and on day 20 were boosted with 2 μg of the same mRNA vaccine. Tumor growth and mouse survival (A) and changes in body weight (B) were recorded. In mice bearing advanced TC-1 tumors, neither the control E7 vaccine nor mtIL12 monotherapy controlled tumor growth. Although the TC-1 tumors continued to grow to approximately 500 mm^3 after two mtIL12-E7 vaccinations, they eventually regressed, resulting in an approximately 80% tumor eradication rate (A). Importantly, the mice treated with the mtIL12-E7 mRNA vaccine did not exhibit significant loss of body weight compared with untreated mice (B).

Figure 11 shows that the mtIL12-based vaccine generated more potent effector T cells in an E7 antigen model. (A) On day 12 after tumor implantation, TC-1 tumor-bearing mice were administered 2.5 μg of mtEGFP-E7 by intramuscular injection. On day 18, the mice were administered 2.5 μg of mtEGFP-E7 or 2.5 μg of mtIL12-E7 by intramuscular injection for immunization. Tumors were collected on day 22 for flow cytometric analysis. (B) Flow cytometric analysis shows the gating of antigen-specific T cells and the expression of selected surface markers. (C) Flow cytometric analysis shows that IL12-mediated immunization promoted the intratumoral enrichment of E7-specific T cells. Compared with T cells generated by mtGFP-E7 immunization, T cells generated by mtIL12-E7 immunization remained to a

greater extent in the effector state (LY108-CD69-) rather than in the exhausted state (LY108-CD69+), and expressed a higher level of the effector molecule GZMB and a lower level of the inhibitory marker PD1.

Figure 12 shows that the mtIL12 adjuvant can be incorporated into an mRNA vaccine directed against a tumor neoantigen derived from an endogenous retrovirus. The P15E protein is expressed from the gp70 retroviral gene and has been detected in many cancers, including the MC38 colon adenocarcinoma cell line (Ye et al., 2020). On day 0, C57BL/6J mice were inoculated subcutaneously with 4×10^5 MC38 tumor cells. After tumor establishment, the mice were administered 2.5 μg of mtIL12-p15E or the control mtEGFP-p15E mRNA-LNP on days 12 and 16 by intramuscular injection, and tumor growth was monitored twice weekly. As shown in Figure 7, the control P15E mRNA vaccine had little inhibitory effect on tumor growth, whereas the mtIL12-P15E treatment induced marked tumor regression and complete tumor eradication (Fig. 7).

Figure 13 shows that the mtIL12-based vaccine generated more potent effector T cells in a p15E antigen model. (A) MC38 tumor-bearing mice were administered 2.5 μg of mtEGFP-p15E mRNA by intramuscular injection on day 12 after tumor inoculation, and were immunized again with 2.5 μg of mtEGFP-p15E mRNA or 2.5 μg of mtIL12-p15E mRNA by intramuscular injection on day 18. Peripheral blood was collected on day 21 for flow cytometric analysis. (B) Flow cytometric analysis showed that mtIL12-p15E immunization generated more p15E antigen-specific T cells. Compared with the T cells generated by mtEGFP-p15E immunization, the T cells generated by mtIL12-p15E immunization expressed higher levels of effector molecules GZMB and IFN-γ and a lower level of the inhibitory marker PD1.

DESCRIPTION OF EMBODIMENTS

The present disclosure provides an mRNA tumor vaccine encoding a membrane-tethered IL-12 cytokine adjuvant, wherein the mRNA comprises three components: an IL-12-encoding sequence (IL12), a membrane-domain-encoding sequence (MD), and a tumor-antigen-encoding sequence (Ag).

In certain embodiments of the present disclosure, the Il-12-encoding sequence (IL12) and the membrane-domain-encoding sequence (MD) are located on the same mRNA, and the

tumor-antigen-encoding sequence (Ag) is located on another mRNA; or the IL-12-encoding sequence (IL12), the membrane-domain-encoding sequence (MD), and the tumor-antigen-encoding sequence (Ag) are on the same mRNA, arranged in the order of IL12-L1-MD-L2-Ag or Ag-L2-IL12-L1-MD; wherein L1 is a Glycine/Serine-based polypeptide linker, and L2 is an IRES sequence, a Glycine/Serine-based polypeptide linker, or a 2A polypeptide linker.

Preferably, the IL-12-encoding sequence (IL12), the membrane-domain-encoding sequence (MD), and the tumor-antigen-encoding sequence (Ag) are located on the same mRNA. This is because a single mRNA can ensure simultaneous expression of the IL12, MD, and Ag in the same cell, thereby avoiding the possibility that some cells express only IL-12-MD or Ag due to a difference in transfection efficiency between two separate mRNAs. This design enables more precise concomitant expression of the three components, ensures their coordination at both expression levels and timing, and facilitates their synergistic effects. It also simplifies the manufacturing process and reduces production cost. The cost of synthesis, purification, and quality control of a single mRNA are substantially lower than those of two separate mRNAs produced in parallel. In practical applications such as gene therapy, delivery of only one type of mRNA into cells is simpler in operation and has higher delivery efficiency than delivery of two types of mRNAs, and reduces issues such as a limited loading capacity and difference in cellular uptake associated with delivery of multiple mRNAs, thereby improving the feasibility and practical operability of the treatment. A single-molecule construct also provides greater stability, for example, by avoiding differences in the degradation rate between two separate mRNAs, and improves batch-to-batch consistency.

The tumor antigen described herein may include a mutation-derived tumor neoantigen, such as KRAS G12C, KRAS G12D, EGFRvIII, and $BRAF^{V600E}$; the a virus-derived tumor-specific antigen, such as HPV E6/E7, the endogenous retroviral antigen hERT, LMP1, and LMP2; and a tumor-associated antigen highly expressed in tumors, such as WT1, MAGE-A3, GP100, NY-ESO-1, HER2/Neu, Claudin18.2, mesothelin, and MUC. The antigen may be selected according to the tumor-specific antigen. Ovalbumin (OVA) is a protein antigen widely used to elicit cellular and humoral immune responses in cancer immunotherapy, and is commonly used as a model tumor antigen in the art. In examples of

the present disclosure, OVA is used as an exemplary tumor-specific antigen.

Interleukin-12 (IL-12) is a pleiotropic cytokine encoded by two separate genes, IL-12A (p35) and IL-12B (p40), and exists in the form of an active heterodimer (p70) or a p40 homodimer (p80). In the present disclosure, IL-12B and IL-12A are linked via a peptide linker. The linker comprises a Gly/Ser-based linker, including (GnS)m, wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20, and m is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20. In examples of the present disclosure, the two subunits of IL-12 are linked via a $(G4S)_3$ linker.

The membrane domain of the present disclosure may comprise a transmembrane domain and an intracellular domain. The transmembrane domain comprises a CD80 transmembrane domain, a CD86 transmembrane domain, a CD4 transmembrane domain, a CD8 transmembrane domain, a VEGFR transmembrane domain, or a PDGF-RB transmembrane domain, preferably a CD80 transmembrane domain; the intracellular domain comprises a CD80 intracellular domain, a CD86 intracellular domain, a CD4 intracellular domain, a CD8 intracellular domain, a VEGFR intracellular domain, a PDGF-RB intracellular domain, or a truncated PDGF-RB intracellular domain. The intracellular domain and the transmembrane domain may be derived from the same gene or from different genes. For example, the following transmembrane domain and intracellular domain sequences may be used in combination, although the present disclosure is not limited thereto:

1. Human CD8A transmembrane domain, IYIWAPLAGTCGVLLLSLVITLYCY (SEQ ID NO: 85)

2. Human PDGF-RB transmembrane domain, VVISAILALVVLTIISLIILI (SEQ ID NO: 3)

3. Human CD80 transmembrane domain, LLPSWAITLISVNGIFVICCL (SEQ ID NO: 5)

4. Human CD86 transmembrane domain, WITAVLPTVIICVMVFCLILW (SEQ ID NO: 7)

5. Murine CD80 transmembrane domain, TLVLFGAGAVITVVVIVVII (SEQ ID NO: 9)

6. Human CD8A intracellular domain (ICD), LYCNHRNRRRVCKCPRPVVKSGDKPSLSARYV (SEQ ID NO: 11)

7. Human PDGF-RB intracellular domain (ICD), QKKPRYEIRWKVIESVSSDGHEYIYVDPMQLPYDSTWELPRDQLVLGRTLGSGAF

GQWEATAHGLSHSQATMKVAVKMLKSTARSSEKQALMSELKIMSHLGPHLNWNLLG
ACTKGGPIYIITEYCRYGDLVDYLHRNKHTFLQHHSDKRRPPSAELYSNALPVGLPLPS
HVSLTGESDGGYMDMSKDESVDYVPMLDMKGDVKYADIESSNYMAPYDNYVPSAP
ERTCRATLINESPVLSYMDLVGFSYQVANGMEFLASKNCVHRDLAARNVLICEGKLV
KICDFGLARDIMRDSNYISKGSTFLPLKWMAPESIFNSLYTTLSDVWSFGILLWEIFTLG
GTPYPELPMNEQFYNAIKRGYRMAQPAHASDEIYEIMQKCWEEKFEIRPPFSQLVLLL
ERLLGEGYKKKYQQVDEEFLRSDHPAILRSQARLPGFHGLRSPLDTSSVLYTAVQPNE
GDNDYIIPLPDPKPEVADEGPLEGSPSLASSTLNEVNTSSTISCDSPLEPQDEPEPEPQLE
LQVEPEPELEQLPDSGCPAPRAEAEDSFL (SEQ ID NO: 13)

8. Truncated human PDGF-RB intracellular domain (ICD)

QKKPRYEIRWKVIESVSSDGHE (SEQ ID NO: 15)

9. Human CD80 intracellular domain (ICD)

TYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO: 17)

10. Human CD86 intracellular domain (ICD)

KWKKKKRPRNSYKCGTNTMEREESEQTKKREKIHIPERSDEAQRVFKSSKTSSC
DKSDTCF (SEQ ID NO: 19)

11. Murine CD80 intracellular domain (ICD)

KCFCKHRSCFRRNEASRETNNSLTFGPEEALAEQTVFL (SEQ ID NO: 21)

The present disclosure is described in further detail below with reference to specific examples. These examples are provided for illustrative purposes only and are not intended to limit the present disclosure. Unless otherwise specified, the experimental methods used in the following examples were carried out under conventional conditions. Unless otherwise specified, the materials, reagents, and other products used in the examples were commercially available.

EXAMPLES

We investigated whether T-cell-stimulatory cytokines can rapidly expand antigen-primed CTLs and effectively enhance antitumor immune responses. Although IL-2 is essential for T-cell activation and IL-15 efficiently expands T-cell populations (Guo et al., 2021; Zou et al., 2024), we found that these cytokines provided only limited enhancement of antitumor efficacy of mRNA vaccines. IL-12 can induce a Th1-type immune microenvironment and promote the differentiation into tumoricidal CD8+ T cells (Hewitt et al., 2020; Tucker et al.,

2020; Tugues et al., 2015; Zou et al., 2024). Notably, we have found that IL-12 is the most effective cytokine adjuvant in enhancing antitumor efficacy of an mRNA tumor vaccine. To optimize the stimulatory effect of IL-12 while reducing its peripheral toxicity, we designed an mRNA vaccine that co-expresses a tumor antigen and a membrane-tethered IL-12 as an adjuvant. Our findings show that the IL-12 induces and expands an understudied pre-effector T-cell subset. We further investigated how mtIL-12 elicits a potent antitumor response while minimizing toxicity.

MATERIALS AND METHODS

Mice and Cell Lines:

C57BL/6J, BALB/c, and C57BL/6J-Tg(TcraTcrb)1100Mjb/J (OT1 TCR-transgenic) mice, 6-8 weeks old, were purchased from Vital River Laboratory Animal Technology or the Jackson Laboratory. Mice were housed under specific pathogen-free (SPF) conditions. All animal experiments were conducted in accordance with the Rules for the Administration of Laboratory Animals of Tsinghua University.

MC38, B16F10, TC-1, and DC2.4 cell lines were purchased from the American Type Culture Collection (ATCC). Freestyle 293-F cells (R79007) were purchased from Invitrogen. The B16F10-OVA cell line was generated by lentiviral expression of OVA, followed by single-cell cloning and selection. All cell lines were routinely tested for mycoplasma contamination. MC38, B16F10, and TC-1 cells were cultured at 37°C with 5% CO_2 in Dulbecco's modified Eagle medium supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, and 100 U/mL streptomycin. DC2.4 cells were cultured at 37°C with 5% CO_2 in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mmol/L L-glutamine, 0.1 mmol/L MEM nonessential amino acids, 100 U/mL penicillin, and 100 U/mL streptomycin. 293-F cells were cultured in SMM 293-TI medium (M293TI; Sino Biological).

Reagents

Anti-CD4 (clone GK1.5), anti-CD8 (clone 53-5.8), anti-mCSF1R (clone AFS98), anti-NK1.1 (clone PK136), anti-PD-L1 (clone 10F.9G2), anti-mCTLA4 (clone 4F10), and anti-mIL12p75 (clone JR2-9A5) antibodies were purchased from Bio X Cell. IL2-Fc, IL15-Fc, and IL12-Fc were produced in-house. Specifically, the method was as follows: each cytokine-encoding sequence was linked to a human IgG1 Fc fragment through a GGGGS

linker gene and cloned into a pEE12.4 vector; plasmids encoding the protein were transfected into 293F cells, and the culture supernatants were purified by Protein A affinity chromatography (GE Healthcare) according to established protocols.

Establishment and Treatment of Murine Tumor Models

MC38 cells (4×10^5), TC-1 cells (3×10^5), or B16F10-OVA cells ($2\text{-}5 \times 10^5$) were subcutaneously inoculated into the right dorsal flank of mice. After tumor establishment, tumor length (a) and width (b) were measured three times per week, and tumor volume was calculated as a*b*b/2. Mice were randomized into groups based on tumor size. At specified time points, mice were injected with 1-5 μg of mRNA-LNP complexes containing different coding regions or with empty LNP controls. For immune-cell depletion experiments, 200 μg of anti-CD8, 200 μg of anti-CD4, 200 μg of anti-NK1.1, or 500 μg of anti-CSFIR antibody was intraperitoneally injected every 3 days, starting 1 day before mRNA vaccination. 6 h after mRNA vaccination, 2 μg of IL2-Fc, 3.5 μg of IL15-Fc, or 0.25 μg of IL12-Fc, mixed with sodium alginate, was intramuscularly injected. Beginning on day 6 after the first mRNA vaccination, 200 μg of anti-PD-L1 and 200 μg of anti-CTLA4 antibodies were intraperitoneally injected every 3 days.

Mice were euthanized when the length, width, or height of the tumor exceeded 2 cm, or when the tumor volume exceeded 1,500 mm^3, or when the tumor-bearing mice experienced a body weight loss of more than 20%.

Flow Cytometry

After digestion and centrifugation, cells were re-suspended to obtain cell suspensions, and incubated on ice for 15 min with anti-FcgRIII/II antibody (clone 2.4G2) to block nonspecific binding. The corresponding fluorophore-conjugated antibodies were then added, and the cells were stained for 30 min at 4°C in the dark. Fixable viability Dye eFluorTM 506 or Dye eFluorTM 780 straining was used to exclude dead cells. After staining, the cells were washed with PBS to remove unbound antibodies. Flow cytometry was performed, and the data were analyzed using FlowJo software (Tree Star).

Cytometric Bead Array (CBA) and ELISA Analysis of Serum and Tissue Samples

Cytokine levels in mouse serum and tumor homogenates were measured using the CBA Mouse Th1/Th2/Th17 Kit (BD Biosciences) according to the manufacturer's instructions. For ELISA, a 96-well microplate (Corning Costar) was coated overnight at 4°C with capture

antibody at 2 μg/mL (100 μL/well). The plate was washed with PBS and blocked with a blocking buffer (PBS containing 0.05% TWEEN-20 and 5% nonfat milk). Serum or tumor homogenate diluted in the blocking buffer was added and incubated at 37°C for 1.5 hours. After washing with PBST, alkaline phosphatase-conjugated goat anti-human IgG secondary antibody was added and incubated at 37°C for 50 min. After another wash with PBST, 100 μL of p-nitrophenyl phosphate substrate was added for color development, and then absorbance at 405 nm was measured using a SPECTROstar Nano plate reader (BMG LABTECH).

In Vitro T-Cell Culture and Adoptive Cell Transfer

To generate activated OT-I cytotoxic T lymphocytes (CTLs) *in vitro*, mouse splenocytes at 10^8 cells/mL were stimulated with 1 mM SIINFEKL peptide (New England Peptide). After a 2-hour peptide pulse, the spleen cells were washed to remove the peptide and cultured at 10^6 cells/mL in a T-cell culture medium supplemented daily with either 5 ng/mL mouse recombinant IL-2 (mrIL-2) + 10 ng/mL mrIL-12 (R&D), or 5 ng/mL mrIL-2 alone. On day 4, 2×10^6 viable OT-1 cells isolated by Ficoll were adoptively transferred into B16-OVA tumor-bearing mice via intravenous injection.

For *in vivo* cell-transfer experiments, 10^5 naive OT1 cells were transferred into naive mice. 24 hours after cell transfer, the mice were immunized with 10 μg of the mtEGFP-OVA or mtIL12-OVA mRNA vaccine. On day 7 after immunization, activated OT-1 cells were isolated from the spleens and purified, and 5×10^5 OT-1 cells were transferred into B16-OVA tumor-bearing mice via intravenous injection.

mRNA Production

DNA plasmids containing the full-length mRNA sequence and a poly(A) sequence were linearized by restriction digestion to prepare templates for *in vitro* transcription. The templates were transcribed and co-transcriptionally capped using the HiSynth® T7 Co-transcription RNA Synthesis Kit (C3111). Double-stranded RNA was removed from the *in vitro* transcribed mRNA by cellulose purification, and the mRNA was stored at -80°C until use.

mRNA-LNP Encapsulation

The mRNA was dissolved in 100 mM sodium citrate buffer at pH 4.0. DSPC, cholesterol, DMG-PEG2000, and SM102 were dissolved in anhydrous ethanol at a molar ratio of 38.5:10:1.5:50. The LNP-to-RNA mass ratio was adjusted to 30:1-50:1. The RNA dissolved in the sodium citrate solution and the lipid mixture dissolved in anhydrous ethanol were then

combined at a 3:1 volume ratio using a microfluidic system to produce mRNA-LNPs. The mRNA-LNP solution was dialyzed against PBS, after which the resulting mRNA-LNPs were suitable for *in vitro* and *in vivo* animal experiments.

Data Analysis

Before treatment, tumor-bearing mice were randomized into groups based on tumor volume. Data were analyzed using GraphPad Prism and are presented as the mean ± SEM. P values for tumor growth curves were calculated using two-way ANOVA, P values for mouse survival curves were calculated using the log-rank test, and all other data were analyzed using unpaired two-tailed t-tests. $P < 0.05$ indicates statistically significant.

The results are presented in the following examples.

Example 1. IL-12 Enhances Efficacy of mRNA Tumor Vaccine and Enables Superior Tumor Control

Efficient induction, activation, and differentiation of CD8+ T cells require a third signal in addition to TCR-pMHC recognition and costimulatory signaling (Tugues et al., 2015). Although current mRNA-LNP vaccines can expand large numbers of tumor-specific CD8+ T cells (Fig. 1A-B; sequence S1 (SEQ ID NO: 69)), they only partially inhibit tumor growth (Fig. 1C-E). These mRNA vaccines are known to induce high levels of IL-1β, IL-6, and type I interferons, thereby promoting immune activation (Li et al., 2022). However, these cytokines may be insufficient to effectively activate CD8+ T cells to achieve optimal tumor control. To identify a key cytokine signal capable of acting synergistically with an mRNA vaccine, we screened several T-lymphocyte-stimulatory cytokines, including IL2, IL15, and IL12, for tumor treatment. These cytokines are known to promote T-cell proliferation, activation, or effector differentiation through different downstream transcription factors (Propper and Balkwill, 2022). In order to deliver these cytokines to the draining lymph nodes (dLNs) to support T-cell priming, the specific Fc-fused cytokines were co-administered intramuscularly with the mRNA vaccine to the same site in tumor-bearing mice (Fig. 1A). While IL-2 and IL-15 did not further enhance the antitumor efficacy of the mRNA vaccine (Fig. 1C-D), IL-12 markedly increased the vaccine efficacy and substantially inhibited tumor growth (Fig. 1E). These results prompted us to incorporate IL-12 as an adjuvant into an mRNA vaccine to improve activation of antigen-specific T cells and tumor control.

Example 2. Membrane-Tethered IL-12-Based mRNA Vaccine Achieves Superior Antitumor Efficacy with Limited Toxicity

To investigate the potential of IL-12 as an mRNA vaccine adjuvant, we constructed an IL12-OVA fusion mRNA vaccine for treating an OVA-expressing B16F10-OVA tumor model. In this construct, the coding sequences for secreted IL-12 and the OT1 epitope were linked via a 2A linker (sIL12-OVA) (Fig. 2A, top; sequence 19, (SEQ ID NO: 67)). For the control mRNA vaccine, the IL-12 coding sequence was replaced with less immunogenic EGFP (Skelton et al., 2001). IL12 is known to activate CD4, CD8, and NK cells and to induce robust IFN-γ secretion (Guo et al., 2012). We hypothesized that systemic dissemination of the IL-12 protein translated from the sIL12-OVA mRNA vaccine may lead to observed toxicity (Hewitt et al., 2020). To address this issue, we further developed a membrane-tethered IL12 (mtIL12)-adjuvanted mRNA vaccine (mtIL12-OVA) intended to restrict IL-12 to the surface of antigen-presenting cells in the dLNs or spleen. In this construct, IL-12 was fused to the transmembrane domain of CD80 and then linked to the tumor antigen via a 2A linker (Fig. 2A, bottom; sequence 10 (SEQ ID NO: 58)). *In vitro* transfection of DC2.4 cells confirmed that mtIL12-OVA mRNA after transfection can result in simultaneous cell surface presentation of the OT-1 peptide/MHC-I complex and the IL-12 cytokine (Fig. 2B; sequence 10 (SEQ ID NO: 58)). *In vitro* transfection with the sIL12-OVA mRNA resulted in IL-12 secretion into the culture supernatant (Fig. 2C; sequence 19 (SEQ ID NO: 67)). By contrast, following transfection with the mtIL12-OVA mRNA, IL-12 was anchored on the cell surface (Fig. 2B; sequence 10 (SEQ ID NO: 58)), with almost no leakage into the supernatant (Fig. 2B-C).

In mice bearing B16-OVA tumors, the sIL12-OVA vaccine significantly improved therapeutic efficacy and prolonged tumor control as compared with the control mRNA vaccine (Fig. 3A; sIL12-OVA sequence 19 (SEQ ID NO: 67); control sequence S1 (SEQ ID NO: 69)). However, the sIL12-OVA mRNA vaccine caused significant loss of body weight in vaccinated mice (Fig. 3B), indicating systemic toxicity. In vivo, mtIL12-OVA inhibited tumor growth and prolonged survival to an extent similar to sIL12-OVA (Fig. 3A; mtIL12-OVA sequence 10 (SEQ ID NO: 58); sIL12-OVA sequence 19 (SEQ ID NO: 67)). Notably, mtIL12-OVA administration did not exacerbate the toxicity associated with soluble IL12; similar to mice in the control OVA mRNA treatment group, the mice exhibited a transient

decrease in body weight followed by rapid recovery (Fig. 3B). Compared with sIL12-OVA, mtIL12-OVA induced markedly lower serum levels of IL-12, IFN-γ, and MCP-1 (Fig. 2C). Moreover, IL-12 was detected only on the surface of monocytes, dendritic cells, and macrophages within the dLNs (Fig. 4A). These results demonstrate that membrane-tethered IL12 effectively restricts IL-12 to the surface of APCs and prevents its release into peripheral tissues.

To further clarify how the mtIL12-adjuvanted vaccine can effectively isolate toxic adverse effects from enhanced antitumor efficacy, 1×10^6 OT-1 TCR-transgenic CD8 T cells were sorted and transferred into wild-type (WT) mice, which were then immunized with the control OVA, sIL12-OVA, or mtIL12-OVA mRNA vaccines (Fig. 4B-C; sequences S1 (SEQ ID NO: 69), 10 (SEQ ID NO: 58), 19 (SEQ ID NO: 67)). Compared with the control OVA vaccine, the sIL12-OVA and mtIL12-OVA vaccines induced comparable numbers of antigen-specific IFN-γ-positive cells in the dLNs (Fig. 4B). However, the sIL12-OVA vaccine further induced greater numbers of IFN-γ-positive bystander CD8+ T cells, CD4+ T cells, and NK cells in the dLNs (Fig. 4B) and peripheral blood (Fig. 4C), whereas this effect was not observed in the mtIL12-OVA group. This difference may explain the higher serum IFN-γ level and peripheral toxicity in the sIL12-OVA group, neither of which was observed in the mtIL12-OVA group. Taken together, these results show that the membrane-tethered IL-12 cytokine-adjuvanted mRNA vaccine can substantially enhance antitumor efficacy while minimizing adverse effects.

In vivo, the mtIL12-OVA mRNA vaccine was therapeutically more effective than either OVA mRNA alone or mtIL12 mRNA alone (Fig. 5; sequence S1 (SEQ ID NO: 69); sequence 19 (SEQ ID NO: 67); sequence S15 (SEQ ID NO: 83)), indicating that the simultaneous presentation of the IL-12 cytokine and the antigen synergistically enhances antitumor efficacy.

Example 3. mtIL12-Based mRNA Vaccine Depends on CD8 T Cells to Control Tumor and Induce Distinct Pre-Effector CD8 T-Cell Subset

IL-12-based therapies can act on various immune cell types, including CD4+ T cells, CD8+ T cells, NK cells, and macrophages, to optimize tumor control (Xue et al., 2022). To determine which cell subsets are essential for the tumor control mediated by the mtIL12-OVA mRNA vaccine, we selectively depleted specific immune cell subsets using antibodies.

Depletion of NK cells, CD4+ T cells, or macrophages did not affect the antitumor efficacy of the vaccine (Fig.6A-B; sequence 19 (SEQ ID NO: 67)). In contrast, depletion of CD8+ T cells markedly reduced vaccine-mediated tumor inhibition and the survival benefit in mice, demonstrating that the CD8+ T-cell response is essential for the antitumor efficacy of the vaccine.

We next investigated the cellular and molecular mechanisms by which the mtIL12-OVA vaccine enhances antitumor efficacy. Compared with the control OVA mRNA vaccine, the mtIL12-OVA after vaccination induced higher proportions of antigen-specific CD8+ T cells in both the draining lymph nodes and spleen (Fig. 6C).

We further analyzed the T-cell differentiation states by flow cytometry using a gating strategy based on Ly108 and CD69 that can elucidate the differentiation trajectory of antigen-specific T cells in a chronic infection model or a cancer model (Beltra et al., 2020). C57BL/6J mice were administered 2.5 μg of mtEGFP-OVA or mtIL12-OVA RNA by intramuscular injection on days 0 and 7, and blood was collected on day 14 for flow cytometric analysis (Fig. 7A). The control OVA mRNA primarily induced the Ly108+ CD69- T-cell subset (progenitor state), whereas the mtIL12-OVA vaccine primarily induced the Ly108- CD69- T-cell subset (effector state) in peripheral blood (Fig. 7B). Compared with the OVA mRNA control group, the mtIL12-OVA vaccine group showed significantly greater proportions of granzyme B-positive or IFN-γ-positive OVA-specific CD8+ T cells in PBMCs (Fig. 7C). In addition, in the mtIL12-OVA group, the proportion of OVA-specific CD8+ T cells expressing the effector T-cell transcription factor T-bet was increased, whereas the proportion of those expressing the inhibitory molecule PD-1 was decreased (Fig. 7C). To further verify the functionality of these cells, we isolated splenocytes from mice immunized with mtIL12-OVA or the control OVA mRNA vaccine, stimulated them with serial dilutions of the SIINFEKL peptide, and analyzed IFN-γ secretion by ELISA. Baseline IFN-γ secretion was low in both groups. After peptide stimulation, splenocytes in both groups secreted IFN-γ in a dose-dependent manner, with the amount detected in the mtIL12-OVA group significantly higher (Fig. 7D). These results demonstrate that the peripheral antigen-specific T cells induced by the mtIL12-OVA vaccine are highly potent pre-effector T cells. Upon re-encountering the antigen, they exhibit greater sensitivity and a stronger effector response, resulting in enhanced tumor inhibition compared with a conventional OVA mRNA vaccine.

We next examined whether the tandem linkage of mtIL12 and the OVA antigen (mtIL12-OVA) provides greater immunogenicity than a simple mixture of them (OVA + mtIL12). On days 0 and 7, B6 mice were administered 2 μg of mtEGFP-OVA, 2 μg of mtEGFP-OVA + 1 μg of mtIL12, or 1 μg of mtEGFP-OVA + 1 μg of mtIL12-OVA RNA-LNP by intravenous injection. Blood samples were collected on day 14 for flow cytometric analysis (Fig. 8A). Flow cytometry showed that mice receiving cis delivery of IL12 and antigen (OVA + mtIL12-OVA) produced a higher proportion and a greater number of antigen-specific T cells in peripheral blood (Fig. 8B; sequence S1 (SEQ ID NO: 69); sequence 19 (SEQ ID NO: 67); sequence S15 (SEQ ID NO: 83)).

Example 4. Membrane-Tethered IL12-Adjuvanted mRNA Vaccine Overcomes Resistance to Immune Checkpoint Blockade

The Immune checkpoint blockade (ICB) therapy has produced promising clinical outcomes (Hargadon et al., 2018), but only a minority of patients have shown an effective response (Sharma et al., 2017). One mechanism underlying the drug resistance is the insufficiency of tumor-infiltrating lymphocytes (TILs) in immunologically "cold" tumors. We reasoned that the mtIL12-adjuvanted mRNA cancer vaccine can overcome the resistance to ICB therapy by providing sufficient antigen-specific TILs for immunologically cold tumors. To confirm this hypothesis, mice bearing well-established B16-OVA tumors were treated with mtIL12-OVA (Fig. 9A; sequence 19 (SEQ ID NO: 67)) or ICB antibodies (anti-PD-L1 and anti-CTLA4). While the ICB therapy alone had little effect on tumor growth or survival, the mtIL12-OVA vaccine markedly inhibited tumor growth. Combining mtIL12-OVA with ICB therapy further improved tumor inhibition and prolonged survival (Fig. 9A).

Example 5. Membrane-Tethered IL12 Adjuvant Is Suitable for Vaccine Design Targeting E6/E7 Antigens of HPV-Associated Tumors

We further investigated whether the membrane-tethered IL12-adjuvanted mRNA vaccine design can be applied to clinically relevant tumor-specific antigens. The E6 and E7 oncoproteins are directly involved in HPV-induced carcinogenesis in both preclinical and clinical settings. Despite the extensive research that has been carried out on therapeutic vaccines targeting E6/E7, progress has been limited (Grunwitz et al., 2019; Peng et al., 2021).

We compared the therapeutic efficacy of a control E7 mRNA vaccine with that of the mtIL12-E7 vaccine in a TC-1 tumor mouse model that mimics HPV-associated cancer (Lin et al., 1996). In mice bearing advanced TC-1 tumors, neither the control E7 vaccine nor the mtIL12 monotherapy controlled tumor growth (Fig. 10A; sequence S15 (SEQ ID NO: 83); sequence S4 (SEQ ID NO: 72); sequence 18 (SEQ ID NO: 66)). Although the TC-1 tumors continued to grow to approximately 500 mm^3 after two mtIL12-E7 vaccinations, they eventually regressed, and in approximately 80% of mice the tumors were completely eradicated (Fig. 10A). Importantly, mice treated with the mtIL12-E7 mRNA vaccine did not exhibit significant loss of body weight as compared with untreated mice (Fig. 10B).

TC-1 tumor-bearing mice were immunized with 2.5 μg of mtEGFP-E7 on day 12 after tumor implantation, and with 2.5 μg of mtEGFP-E7 or 2.5 μg of mtIL12-E7 on day 18. Tumor tissue was collected on day 22 for flow cytometric analysis (Fig. 11A). The results showed that the IL12-mediated immunization promoted intratumoral enrichment of E7-specific T cells. Compared with the T cells generated by mtGFP-E7 immunization, the T cells generated by the mtIL12-E7 immunization more remained in the effector state (LY108-CD69-) rather than the exhausted state (LY108-CD69+), and expressed a higher level of the effector molecule GZMB and a lower level of the inhibitory marker PD-1 (Fig. 11C).

Example 6. Membrane-Tethered IL12 Adjuvant Is Suitable for Vaccine Design Targeting Tumor Neoantigens Derived from Retroviruses

Endogenous retroviral (ERV) genes are integrated into the host genome and generally remain silent in healthy tissues, but can be reactivated under pathological conditions such as cancer (Cherkasova et al., 2013; Smith et al., 2018). These reactivated ERV proteins provide viable targets for cancer vaccine development. The P15E protein is derived from expression of the gp70 retroviral gene and has been detected in many cancers, including the MC38 colon adenocarcinoma cell line (Ye et al., 2020). C57BL/6 mice were inoculated subcutaneously with 4×10^5 MC38 tumor cells. After tumor establishment, the mice were administered 2.5 μg of mtIL12-p15E or control mtEGFP-p15E mRNA-LNPs on days 12 and 16 by intramuscular injection, and tumor growth was monitored twice weekly. As shown in Fig. 12, treatment with the control P15E mRNA vaccine had a minimal effect on tumor growth, whereas treatment with mtIL12-P15E induced marked tumor regression and complete tumor eradication (Fig. 12;

sequence S5 (SEQ ID NO: 73); sequence S9 (SEQ ID NO: 77)).

MC38 tumor-bearing mice were administered 2.5 μg of mtEGFP-p15E on day 12 after tumor implantation by intramuscular injection, and 2.5 μg of either mtEGFP-p15E or mtIL12-p15E on day 18 after tumor implantation by intramuscular injection. Peripheral blood was collected on day 21 for flow cytometric analysis (Fig. 13A). Compared with the mtEGFP-P15E mRNA vaccine, immunization with mtIL12-P15E generated more p15E antigen-specific T cells. Moreover, as compared with the T cells generated by mtGFP-p15E immunization, those generated by the mtIL12-p15E immunization expressed a higher level of the effector molecule IFN-γ and a lower level of the immunoinhibitory molecule PD-1.

In summary, these experiments demonstrate that the mtIL12-based vaccine strategy is suitable for the treatment of cancers expressing multiple clinically relevant antigenic epitopes.

DISCUSSION

Cytokines function as a third signal during T-cell activation. The self-adjuvanting effect of mRNA-LNPs primarily induces proinflammatory cytokines, including IL-1β, IL-6, and type I interferon (Li et al., 2022; Tahtinen et al., 2022), which may not optimally support antigen-specific CD8+ T-cell responses. After screening multiple T-cell-stimulatory cytokines, we found that IL-12 was the most effective cytokine in enhancing the antitumor efficacy of an mRNA vaccine.

Many studies have focused on delivering IL-12 locally to the tumor microenvironment (TME) to enhance antitumor immune responses while reducing systemic toxicity (Nguyen et al., 2020). Current clinical approaches include intratumoral transfection with a plasmid or mRNA encoding IL-12 (Hewitt et al., 2020; Telli et al., 2021). However, these local delivery methods remain major challenges, particularly for deep-seated solid tumors, and IL-12 can still enter the circulation, causing toxicity and limiting the therapeutic window. Our study shows that local injection of mRNA encoding soluble IL-12 results in substantial leakage of IL-12 into the serum, triggering a subsequent IFN-γ response. IFN-γ is a key proinflammatory cytokine closely associated with IL-12-related toxicity. By anchoring IL-12 to the surface of transfected cells through a transmembrane domain, we successfully uncoupled the therapeutic benefits of IL-12 from its systemic toxicity.

T-cell dysfunction enables tumor cells to evade T-cell-mediated cytotoxicity through well-established mechanisms (Zebley et al., 2024). Studies have shown that naive

tumor-specific T cells rapidly acquire a dysfunctional phenotype within hours after entering the TME (Philip et al., 2017; Rudloff et al., 2023). In our study, conventional mRNA vaccines expanded large numbers of progenitor-like antigen-specific T cells, but these cells exhibited limited antitumor activity. We hypothesized that similar mechanisms might prevent the progenitor T cells generated by conventional mRNA vaccines from differentiating into functional effector cells. Introducing IL-12 signaling during the priming phase of mRNA vaccination promoted the differentiation of antigen-specific T cells into a pre-effector phenotype. Interestingly, these pre-effector CD8+ T cells secreted a low level of effector cytokines in the circulation, but displayed greater sensitivity and a stronger effector response upon re-encountering with the tumor antigen. This may explain their limited peripheral toxicity and enhanced antitumor efficacy. These cells also expressed a lower level of the inhibitory molecule PD-1 and exhibited resistance to the suppressive TME, further enhancing their antitumor activity.

In summary, the use of membrane-tethered IL-12 as an adjuvant substantially enhances the therapeutic efficacy of an mRNA tumor vaccine while minimizing toxicity. This strategy is applicable to various clinically relevant tumor antigens and shows a considerable potential for clinical translation.

The sequences according to the present disclosure are as follows:

Sequence information:

No.	Name	Sequence
Transmembrane Domain Sequences		
1	Amino acid sequence of human CD8A transmembrane domain (TM)	IYIWAPLAGTCGVLLLSLVIT (SEQ ID NO: 1)
2	cDNA sequence of human CD8A transmembrane domain (TM)	atttatatttgggcgccgctggcgggcacctgcggcgtgctgctgctgagcctggtgattacc (SEQ ID NO: 2)
3	Amino acid sequence of human PDGF-RB transmembrane domain (TM)	VVISAILALVVLTIISLIILI (SEQ ID NO: 3)
4	cDNA sequence of human PDGF-RB transmembrane domain (TM)	Gtggtgattagcgcgattctggcgctggtggtgctgaccattattagcctgattattctgatt (SEQ ID NO: 4)
5	Amino acid sequence of human CD80 transmembrane domain (TM)	LLPSWAITLISVNGIFVICCL (SEQ ID NO: 5)
6	cDNA sequence of human CD80 transmembrane domain (TM)	Ctgctgccgagctgggcgattaccctgattagcgtgaacggcatttttgtgatttgctgcctg (SEQ ID NO: 6)
7	Amino acid sequence of human CD86 transmembrane domain (TM)	WITAVLPTVIICVMVFCLILW (SEQ ID NO: 7)
8	cDNA sequence of human CD86 transmembrane domain (TM)	Tggattaccgcggtgctgccgaccgtgattatttgcgtgatggtgttttgcctgattctgtgg (SEQ ID NO: 8)
9	Amino acid sequence of murine CD80 transmembrane domain (TM)	TLVLFGAGAVITVVVIVVII (SEQ ID NO: 9)
10	cDNA sequence of murine CD80 transmembrane domain (TM)	Acactggtgctgtttggcgccggcttcggcgccgtgatcacagtggtggtcattgtggtcattatc (SEQ ID NO: 10)
Intracellular Domain Sequences		
1	Amino acid sequence of human CD8A intracellular domain (ICD)	LYCNHRNRRRVCKCPRPVVKSGDKPSLSARYV (SEQ ID NO: 11)
2	cDNA sequence of human CD8A	Ctgtactgcaaccaccggaatcggcggagagtgtgcaagtgccctagacctgtggtcaagagcgg cgacaagcctagcctgagcgccagatatgtt (SEQ ID NO: 12)

	intracellular domain (ICD)	
3	Amino acid sequence of human PDGF-RB intracellular domain (ICD)	QKKPRYEIRWKVIESVSSDGHEYIYVDPMQLPYDSTWELPRDQL VLGRTLGSGAFGQWEATAHGLSHSQATMKVAVKMLKSTARSSE KQALMSELKIMSHLGPHLNWNLLGACTKGGPIYIITEYCRYGDL VDYLHRNKHTFLQHHSDKRRPPSAELYSNALPVGLPLPSHVSLT GESDGGYMDMSKDESVDYVPMLDMKGDVKYADIESSNYMAP YDNYVPSAPERTCRATLINESPVLSYMDLVGFSYQVANGMEFL ASKNCVHRDLAARNVLICEGKLVKICDFGLARDIMRDSNYISK GSTFLPLKWMAPESIFNSLYTTLSDVWSFGILLWEIFTLGGTPYP ELPMNEQFYNAIKRGYRMAQPAHASDEIYEIMQKCWEEKFEIR PPFSQLVLLLERLLGEGYKKKYQQVDEEFLRSDHPAILRSQARL PGFHGLRSPLDTSSVLYTAVQPNEGDNDYIIPLPDPKPEVADEGP LEGSPSLASSTLNEVNTSSTISCDSPLEPQDEPEPEPQLELQVEPE PELEQLPDSGCPAPRAEAEDSFL (SEQ ID NO: 13)
4	cDNA sequence of human PDGF-RB intracellular domain (ICD)	cagaagaagcccagatacgagatccggtggaaagtgatcgagagcgtgtcctccgatggccacga gtacatctacgtggaccccatgcagctgccctacgatagcacatgggagctgcccagagatcagct ggtgctgggaagaacacttggctctggcgcctttggacagtgggaagccacagctcacggactga gccatagccaggccacaatgaaggtggccgtgaagatgctgaagtccaccgccagaagcagcga gaagcaggccctgatgagcgagctgaagatcatgagccacctgggacctcacctgaactggaatct gctgggcgcctgcacaaaaggcggccctatctacatcatcaccgagtactgcagatacggcgacct ggtggactacctgcaccggaacaagcacacctttctgcagcaccacagcgacaagagaaggcctc ctagcgccgagctgtactctaatgctctgcctgtgggactgcccctgcctagccatgtttctctgaccg gcgaatccgacggcggctacatggacatgagcaaggatgagagcgtggactacgtgcccatgctg gacatgaagggcgacgtgaagtacgccgacatcgagagcagcaactacatggccccttacgacaa ttacgtgccaagcgctcccgagcggacctgtagagccacactgatcaatgagagccccgtgctgtc ctacatggatctcgtggggttcagctaccaggtggccaacggcatggaatttctggccagcaagaac tgcgtgcacagagatctggccgccagaaacgtgctgatctgcgagggcaagctggtcaagatctgc gactttggcctggccagagacatcatgcgggactccaactacatcagcaagggcagcaccttcctg cctctgaagtggatggcccctgagtccatcttcaacagcctgtacacaaccctgagcgacgtgtggt ccttcggcattctgctgtgggagatctttaccctcggcggcaccccttatcctgagctgcctatgaacg agcagttctacaacgccatcaagcggggctacagaatggcccagcctgctcatgccagcgacgag atctacgagatcatgcagaagtgttgggaagagaagttcgagatcaggcctccattcagccagctgg tcctgctgctggaaagactgctcggcgagggctacaagaaaaagtaccagcaggtcgacgaagag ttcctgcggagcgatcaccctgccatcctcagatctcaggccagactgcctggattccacggcctga gaagccctctggatacaagcagcgtgctgtacacagccgtgcagcccaacgagggcgacaacgat tacatcatccctctgcctgatcctaagcctgaggtggccgatgagggacctctggaaggatctccaa gcctggcctctagcaccctgaacgaagtgaacaccagctccaccatcagctgcgacagcccactg gaacctcaggatgagcctgagcctgaacctcagctggaactgcaggtcgagcccgagccagaact ggaacagctgcctgattctggctgccctgctcctagagccgaagccgaggatagctttctg (SEQ ID NO: 14)
5	Amino acid sequence of truncated human PDGF-RB intracellular domain (ICD)	QKKPRYEIRWKVIESVSSDGHE (SEQ ID NO: 15)
6	cDNA sequence of truncated human PDGF-RB intracellular domain (ICD)	Cagaagaagcccagatacgagatccggtggaaagtgatcgagagcgtgtcctccgatggccacg aa (SEQ ID NO: 16)
7	Amino acid sequence of human CD80 intracellular domain (ICD)	TYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO: 17)
8	cDNA sequence of human CD80	Acctactgcttcgcccctagatgcagagagcggcggagaaacgaacggctgagaagagaatctgt gcggcccgtt (SEQ ID NO: 18)

	intracellular domain (ICD)	
9	Amino acid sequence of human CD86 intracellular domain (ICD)	KWKKKKRPRNSYKCGTNTMEREESEQTKKREKIHIPERSDEAQ RVFKSSKTSSCDKSDTCF (SEQ ID NO: 19)
10	cDNA sequence of human CD86 intracellular domain (ICD)	Aagtggaagaagaagaaaaggccccggaacagctacaagtgcggcaccaacaccatggaacg ggaagagagcgagcagaccaagaagagagagaagattcacatccccgagcggagcgacgaag cccagagagtgtttaagagcagcaagaccagcagctgcgacaagagcgatacctgcttc (SEQ ID NO: 20)
11	Amino acid sequence of murine CD80 intracellular domain (ICD)	KCFCKHRSCFRRNEASRETNNSLTFGPEEALAEQTVFL (SEQ ID NO: 21)
12	cDNA sequence of murine CD80 intracellular domain (ICD)	Aagtgcttctgcaagcacagatcctgtttcaggagaaatgaggccagcagagagacaaacaacag cctgacatttggccctgaggaggccctggccgagcagacagtgtttctg (SEQ ID NO: 22)
1	hIL12B cDNA sequence	Atgtgccatcagcagctggtgattagctggtttagcctggtgtttctggcgagcccgctggtggcgat ttgggaactgaaaaaagatgtgtatgtggtggaactggattggtatccggatgcgccgggcgaaatg gtggtgctgacctgcgataccccggaagaagatggcattacctggaccctggatcagagcagcga agtgctgggcagcggcaaaaccctgaccattcaggtgaaagaatttggcgatgcgggccagtatac ctgccataaaggcggcgaagtgctgagccatagcctgctgctgctgcataaaaaagaagatggcat ttggagcaccgatattctgaaagatcagaaagaaccgaaaaacaaaaacctttctgcgctgcgaagcg aaaaactatagcggccgctttacctgctggtggctgaccaccattagcaccgatctgacctttagcgt gaaaagcagccgcggcagcagcgatccgcagggcgtgacctgcggcgcggcgaccctgagcg cggaacgcgtgcgcggcgataacaaagaatatgaatatagcgtggaatgccaggaagatagcgcg tgcccggcggcggaagaaagcctgccgattgaagtgatggtggatgcggtgcataaactgaaatat gaaaactataccagcagctttttattcgcgatattattaaaccggatccgccgaaaaacctgcagctg aaaccgctgaaaaacagccgccaggtggaagtgagctgggaatatccggatacctggagcacccc gcatagctattttagcctgacctttgcgtgcaggtgcagggcaaaagcaaacgcgaaaaaaaagat cgcgtgtttaccgataaaaccagcgcgaccgtgatttgccgcaaaaacgcgagcattagcgtgcgc gcgcaggatcgctattatagcagcagctggagcgaatgggcgagcgtgccgtgcagc (SEQ ID NO: 23)
2	mIL12B cDNA sequence	Atgtgtcctcagaagctaaccatctcctggtttgccatcgttttgctggtgtctccactcatggccatgt gggagctggagaaagacgtttatgttgtagaggtggactggactcccgatgcccctggagaaacag tgaacctcacctgtgacacgcctgaagaagatgacatcacctggacctcagaccagagacatggag tcataggctctggaaagaccctgaccatcactgtcaaagagtttctagatgctggccagtacacctgc cacaaaggaggcgagactctgagccactcacatctgctgctccacaagaaggaaaatggaatttgg tccactgaaattttaaaaaatttcaaaaacaagactttcctgaagtgtgaagcaccaaattactccggac ggttcacgtgctcatggctggtgcaaagaaacatggacttgaagttcaacatcaagagcagtagcag ttcccctgactctcgggcagtgacatgtggaatggcgtctctgtctgcagagaaggtcacactggac caaagggactatgagaagtattcagtgtcctgccaggaggatgtcacctgcccaactgccgaggag accctgcccattgaactggcgttggaagcacggcagcagaataaatatgagaactacagcaccagc ttcttcatcagggacatcatcaaaccagacccgcccaagaacttgcagatgaagcctttgaagaactc acaggtggaggtcagctgggagtaccctgactcctggagcactccccattcctacttctccctcaagt tctttgttcgaatccagcgcaagaaagaaaagatgaaggagacagaggaggggtgtaaccagaaa ggtgcgttcctcgtagagaagacatctaccgaagtccaatgcaaaggcgggaatgtctgcgtgcaa gctcaggatcgctattacaattcctcatgcagcaagtgggcatgtgttccctgcagggtccgatcc (SEQ ID NO: 24)
3	hIL12B amino acid sequence	MCHQQLVISWFSLVFLASPLVAIWELKKDVYVVELDWYPDAPG EMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLTIQVKEFGDAG QYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFL RCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAA TLSAERVRGDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVH KLKYENYTSSFFIRDIIKPDPPKNLQLKPLKNSRQVEVSWEYPDT WSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSATVICRKNAS

		ISVRAQDRYYSSSWSEWASVPCS (SEQ ID NO: 25)
4	mIL12B amino acid sequence	MCPQKLTISWFAIVLLVSPLMAMWELEKDVYVVEVDWTPDAP GETVNLTCDTPEEDDITWTSDQRHGVIGSGKTLTITVKEFLDAG QYTCHKGGETLSHSHLLLHKKENGIWSTEILKNFKNKTFLKCE APNYSGRFTCSWLVQRNMDLKFNIKSSSSSPDSRAVTCGMASLS AEKVTLDQRDYEKYSVSCQEDVTCPTAEETLPIELALEARQQN KYENYSTSFFIRDIIKPDPPKNLQMKPLKNSQVEVSWEYPDSWS TPHSYFSLKFFVRIQRKKEKMKETEEGCNQKGAFLVEKTSTEVQ CKGGNVCVQAQDRYYNSSCSKWACVPCRVRS (SEQ ID NO: 26)
5	hIL12A cDNA sequence	Atgtgcccggcgcgcagcctgctgctggtggcgaccctggtgctgctggatcatctgagcctggc gcgcaacctgccggtggcgaccccggatccgggcatgtttccgtgcctgcatcatagccagaacct gctgcgcgcggtgagcaacatgctgcagaaagcgcgccagaccctggaattttatccgtgcaccag cgaagaaattgatcatgaagatattaccaaagataaaaccagcaccgtggaagcgtgcctgccgct ggaactgaccaaaaacgaaagctgcctgaacagccgcgaaaccagctttattaccaacggcagct gcctggcgagccgcaaaaccagctttatgatggcgctgtgcctgagcagcatttatgaagatctgaa aatgtatcaggtggaatttaaaaccatgaacgcgaaactgctgatggatccgaaacgccagatttttct ggatcagaacatgctggcggtgattgatgaactgatgcaggcgctgaactttaacagcgaaaccgtg ccgcagaaaagcagcctggaagaaccggatttttataaaaccaaaattaaactgtgcattctgctgca tgcgtttcgcattcgcgcggtgaccattgatcgcgtgatgagctatctgaacgcgagc (SEQ ID NO: 27)
6	mIL12A cDNA sequence	Agggtcattccagtctctggacctgccaggtgtcttagccagtcccgaaacctgctgaagaccaca gatgacatggtgaagacggccagagaaaaactgaaacattattcctgcactgctgaagacatcgatc atgaagacatcacacgggaccaaaccagcacattgaagacctgtttaccactggaactacacaaga acgagagttgcctggctactagagagacttcttccacaacaagagggagctgcctgccccacaga agacgtctttgatgatgaccctgtgccttggtagcatctatgaggacttgaagatgtaccagacagagt tccaggccatcaacgcagcacttcagaatcacaaccatcagcagatcattctagacaagggcatgct ggtggccatcgatgagctgatgcagtctctgaatcataatggcgagactctgcgccagaaacctcct gtgggagaagcagacccttacagagtgaaaatgaagctctgcatcctgcttcacgccttcagcaccc gcgtcgtgaccatcaacagggtgatgggctatctgagctccgcc (SEQ ID NO: 28)
7	hIL12A amino acid sequence	MCPARSLLLVATLVLLDHLSLARNLPVATPDPGMFPCLHHSQNL LRAVSNMLQKARQTLEFYPCTSEEIDHEDITKDKTSTVEACLPL ELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYEDLKM YQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSE TVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS (SEQ ID NO: 29)
8	mIL12A amino acid sequence	MCQSRYLLFLATLALLNHLSLARVIPVSGPARCLSQSRNLLKTT DDMVKTAREKLKHYSCTAEDIDHEDITRDQTSTLKTCLPLELHK NESCLATRETSSTTRGSCLPPQKTSLMMTLCLGSIYEDLKMYQT EFQAINAALQNHNHQQIILDKGMLVAIDELMQSLNHNGETLRQ KPPVGEADPYRVKMKLCILLHAFSTRVVTINRVMGYLSSA (SEQ ID NO: 30)
9	G4S linker mRNA sequence	Ggcggaggcggatcaggcggcggtggatctgggggtggcggatct (SEQ ID NO: 31)
10	G4S linker amino acid sequence	GGGGSGGGGSGGGGS (SEQ ID NO: 32)
11	OVA cDNA sequence	atgctggagcagctggagtccatcatcaacttcgagaagctgaccgagtggacctcc (SEQ ID NO: 33)
12	OVA amino acid sequence	MLEQLESIINFEKLTEWTS (SEQ ID NO: 34)
Sequence information of mutation-derived tumor neoantigens:		
1	KRAS G12C	MTEYKLVVVGACGVGKSALTIQLIQ (SEQ ID NO: 35)
2	KRAS G12D	MTEYKLVVVGADGVGKSALTIQLIQ (SEQ ID NO: 36)
3	EGFRvIII	LEEKKGNYVVTDHGSCVRACGADSYEMEEDGVRKCKKCEGP CRKVCNGIGIGEFKDSLSINATNIKHFKNCTSISGDLHILPVAFRG DSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENL EIIRGRTKQHGQFSLAVVSLNITSLGLRSLKEISDGDVIISGNKNL CYANTINWKKLFGTSGQKTKIISNRGENSCKATGQVCHALCSPE

		GCWGPEPRDCVSCRNVSRGRECVDKCNLLEGEPREFVENSECI QCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGV MGENNTLVWKYADAGHVCHLCHPNCTYGCTGPGLEGCPTNGP KIPS (SEQ ID NO: 37)
4	BRAFV600E	LTVKIGDFGLATEKSRWSGSHQFEQ (SEQ ID NO: 38)
Information of virus-derived tumor-specific antigens		
1	HPV16 E6	MHQKRTAMFQDPQERPRKLPQLCTELQTTIHDIILECVYCKQQL LRREVYDFAFRDLCIVYRDGNPYAVCDKCLKFYSKISEYRHYCY SLYGTTLEQQYNKPLCDLLIRCINCQKPLCPEEKQRHLDKKQRF HNIRGRWTGRCMSCCRSSRTRRETQL (SEQ ID NO: 39)
2	HPV 18 E7	MARFEDPTRRPYKLPDLCTELNTSLQDIEITCVYCKTVLELTEVF EFAFKDLFVVYRDSIPHAACHKCIDFYSRIRELRHYSDSVYGDT LEKLTNTGLYNLLIRCLRCQKPLNPAEKLRHLNEKRRFHNIAGH YRGQCHSCCNRARQERLQRRRETQV (SEQ ID NO: 40)
3	HPV16 E7	MHGDTPTLHEYMLDLQPETTDLYCYEQLNDSSEEEDEIDGPAG QAEPDRAHYNIVTFCCKCDSTLRLCVQSTHVDIRTLEDLLMGTL GIVCPICSQKP (SEQ ID NO: 41)
4	HPV18 E7	MHGPKATLQDIVLHLEPQNEIPVDLLCHEQLSDSEEENDEIDGV NHQHLPARRAEPQRHTMLCMCCKCEARIKLVVESSADDLRAFQ QLFLNTLSFVCPWCASQQ (SEQ ID NO: 42)
5	Endogenous retrovirus-derived antigens (hERT)	TFLGSLTWK, ATFLGSLTWK, TFLGSLTWKR, PATFLGSLTWK (SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, and SEQ ID NO: 46, respectively)
6	LMP1 (latent membrane protein 1)	MEHDLERGPPGPRRPPRGPPLSSSLGLALLLLLLALLFWLYIVM SDWTGGALLVLYSFALMLIIIILIIFIFRRDLLCPLGALCILLLMITL LLIALWNLHGQALFLGIVLFIFGCLLVLGIWIYLLEMLWRLGATI WQLLAFFLAFFLDLILLIIALYLQQNWWTLLVDLLWLLLFLAILI WMYYHGQRHSDEHHHDDSLPHPQQATDDSGHESDSNSNEGRH HLLVSGAGDGPPLCSQNLGAPGGGPDNGPQDPDNTDDNGPQD PDNTDDNGPHDPLPQDPDNTDDNGPQDPDNTDDNGPHDPLPH SPSDSAGNDGGPPQLTEEVENKGGDQGPPLMTDGGGGHSHDS GHGGGDPHLPTLLLGSSGSGGDDDDPHGPVQLSYYD (SEQ ID NO: 47)
7	LMP2 (latent membrane protein 2)	MGSLEMVPMGAGPPSPGGDPDGYDGGNNSQYPSASGSSGNTP TPPNDEERESNEEPPPPYEDPYWGNGDRHSDYQPLGTQDQSLY LGLQHDGNDGLPPPPYSPRDDSSQHIYEEAGRGSMNPVCLPVIV APYLFWLAAIAASCFTASVSTVVTATGLALSLLLLAAVASSYAA AQRKLLTPVTVLTAVVTFFAICLTWRIEDPPFNSLLFALLAAAGG LQGIYVLVMLVLLILAYRRRWRRLTVCGGIMFLACVLVLIVDAV LQLSPLLGAVTVVSMTLLLLAFVLWLSSPGGLGTLGAALLTLAA ALALLASLILGTLNLTTMFLLMLLWTLVVLLICSSCSSCPLSKILL ARLFLYALALLLLASALIAGGSILQTNFKSLSSTEFIPNLFCMLLL IVAGILFILAILTEWGSGNRTYGPVFMCLGGLLTMVAGAVWLTV MSNTLLSAWILTAGFLIFLIGFALFGVIRCCRYCCYYCLTLESEER PPTPYRNTV (SEQ ID NO: 48)
Sequence information of tumor-associated antigens highly expressed in tumors		
1	WT1	MGSDVRDLNALLPAVPSLGGGGGCALPVSGAAQWAPVLDFAPP GASAYGSLGGPAPPPAPPPPPPPPHSFIKQEPSWGGAEPHEEQC LSAFTVHFSGQFTGTAGACRYGPFGPPPPSQASSGQARMFPNAP YLPSCLESQPAIRNQGYSTVTFDGTPSYGHTPSHHAAQFPNHSF KHEDPMGQQGSLGEQQYSVPPPVYGCHTPTDSCTGSQALLLRT PYSSDNLYQMTSQLECMTWNQMNLGATLKGVAAGSSSSVKWT EGQSNHSTGYESDNHTTPILCGAQYRIHTHGVFRGIQDVRRVPG VAPTLVRSASETSEKRPFMCAYPGCNKRYFKLSHLQMHSRKHT GEKPYQCDFKDCERRFSRSDQLKRHQRRHTGVKPFQCKTCQR KFSRSDHLKTHTRTHTGKTSEKPFSCRWPSCQKKFARSDELVRH HNMHQRNMTKLQLAL (SEQ ID NO: 49)

2	MAGE-A3	MPLEQRSQHCKPEEGLEARGEALGLVGAQAPATEEQEAASSSST LVEVTLGEVPAAESPDPPQSPQGASSLPTTMNYPLWSQSYEDSS NQEEEGPSTFPDLESEFQAALSRKVAELVHFLLLKYRAREPVTK AEMLGSVVGNWQYFFPVIFSKASSSLQLVFGIELMEVDPIGHLYI FATCLGLSYDGLLGDNQIMPKAGLLIIVLAIIAREGDCAPEEKIW EELSVLEVFEGREDSILGDPKKLLTQHFVQENYLEYRQVPGSDP ACYEFLWGPRALVETSYVKVLHHMVKISGGPHISYPPLHEWVL REGEE (SEQ ID NO: 50)
3	GP100(Pmel)	MDLVLKRCLLHLAVIGALLAVGATKVPRNQDWLGVSRQLRTKA WNRQLYPEWTEAQRLDCWRGGQVSLKVSNDGPTLIGANASFSI ALNFPGSQKVLPDGQVIWVNNTIINGSQVWGGQPVYPQETDDA CIFPDGGPCPSGSWSQKRSFVYVWKTWGQYWQVLGGPVSGLS IGTGRAMLGTHTMEVTVYHRRGSRSYVPLAHSSSAFTITDQVPF SVSVSQLRALDGGNKHFLRNQPLTFALQLHDPSGYLAEADLSY TWDFGDSSGTLISRALVVTHTYLEPGPVTAQVVLQAAIPLTSCG SSPVPGTTDGHRPTAEAPNTTAGQVPTTEVVGTTPGQAPTAEPS GTTSVQVPTTEVISTAPVQMPTAESTGMTPEKVPVSEVMGTTLA EMSTPEATGMTPAEVSIVVLSGTTAAQVTTTEWVETTARELPIPE PEGPDASSIMSTESITGSLGPLLDGTATLRLVKRQVPLDCVLYRY GSFSVTLDIVQGIESAEILQAVPSGEGDAFELTVSCQGGLPKEAC MEISSPGCQPPAQRLCQPVLPSPACQLVLHQILKGGSGTYCLNVS LADTNSLAVVSTQLIMPGQEAGLGQVPLIVGILLVLMAVVLASL IYRRRLMKQDFSVPQLPHSSSHWLRLPRIFCSCPIGENSPLLSGQ QV (SEQ ID NO: 51)
4	NY-ESO-I	MQAEGRGTGGSTGDADGPGGPGIPDGPGGNAGGPGEAGATGG RGPRGAGAARASGPGGGAPRGPHGGAASGLNGCCRCGARGPE SRLLEFYLAMPFATPMEAELARRSLAQDAPPLPVPGVLLKEFTV SGNILTIRLTAADHRQLQLSISSCLQQLSLLMWITQCFLPVFLAQP PSGQRR (SEQ ID NO: 52)
5	HER2/Neu	MELAALCRWGLLLALLPPGAASTQVCTGTDMKLRLPASPETHL DMLRHLYQGCQVVQGNLELTYLPTNASLSFLQDIQEVQGYVLI AHNQVRQVPLQRLRIVRGTQLFEDNYALAVLDNGDPLNNTTPV TGASPGGLRELQLRSLTEILKGGVLIQRNPQLCYQDTILWKDIFH KNNQLALTLIDTNRSRACHPCSPMCKGSRCWGESSEDCQSLTRT VCAGGCARCKGPLPTDCCHEQCAAGCTGPKHSDCLACLHFNH SGICELHCPALVTYNTDTFESMPNPEGRYTFGASCVTACPYNYL STDVGSCTLVCPLHNQEVTAEDGTQRCEKCSKPCARVCYGLGM EHLREVRAVTSANIQEFAGCKKIFGSLAFLPESFDGDPASNTAPL QPEQLQVFETLEEITGYLYISAWPDSLPDLSVFQNLQVIRGRILH NGAYSLTLQGLGISWLGLRSLRELGSGLALIHHNTHLCFVHTVP WDQLFRNPHQALLHTANRPEDECVGEGLACHQLCARGHCWG PGPTQCVNCSQFLRGQECVEECRVLQGLPREYVNARHCLPCHP ECQPQNGSVTCFGPEADQCVACAHYKDPPFCVARCPSGVKPDL SYMPIWKFPDEEGACQPCPINCTHSCVDLDDKGCPAEQRASPLT SIISAVVGILLVVVLGVVFGILIKRRQQKIRKYTMRRLLQETELVE PLTPSGAMPNQAQMRILKETELRKVKVLGSGAFGTVYKGIWIP DGENVKIPVAIKVLRENTSPKANKEILDEAYVMAGVGSPYVSRL LGICLTSTVQLVTQLMPYGCLLDHVRENRGRLGSQDLLNWCM QIAKGMSYLEDVRLVHRDLAARNVLVKSPNHVKITDFGLARLL DIDETEYHADGGKVPIKWMALESILRRRFTHQSDVWSYGVTV WELMTFGAKPYDGIPAREIPDLLEKGERLPQPPICTIDVYMIMV KCWMIDSECRPRFRELVSEFSRMARDPQRFVVIQNEDLGPASPL DSTFYRSLLEDDDMGDLVDAEEYLVPQQGFFCPDPAPGAGGMV HHRHRSSSTRSGGGDLTLGLEPSEEEAPRSPLAPSEGAGSDVFD GDLGMGAAKGLQSLPTHDPSPLQRYSEDPTVPLPSETDGYVAPL TCSPQPEYVNQPDVRPQPPSPREGPLPAARPAGATLERPKTLSPG KNGVVKDVFAFGGAVENPEYLTPQGGAAPQPHPPPAFSPAFDNL YYWDQDPPERGAPPSTFKGTPTAENPEYLGLDVPV (SEQ ID

		NO: 53)
6	MUC1	MTPGTQSPFFLLLLLTVLTVVTGSGHASSTPGGEKETSATQRSSV PSSTEKNAVSMTSSVLSSHSPGSGSSTTQGQDVTLAPATEPASGS AATWGQDVTSVPVTRPALGSTTPPAHDVTSAPDNKPAPGSTAPP AHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGV TSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPD TRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAP GSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAP PAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGV TSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPD TRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAP GSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAP PAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGV TSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPD TRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAP GSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAP PAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGV TSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPD NRPALGSTAPPVHNVTSASGSASGSASTLVHNGTSARATTTPAS KSTPFSIPSHHSDTPTTLASHSTKTDASSTHHSSVPPLTSSNHSTS PQLSTGVSFFFLSFHISNLQFNSSLEDPSTDYYQELQRDISEMFL QIYKQGGFLGLSNIKFRPGSVVVQLTLAFREGTINVHDVETQFN QYKTEAASRYNLTISDVSVSDVPFPFSAQSGAGVPGWGIALLVL VCVLVALAIVYLIALAVCQCRRKNYGQLDIFPARDTYHPMSEYP TYHTHGRYVPPSSTDRSPYEKVSAGNGGSSLSYTNPAVAATSAN L (SEQ ID NO: 54)
7	mIL12A-(G4S)3-IL 12B cDNA sequence	atgtgtcctcagaagctaaccatctcctggtttgccatcgttttgctggtgtctccactcatggccatgtg ggagctggagaaagacgtttatgttgtagaggtggactggactcccgatgcccctggagaaacagt gaacctcacctgtgacacgcctgaagaagatgacatcacctggacctcagaccagagacatggagt cataggctctggaaagaccctgaccatcactgtcaaagagtttctagatgctggccagtacacctgcc acaaaggaggcgagactctgagccactcacatctgctgctccacaagaaggaaaatggaatttggt ccactgaaattttaaaaaatttcaaaaacaagactttcctgaagtgtgaagcaccaaattactccggac ggttcacgtgctcatggctggtgcaaagaaacatggacttgaagttcaacatcaagagcagtagcag ttcccctgactctcgggcagtgacatgtggaatggcgtctctgtctgcagagaaggtcacactggac caaagggactatgagaagtattcagtgtcctgccaggaggatgtcacctgcccaactgccgaggag accctgcccattgaactggcgttggaagcacggcagcagaataaatatgagaactacagcaccagc ttcttcatcagggacatcatcaaaccagacccgcccaagaacttgcagatgaagcctttgaagaactc acaggtggaggtcagctgggagtaccctgactcctggagcactccccattcctacttctccctcaagt tctttgttcgaatccagcgcaagaaagaaaagatgaaggagacagaggaggggtgtaaccagaaa ggtgcgttcctcgtagagaagacatctaccgaagtccaatgcaaaggcgggaatgtctgcgtgcaa gctcaggatcgctattacaattcctcatgcagcaagtgggcatgtgttccctgcagggtccgatccgg cggaggcggatcaggcggcggtggatctgggggtggcggatcttcgcgaagggtcattccagtct ctggacctgccaggtgtcttagccagtcccgaaacctgctgaagaccacagatgacatggtgaaga cggccagagaaaaactgaaacattattcctgcactgctgaagacatcgatcatgaagacatcacacg gaccaaaccagcacattgaagacctgtttaccactggaactacacaagaacgagagttgcctggct actagagagacttcttccacaacaagagggagctgcctgcccccacagaagacgtctttgatgatga ccctgtgccttggtagcatctatgaggacttgaagatgtaccagacagagttccaggccatcaacgc agcacttcagaatcacaaccatcagcagatcattctagacaagggcatgctggtggccatcgatgag ctgatgcagtctctgaatcataatggcgagactctgcgccagaaacctcctgtgggagaagcagacc cttacagagtgaaaatgaagctctgcatcctgcttcacgccttcagcacccgcgtcgtgaccatcaac agggtgatgggctatctgagctccgcc (SEQ ID NO: 55)

8	mIL12A-(G4S)3-IL 12B amino acid sequence	MCPQKLTISWFAIVLLVSPLMAMWELEKDVYVVEVDWTPDAP GETVNLTCDTPEEDDITWTSDQRHGVIGSGKTLTITVKEFLDAG QYTCHKGGETLSHSHLLLHKKENGIWSTEILKNFKNKTFLKCE APNYSGRFTCSWLVQRNMDLKFNIKSSSSSPDSRAVTCGMASLS AEKVTLDQRDYEKYSVSCQEDVTCPTAEETLPIELALEARQQN KYENYSTSFFIRDIIKPDPPKNLQMKPLKNSQVEVSWEYPDSWS TPHSYFSLKFFVRIQRKKEKMKETEEGCNQKGAFLVEKTSTEVQ CKGGNVCVQAQDRYYNSSCSKWACVPCRVRSGGGGSGGGGS GGGGSSRRVIPVSGPARCLSQSRNLLKTTDDMVKTAREKLKHY SCTAEDIDHEDITRDQTSTLKTCLPLELHKNESCLATRETSSTTR GSCLPPQKTSLMMTLCLGSIYEDLKMYQTEFQAINAALQNHNH QQIILDKGMLVAIDELMQSLNHNGETLRQKPPVGEADPYRVKM KLCILLHAFSTRVVTINRVMGYLSSA (SEQ ID NO: 56)
9	mIL12-mCD80TM-T2A-OVA cDNA sequence	atgtgtcctcagaagctaaccatctcctggtttgccatcgttttgctggtgtctccactcatggccatgtg ggagctggagaaagacgtttatgttgtagaggtggactggactcccgatgcccctggagaaacagt gaacctcacctgtgacacgcctgaagaagatgacatcacctggacctcagaccagagacatggagt cataggctctggaaagaccctgaccatcactgtcaaagagtttctagatgctggccagtacacctgcc acaaaggaggcgagactctgagccactcacatctgctgctccacaagaaggaaaatggaatttggt ccactgaaattttaaaaaatttcaaaaacaagactttcctgaagtgtgaagcaccaaattactccggac ggttcacgtgctcatggctggtgcaaagaaacatggacttgaagttcaacatcaagagcagtagcag ttcccctgactctcgggcagtgacatgtggaatggcgtctctgtctgcagagaaggtcacactggac caaagggactatgagaagtattcagtgtcctgccaggaggatgtcacctgcccaactgccgaggag accctgcccattgaactggcgttggaagcacggcagcagaataaatatgagaactacagcaccagc ttcttcatcagggacatcatcaaaccagacccgcccaagaacttgcagatgaagcctttgaagaactc acaggtggaggtcagctgggagtaccctgactcctggagcactccccattcctacttctccctcaagt tctttgttcgaatccagcgcaagaaagaaaagatgaaggagacagaggaggggtgtaaccagaaa ggtgcgttcctcgtagagaagacatctaccgaagtccaatgcaaaggcgggaatgtctgcgtgcaa gctcaggatcgctattacaattcctcatgcagcaagtgggcatgtgttccctgcagggtccgatccgg cggaggcggatcaggcggcggtggatctgggggtggcggatcttcgcgaagggtcattccagtct ctggacctgccaggtgtcttagccagtcccgaaacctgctgaagaccacagatgacatggtgaaga cggccagagaaaaactgaaacattattcctgcactgctgaagacatcgatcatgaagacatcacacg ggaccaaaccagcacattgaagacctgtttaccactggaactacacaagaacgagagttgcctggct actagagagacttcttccacaacaagagggagctgcctgcccccacagaagacgtctttgatgatga ccctgtgccttggtagcatctatgaggacttgaagatgtaccagacagagttccaggccatcaacgc agcacttcagaatcacaaccatcagcagatcattctagacaagggcatgctggtggccatcgatgag ctgatgcagtctctgaatcataatggcgagactctgcgccagaaacctcctgtgggagaagcagacc cttacagagtgaaaatgaagctctgcatcctgcttcacgccttcagcacccgcgtcgtgaccatcaac agggtgatgggctatctgagctccgcctccggaggaggctccggaggaggaggcagcggagga ggaggatccggcggaggaggctctggcggaggaagcggcgatgcccacgtgagcgaggatttc acatgggagaagccccccgaggaccctcccgacagcaagaacacactggtgctgtttggcgccg gcttcggcgccgtgatcacagtggtggtcattgtggtcattatcaagtgcttctgcaagcacagatcct gtttcaggagaaatgaggccagcagagagacaaacaacagcctgacatttggccctgaggaggcc ctggccgagcagacagtgtttctggagggcaggggcagcctgctgacctgcggagacgtggagg agaaccctggccccatgctggagcagctggagtccatcatcaacttcgagaagctgaccgagtgga cctcctga (SEQ ID NO: 57)
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		TVVVIVVIIKCFCKHRSCFRRNEASRETNNSLTFGPEEALAEQTV FLEGRGSLLTCGDVEENPGPMLEQLESIINFEKLTEWTS (SEQ ID NO: 58)
11	mIL12-mCD80TM-T2A-HPV16 E6 cDNA sequence	atgtgcccgcagaaactgaccattagctggtttgcgattgtgctgctggtgagcccgctgatggcgat gtgggaactggaaaaagatgtgtatgtggtggaagtggattggaccccggatgcgccgggcgaaa ccgtgaacctgacctgcgatacccccggaagaagatgatattacctggaccagcgatcagcgccatg gcgtgattggcagcggcaaaaccctgaccattaccgtgaaagaatttctggatgcgggccagtatac ctgccataaaggcggcgaaaccctgagccatagccatctgctgctgcataaaaaagaaaacggcat ttggagcaccgaaattctgaaaaactttaaaaacaaaaccttttctgaaatgcgaagcgccgaactata gcggccgctttacctgcagctggctggtgcagcgcaacatggatctgaaatttaacattaaaagcag cagcagcagcccggatagccgcgcggtgacctgcggcatggcgagcctgagcgcggaaaaagt gaccctggatcagcgcgattatgaaaaatatagcgtgagctgccaggaagatgtgacctgcccgac cgcggaagaaaccctgccgattgaactggcgctggaagcgcgccagcagaacaaatatgaaaact atagcaccagctttttattcgcgatattattaaaccggatccgccgaaaaacctgcagatgaaaccgc tgaaaaacagccaggtggaagtgagctgggaatatccggatagctggagcaccccgcatagctatt ttagcctgaaatttttgtgcgcattcagcgcaaaaaagaaaaaatgaaagaaaccgaagaaggctg caaccagaaaggcgcgtttctggtggaaaaaaccagcaccgaagtgcagtgcaaaggcggcaac gtgtgcgtgcaggcgcaggatcgctattataacagcagctgcagcaaatgggcgtgcgtgccgtgc cgcgtgcgcagcggcggcggcggcagcggcggcggcggcagcggcggcggcggcagcagc cgccgcgtgattccggtgagcggcccggcgcgctgcctgagccagagccgcaacctgctgaaaa ccaccgatgatatggtgaaaaccgcgcgcgaaaaactgaaacattatagctgcaccgcggaagata ttgatcatgaagatattacccgcgatcagaccagcaccctgaaaacctgcctgccgctggaactgca taaaaacgaaagctgcctggcgacccgcgaaaccagcagcaccacccgcggcagctgcctgccg ccgcagaaaaccagcctgatgatgaccctgtgcctgggcagcatttatgaagatctgaaaatgtatca gaccgaatttcaggcgattaacgcggcgctgcagaaccataaccatcagcagattattctggataaa ggcatgctggtggcgattgatgaactgatgcagagcctgaaccataacggcgaaaccctgcgcca gaaaccgccggtgggcgaagcggatccgtatcgcgtgaaaatgaaactgtgcattctgctgcatgc gtttagcacccgcgtggtgaccattaaccgcgtgatgggctatctgagcagcgcgagcggcggcg gcagcggcggcggcggcagcggcggcggcggcagcggcggcggcggcagcggcggcggca gcggcgatgcgcatgtgagcgaagattttacctgggaaaaaccgccggaagatccgccggatagc aaaaacaccctggtgctgtttggcgcgggctttggcgcggtgattaccgtggtggtgattgtggtgatt attaaatgcttttgcaaacatcgcagctgctttcgccgcaacgaagcgagccgcgaaaccaacaaca gcctgaccttggcccggaagaagcgctggcggaacagaccgtgtttctggaaggccgcggcagc ctgctgacctgcggcgatgtggaagaaaacccgggcccgatgcatcagaaacgcaccgcgatgttt caggatccgcaggaacgcccgcgcaaactgccgcagctgtgcaccgaactgcagaccaccattca tgatattattctggaatgcgtgtattgcaaacagcagctgctgcgccgcgaagtgtatgattttgcgtttc gcgatctgtgcattgtgtatcgcgatggcaacccgtatgcggtgtgcgataaatgcctgaaattttata gcaaaattagcgaatatcgccattattgctatagcctgtatggcaccaccctggaacagcagtataac aaaccgctgtgcgatctgctgattcgctgcattaactgccagaaaccgctgtgcccggaagaaaaac agcgccatctggataaaaaacagcgctttcataacattcgcggccgctggaccggccgctgcatga gctgctgccgcagcagccgcacccgccgcgaaacccagctg (SEQ ID NO: 59)
12	mIL12-mCD80TM-T2A-HPV16 E6 amino acid sequence	MCPQKLTISWFAIVLLVSPLMAMWELEKDVYVVEVDWTPDAP GETVNLTCDTPEEDDITWTSDQRHGVIGSGKTLTITVKEFLDAG QYTCHKGGETLSHSHLLLHKKENGIWSTEILKNFKNKTFLKCE APNYSGRFTCSWLVQRNMDLKFNIKSSSSSPDSRAVTCGMASLS AEKVTLDQRDYEKYSVSCQEDVTCPTAEETLPIELALEARQQN KYENYSTSFFIRDIIKPDPPKNLQMKPLKNSQVEVSWEYPDSWS TPHSYFSLKFFVRIQRKKEKMKETEEGCNQKGAFLVEKTSTEVQ CKGGNVCVQAQDRYYNSSCSKWACVPCRVRSGGGGSGGGGS GGGGSSRRVIPVSGPARCLSQSRNLLKTTDDMVKTAREKLKHY SCTAEDIDHEDITRDQTSTLKTCLPLELHKNESCLATRETSSTTR GSCLPPQKTSLMMTLCLGSIYEDLKMYQTEFQAINAALQNHNH QQIILDKGMLVAIDELMQSLNHNGETLRQKPPVGEADPYRVKM KLCILLHAFSTRVVTINRVMGYLSSASGGGSGGGGSGGGGSGG GGSGGGSGDAHVSEDFTWEKPPEDPPDSKNTLVLFGAGFGAVI TVVVIVVIIKCFCKHRSCFRRNEASRETNNSLTFGPEEALAEQTV FLEGRGSLLTCGDVEENPGPMHQKRTAMFQDPQERPRKLPQLC TELQTTIHDIILECVYCKQQLLRREVYDFAFRDLCIVYRDGNPYA VCDKCLKFYSKISEYRHYCYSLYGTTLEQQYNKPLCDLLIRCIN

		CQKPLCPEEKQRHLDKKQRFHNIRGRWTGRCMSCCRSSRTRRETQL (SEQ ID NO: 60)
13	mIL12-mCD80TM-T2A-HPV18 E6 cDNA Sequence	atgtgcccgcagaaactgaccattagctggtttgcgattgtgctgctggtgagcccgctgatggcgatgtgggaactggaaaaagatgtgtatgtggtggaagtggattggaccccggatgcgccgggcgaaaccgtgaacctgacctgcgatacccccggaagaagatgatattacctggaccagcgatcagcgccatggcgtgattggcagcggcaaaaccctgaccattaccgtgaaagaatttctggatgcgggccagtatacctgccataaaggcggcgaaaccctgagccatagccatctgctgctgcataaaaaagaaaacggcatttggagcaccgaaattctgaaaaactttaaaaacaaaaccttctgaaatgcgaagcgccgaactatagcggccgctttacctgcagctggctggtgcagcgcaacatggatctgaaatttaacattaaaagcagcagcagcccggatagccgcgcggtgacctgcggcatggcgagcctgagcgcggaaaaagtgaccctggatcagcgcgattatgaaaaatatagcgtgagctgccaggaagatgtgacctgcccgaccgcggaagaaaccctgccgattgaactggcgctggaagcgcgccagcagaacaaatatgaaaactatagcaccagctttttattcgcgatattattaaaccggatccgccgaaaaacctgcagatgaaaccgctgaaaaacagccaggtggaagtgagctgggaatatccggatagctggagcaccccgcatagctatttttagcctgaaatttttgtgcgcattcagcgcaaaaaagaaaaaatgaaagaaaccgaagaaggctgcaaccagaaaggcgcgtttctggtggaaaaaaccagcaccgaagtgcagtgcaaaggcggcaacgtgtgcgtgcaggcgcaggatcgctattataacagcagctgcagcaaatgggcgtgcgtgccgtgccgcgtgcgcagcggcggcggcggcagcggcggcggcggcggcagcggcggcggcggcagcagccgccgcgtgattccggtgagcggcccggcgcgctgcctgagccagagccgcaacctgctgaaaaccaccgatgatatggtgaaaaccgcgcgcgaaaaactgaaacattatagctgcaccgcggaagatattgatcatgaagatattacccgcgatcagaccagcaccctgaaaacctgcctgccgctggaactgcataaaaacgaaagctgcctggcgacccgcgaaaccagcagcaccacccgcggcagctgcctgccgccgcagaaaaccagcctgatgatgaccctgtgcctgggcagcatttatgaagatctgaaaatgtatcagaccgaatttcaggcgattaacgcggcgctgcagaaccataaccatcagcagattattctggataaaggcatgctggtggcgattgatgaactgatgcagagcctgaaccataacggcgaaaccctgcgccagaaaccgccggtgggcgaagcggatccgtatcgcgtgaaaatgaaactgtgcattctgctgcatgcgtttagcacccgcgtggtgaccattaaccgcgtgatgggctatctgagcagcgcgagcggcggcggcagcggcggcggcagcggcggcggcagcggcggcggcagcggcggcggcagcggcggcggcagcggcggcggcagcggcgatgcgcatgtgagcgaagattttacctgggaaaaaccgccggaagatccgccggatagcaaaaacaccctggtgctgtttggcgcgggctttggcgcggtgattaccgtggtggtgattgtggtgattattaaatgcttttgcaaacatcgcagctgctttcgccgcaacgaagcgagccgcgaaaccaacaacagcctgaccttggcccggaagaagcgctggcggaacagaccgtgtttctggaaggccgcggcagcctgctgacctgcggcgatgtggaagaaaacccgggcccgatggcgcgctttgaagatccgacccgccgcccgtataaactgccggatctgtgcaccgaactgaacaccagcctgcaggatattgaaattaccctgcgtgtattgcaaaaccgtgctggaactgaccgaagtgtttgaatttgcgtttaaagatctgtttgtggtgtatcgcgatagcattccgcatgcggcgtgccataaatgcattgatttttatagccgcattcgcgaactgcgccattatagcgatagcgtgtatggcgataccctggaaaaactgaccaacaccggcctgtataacctgctgattcgctgcctgcgctgccagaaaccgctgaacccggcggaaaaactgcgccatctgaacgaaaaacgccgctttcataacattgcgggccattatcgcggccagtgccatagctgctgcaaccgcgcgcgccaggaacgcctgcagcgccgccgcgaaacccaggtg (SEQ ID NO: 61)
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		AEKLRHLNEKRRFHNIAGHYRGQCHSCCNRARQERLQRRRET QV (SEQ ID NO: 62)
15	mIL12-mCD80TM-T2A-HPV16 E7 cDNA Sequence	atgtgcccgcagaaactgaccattagctggtttgcgattgtgctgctggtgagcccgctgatggcgat gtgggaactggaaaaagatgtgtatgtggtggaagtggattggaccccggatgcgccgggcgaaa ccgtgaacctgacctgcgatacccc ggaagaagatgatattacctggaccagcgatcagcgccatg gcgtgattggcagcggcaaaaccctgaccattaccgtgaaagaatttctggatgcgggccagtatac ctgccataaaggcggcgaaaccctgagccatagccatctgctgctgcataaaaaagaaaacggcat ttggagcaccgaaattctgaaaaactttaaaaacaaaaccttt ctgaaatgcgaagcgccgaactata gcggccgctttacctgcagctggctggtgcagcgcaacatggatctgaaatttaacattaaaagcag cagcagcagcccggatagccgcgcggtgacctgcggcatggcgagcctgagcgcggaaaaagt gaccctggatcagcgcgattatgaaaaatatagcgtgagctgccaggaagatgtgacctgcccgac cgcggaagaaaccctgccgattgaactggcgctggaagcgcgccagcagaacaaatatgaaaact atagcaccagctttttattcgcgatattattaaaccggatccgccgaaaaacctgcagatgaaaccgc tgaaaaacagccaggtggaagtgagctgggaatatccggatagctggagcaccccgcatagctatt ttagcctgaaatttttgtgcgcattcagcgcaaaaaagaaaaaatgaaagaaaccgaagaaggctg caaccagaaaggcgcgtttctggtggaaaaaaccagcaccgaagtgcagtgcaaaggcggcaac gtgtgcgtgcaggcgcaggatcgctattataacagcagctgcagcaaatgggcgtgcgtgccgtgc cgcgtgcgcagcggcggcggcggcagcggcggcggcggcagcggcggcggcggcagcagc cgccgcgtgattccggtgagcggcccggcgcgctgcctgagccagagccgcaacctgctgaaaa ccaccgatgatatggtgaaaaccgcgcgcgaaaaactgaaacattatagctgcaccgcggaagata ttgatcatgaagatattacccgcgatcagaccagcaccctgaaaacctgcctgccgctggaactgca taaaaacgaaagctgcctggcgacccgcgaaaccagcagcaccacccgcggcagctgcctgccg ccgcagaaaaccagcctgatgatgaccctgtgcctgggcagcatttatgaagatctgaaaatgtatca gaccgaatttcaggcgattaacgcggcgctgcagaaccataaccatcagcagattattctggataaa ggcatgctggtggcgattgatgaactgatgcagagcctgaaccataacggcgaaaccctgcgcca gaaaccgccggtgggcgaagcggatccgtatcgcgtgaaaatgaaactgtgcattctgctgcatgc gtttagcacccgcgtggtgaccattaaccgcgtgatgggctatctgagcagcgcgagcggcggcg gcagcggcggcggcggcagcggcggcggcggcagcggcggcggcggcagcggcggcggca gcggcgatgcgcatgtgagcgaagattttacctgggaaaaaccgccggaagatccgccggatagc aaaaacaccctggtgctgtttggcgcgggctttggcgcggtgattaccgtggtggtgattgtggtgatt attaaatgcttttgcaaacatcgcagctgctttcgccgcaacgaagcgagccgcgaaaccaacaaca gcctgaccttt ggcccggaagaagcgctggcggaacagaccgtgtttctggaaggccgcggcagc ctgctgacctgcggcgatgtggaagaaaacccgggcccgatgcatggcgataccccgaccctgca tgaatatatgctggatctgcagccggaaaccaccgatctgtattgctatgaacagctgaacgatagca gcgaagaagaagatgaaattgatggcccggcgggccaggcggaaccggatcgcgcgcattataa cattgtgacctttt gctgcaaatgcgatagcaccctgcgcctgtgcgtgcagagcacccatgtggata ttcgcaccctggaagatctgctgatgggcaccctgggcattgtgtgcccgatttgcagccagaaacc g (SEQ ID NO: 63)
16	mIL12-mCD80TM-T2A-HPV16 E7 amino acid sequence	MCPQKLTISWFAIVLLVSPLMAMWELEKDVYVVEVDWTPDAP GETVNLTCDTPEEDDITWTSDQRHGVIGSGKTLTITVKEFLDAG QYTCHKGGETLSHSHLLLHKKENGIWSTEILKNFKNKTFLKCE APNYSGRFTCSWLVQRNMDLKFNIKSSSSSPDSRAVTCGMASLS AEKVTLDQRDYEKYSVSCQEDVTCPTAEETLPIELALEARQQN KYENYSTSFFIRDIIKPDPPKNLQMKPLKNSQVEVSWEYPDSWS TPHSYFSLKFFVRIQRKKEKMKETEEGCNQKGAFLVEKTSTEVQ CKGGNVCVQAQDRYYNSSCSKWACVPCRVRSGGGGSGGGGS GGGGSSRRVIPVSGPARCLSQSRNLLKTTDDMVKTAREKLKHY SCTAEDIDHEDITRDQTSTLKTCLPLELHKNESCLATRETSSTTR GSCLPPQKTSLMMTLCLGSIYEDLKMYQTEFQAINAALQNHNH QQIILDKGMLVAIDELMQSLNHNGETLRQKPPVGEADPYRVKM KLCILLHAFSTRVVTINRVMGYLSSASGGGSGGGGSGGGGSGG GGSGGGSGDAHVSEDFTWEKPPEDPPDSKNTLVLFGAGFGAVI TVVVIVVIIKCFCKHRSCFRRNEASRETNNSLTFGPEEALAEQTV FLEGRGSLLTCGDVEENPGPMHGDTPTLHEYMLDLQPETTDLY CYEQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFCCKCDSTLRL CVQSTHVDIRTLEDLLMGTLGIVCPICSQKP (SEQ ID NO: 64)

17	mIL12-mCD80TM-T2A-HPV18 E7 cDNA Sequence	atgtgcccgcagaaactgaccattagctggtttgcgattgtgctgctggtgagcccgctgatggcgat gtgggaactggaaaaagatgtgtatgtggtggaagtggattggaccccggatgcgccgggcgaaa ccgtgaacctgacctgcgatacccggaagaagatgatattacctggaccagcgatcagcgccatg gcgtgattggcagcggcaaaaccctgaccattaccgtgaaagaatttctggatgcgggccagtatac ctgccataaaggcggcgaaaccctgagccatagccatctgctgctgcataaaaaagaaaacggcat ttggagcaccgaaattctgaaaaactttaaaaacaaaaccctttctgaaatgcgaagcgccgaactata gcggccgctttacctgcagctggctggtgcagcgcaacatggatctgaaatttaacattaaaagcag cagcagcagcccggatagccgcgcggtgacctgcggcatggcgagcctgagcgcggaaaaagt gaccctggatcagcgcgattatgaaaaatatagcgtgagctgccaggaagatgtgacctgcccgac cgcggaagaaaccctgccgattgaactggcgctggaagcgcgccagcagaacaaatatgaaaact atagcaccagctttttattcgcgatattattaaaccggatccgccgaaaaacctgcagatgaaaccgc tgaaaaacagccaggtggaagtgagctgggaatatccggatagctggagcaccccgcatagctatt ttagcctgaaatttttgtgcgcattcagcgcaaaaaagaaaaaatgaaagaaaccgaagaaggctg caaccagaaaggcgcgtttctggtggaaaaaaccagcaccgaagtgcagtgcaaaggcggcaac gtgtgcgtgcaggcgcaggatcgctattataacagcagctgcagcaaatgggcgtgcgtgccgtgc cgcgtgcgcagcggcggcggcggcagcggcggcggcggcagcggcggcggcggcagcagc cgccgcgtgattccggtgagcggcccggcgcgctgcctgagccagagccgcaacctgctgaaaa ccaccgatgatatggtgaaaaccgcgcgcgaaaaactgaaacattatagctgcaccgcggaagata ttgatcatgaagatattacccgcgatcagaccagcaccctgaaaacctgcctgccgctggaactgca taaaaacgaaagctgcctggcgacccgcgaaaccagcagcaccacccgcggcagctgcctgccg ccgcagaaaaccagcctgatgatgaccctgtgcctgggcagcatttatgaagatctgaaaatgtatca gaccgaatttcaggcgattaacgcggcgctgcagaaccataaccatcagcagattattctggataaa ggcatgctggtggcgattgatgaactgatgcagagcctgaaccataacggcgaaaccctgcgcca gaaaccgccggtgggcgaagcggatccgtatcgcgtgaaaatgaaactgtgcattctgctgcatgc gtttagcacccgcgtggtgaccattaaccgcgtgatgggctatctgagcagcgcgagcggcggcg gcagcggcggcggcggcagcggcggcggcggcagcggcggcggcggcagcggcggcggca gcggcgatgcgcatgtgagcgaagattttacctgggaaaaaccgccggaagatccgccggatagc aaaaacaccctggtgctgtttggcgcgggctttggcgcggtgattaccgtggtggtgattgtggtgatt attaaatgcttttgcaaacatcgcagctgctttcgccgcaacgaagcgagccgcgaaaccaacaaca gcctgaccttggcccggaagaagcgctggcggaacagaccgtgtttctggaaggccgcggcagc ctgctgacctgcggcgatgtggaagaaaacccgggcccgatgcatggcccgaaagcgaccctgc aggatattgtgctgcatctggaaccgcagaacgaaattccggtggatctgctgtgccatgaacagct gagcgatagcgaagaagaaaacgatgaaattgatggcgtgaaccatcagcatctgccggcgcgcc gcgcggaaccgcagcgccataccatgctgtgcatgtgctgcaaatgcgaagcgcgcattaaactgg tggtggaaagcagcgcggatgatctgcgcgcgtttcagcagctgtttctgaacaccctgagctttgtg tgcccgtggtgcgcgagccagcag (SEQ ID NO: 65)
18	mIL12-mCD80TM-T2A-HPV18 E7 amino acid sequence	MCPQKLTISWFAIVLLVSPLMAMWELEKDVYVVEVDWTPDAP GETVNLTCDTPEEDDITWTSDQRHGVIGSGKTLTITVKEFLDAG QYTCHKGGETLSHSHLLLHKKENGIWSTEILKNFKNKTFLKCE APNYSGRFTCSWLVQRNMDLKFNIKSSSSSPDSRAVTCGMASLS AEKVTLDQRDYEKYSVSCQEDVTCPTAEETLPIELALEARQQN KYENYSTSFFIRDIIKPDPPKNLQMKPLKNSQVEVSWEYPDSWS TPHSYFSLKFFVRIQRKKEKMKETEEGCNQKGAFLVEKTSTEVQ CKGGNVCVQAQDRYYNSSCSKWACVPCRVRSGGGGSGGGGS GGGGSSRRVIPVSGPARCLSQSRNLLKTTDDMVKTAREKLKHY SCTAEDIDHEDITRDQTSTLKTCLPLELHKNESCLATRETSSTTR GSCLPPQKTSLMMTLCLGSIYEDLKMYQTEFQAINAALQNHNH QQIILDKGMLVAIDELMQSLNHNGETLRQKPPVGEADPYRVKM KLCILLHAFSTRVVTINRVMGYLSSASGGGSGGGGSGGGGSGG GGSGGGSGDAHVSEDFTWEKPPEDPPDSKNTLVLFGAGFGAVI TVVVIVVIIKCFCKHRSCFRRNEASRETNNSLTFGPEEALAEQTV FLEGRGSLLTCGDVEENPGPMHGPKATLQDIVLHLEPQNEIPVD LLCHEQLSDSEEENDEIDGVNHQHLPARRAEPQRHTMLCMCCK CEARIKLVVESSADDLRAFQQLFLNTLSFVCPWCASQQ (SEQ ID NO: 66)

19	mIL12-T2A-OVA cDNA Sequence	atgtgtcctcagaagctaaccatctcctggtttgccatcgttttgctggtgtctccactcatggccatgtg ggagctggagaaagacgtttatgttgtagaggtggactggactcccgatgcccctggagaaacagt gaacctcacctgtgacacgcctgaagaagatgacatcacctggacctcagaccagagacatggagt cataggctctggaaagaccctgaccatcactgtcaaagagtttctagatgctggccagtacacctgcc acaaaggaggcgagactctgagccactcacatctgctgctccacaagaaggaaaatggaatttggt ccactgaaatttaaaaaatttcaaaaacaagactttcctgaagtgtgaagcaccaaattactccggac ggttcacgtgctcatggctggtgcaaagaaacatggacttgaagttcaacatcaagagcagtagcag ttcccctgactctcgggcagtgacatgtggaatggcgtctctgtctgcagagaaggtcacactggac caaagggactatgagaagtattcagtgtcctgccaggaggatgtcacctgcccaactgccgaggag accctgcccattgaactggcgttggaagcacggcagcagaataaatatgagaactacagcaccagc ttcttcatcagggacatcatcaaaccagacccgcccaagaacttgcagatgaagcctttgaagaactc acaggtggaggtcagctgggagtaccctgactcctggagcactccccattcctacttctccctcaagt tctttgttcgaatccagcgcaagaaagaaaagatgaaggagacagaggaggggtgtaaccagaaa ggtgcgttcctcgtagagaagacatctaccgaagtccaatgcaaaggcgggaatgtctgcgtgcaa gctcaggatcgctattacaattcctcatgcagcaagtgggcatgtgttccctgcagggtccgatccgg cggaggcggatcaggcggcggtggatctgggggtggcggatcttcgcgaagggtcattccagtct ctggacctgccaggtgtcttagccagtcccgaaacctgctgaagaccacagatgacatggtgaaga cggccagagaaaaactgaaacattattcctgcactgctgaagacatcgatcatgaagacatcacacg ggaccaaaccagcacattgaagacctgtttaccactggaactacacaagaacgagagttgcctggct actagagagacttcttccacaacaagagggagctgcctgcccccacagaagacgtctttgatgatga ccctgtgccttggtagcatctatgaggacttgaagatgtaccagacagagttccaggccatcaacgc agcacttcagaatcacaaccatcagcagatcattctagacaagggcatgctggtggccatcgatgag ctgatgcagtctctgaatcataatggcgagactctgcgccagaaacctcctgtgggagaagcagacc cttacagagtgaaaatgaagctctgcatcctgcttcacgccttcagcacccgcgtcgtgaccatcaac agggtgatgggctatctgagctccgccgagggcaggggcagcctgctgacctgcggagacgtgg aggagaaccctggccccatgctggagcagctggagtccatcatcaacttcgagaagctgaccgagt ggacctcctga (SEQ ID NO: 67)
20	mIL12-T2A-OVA amino acid sequence	MCPQKLTISWFAIVLLVSPLMAMWELEKDVYVVEVDWTPDAP GETVNLTCDTPEEDDITWTSDQRHGVIGSGKTLTITVKEFLDAG QYTCHKGGETLSHSHLLLHKKENGIWSTEILKNFKNKTFLKCE APNYSGRFTCSWLVQRNMDLKFNIKSSSSSPDSRAVTCGMASLS AEKVTLDQRDYEKYSVSCQEDVTCPTAEETLPIELALEARQQN KYENYSTSFFIRDIIKPDPPKNLQMKPLKNSQVEVSWEYPDSWS TPHSYFSLKFFVRIQRKKEKMKETEEGCNQKGAFLVEKTSTEVQ CKGGNVCVQAQDRYYNSSCSKWACVPCRVRSGGGGSGGGGS GGGGSSRRVIPVSGPARCLSQSRNLLKTTDDMVKTAREKLKHY SCTAEDIDHEDITRDQTSTLKTCLPLELHKNESCLATRETSSTTR GSCLPPQKTSLMMTLCLGSIYEDLKMYQTEFQAINAALQNHNH QQIILDKGMLVAIDELMQSLNHNGETLRQKPPVGEADPYRVKM KLCILLHAFSTRVVTINRVMGYLSSAEGRGSLLTCGDVEENPGP MLEQLESIINFEKLTEWTS (SEQ ID NO: 68)
S1	mtGFP-T2A-OVA cDNA Sequence	ATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGG GTTCCAGGTTCCACTGGTTCGCGAatggtgagcaagggcgaggagctgttca ccggggtggtgcccatcctggtcgagctggacggcgacgtaaacggccacaagttcagcgtgtcc ggcgagggcgagggcgatgccacctacggcaagctgaccctgaagttcatctgcaccaccggca agctgcccgtgccctggcccaccctcgtgaccaccctgacctacggcgtgcagtgcttcagccgct accccgaccacatgaagcagcacgacttcttcaagtccgccatgcccgaaggctacgtccaggag cgcaccatcttcttcaaggacgacggcaactacaagacccgcgccgaggtgaagttcgagggcga caccctggtgaaccgcatcgagctgaagggcatcgacttcaaggaggacggcaacatcctggggc acaagctggagtacaactacaacagccacaacgtctatatcatggccgacaagcagaagaacggc atcaaggtgaacttcaagatccgccacaacatcgaggacggcagcgtgcagctcgccgaccacta ccagcagaacaccccatcggcgacggccccgtgctgctgcccgacaaccactacctgagcacc cagtccgccctgagcaaagaccccaacgagaagcgcgatcacatggtcctgctggagttcgtgac cgccgccgggatcactctcggcatggacgagctgtacaagTCCGGAGGAGGCTCCG GAGGAGGAGGCAGCGGAGGAGGAGGATCCGGCGGAGGAGG CTCTGGCGGAGGAAGCGGCGATGCCCACGTGAGCGAGGATT TCACATGGGAGAAGCCCCCCGAGGACCCTCCCGACAGCAAG AACACACTGGTGCTGTTTGGCGCCGGCTTCGGCGCCGTGATC ACAGTGGTGGTCATTGTGGTCATTATCAAGTGCTTCTGCAAG

		CACAGATCCTGTTTCAGGAGAAATGAGGCCAGCAGAGAGAC AAACAACAGCCTGACATTTGGCCCTGAGGAGGCCCTGGCCG AGCAGACAGTGTTTCTGGAGGGCAGGGGCAGCCTGCTGACC TGCGGAGACGTGGAGGAGAACCCTGGCCCCATGCTGGAGCA GCTGGAGTCCATCATCAACTTCGAGAAGCTGACCGAGTGGAC CTCCTGA (SEQ ID NO: 69)
S2	mtGFP-T2A-OVA amino acid sequence	METDTLLLWVLLLWVPGSTGSRMVSKGEELFTGVVPILVELDG DVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTL TYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNY KTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHN VYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGP VLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDE LYKSGGGSGGGGSGGGGSGGGGSGGGSGDAHVSEDFTWEKPP EDPPDSKNTLVLFGAGFGAVITVVVIVVIIKCFCKHRSCFRRNEA SRETNNSLTFGPEEALAEQTVFLEGRGSLLTCGDVEENPGPMLE QLESIINFEKLTEWTS* (SEQ ID NO: 70)
S3	mtGFP-T2A-E7 cDNA Sequence	ATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGG GTTCCAGGTTCCACTGGTTCGCGAatggtgagcaagggcgaggagctgttca ccggggtggtgcccatcctggtcgagctggacggcgacgtaaacggccacaagttcagcgtgtcc ggcgagggcgagggcgatgccacctacggcaagctgaccctgaagttcatctgcaccaccggca agctgcccgtgccctggcccaccctcgtgaccaccctgacctacggcgtgcagtgcttcagccgct accccgaccacatgaagcagcacgacttcttcaagtccgccatgcccgaaggctacgtccaggag cgcaccatcttcttcaaggacgacggcaactacaagacccgcgccgaggtgaagttcgagggcga caccctggtgaaccgcatcgagctgaagggcatcgacttcaaggaggacggcaacatcctggggc acaagctggagtacaactacaacagccacaacgtctatatcatggccgacaagcagaagaacggc atcaaggtgaacttcaagatccgccacaacatcgaggacggcagcgtgcagctcgccgaccacta ccagcagaacaccccatcggcgacggccccgtgctgctgcccgacaaccactacctgagcacc cagtccgccctgagcaaagaccccaacgagaagcgcgatcacatggtcctgctggagttcgtgac cgccgccgggatcactctcggcatggacgagctgtacaagTCCGGAGGAGGCTCCG GAGGAGGAGGCAGCGGAGGAGGAGGATCCGGCGGAGGAGG CTCTGGCGGAGGAAGCGGCGATGCCCACGTGAGCGAGGATT TCACATGGGAGAAGCCCCCCGAGGACCCTCCCGACAGCAAG AACACACTGGTGCTGTTTGGCGCCGGCTTCGGCGCCGTGATC ACAGTGGTGGTCATTGTGGTCATTATCAAGTGCTTCTGCAAG CACAGATCCTGTTTCAGGAGAAATGAGGCCAGCAGAGAGAC AAACAACAGCCTGACATTTGGCCCTGAGGAGGCCCTGGCCG AGCAGACAGTGTTTCTGGAGGGCAGGGGCAGCCTGCTGACC TGCGGAGACGTGGAGGAGAACCCTGGCCCCATGCTGGAGCA GCTGGAGCGGGCCCACTATAACATCGTCACTTTTACCGAGTG GACCTCC (SEQ ID NO: 71)
S4	mtGFP-T2A-E7 amino acid sequence	METDTLLLWVLLLWVPGSTGSRMVSKGEELFTGVVPILVELDG DVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTL TYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNY KTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHN VYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGP VLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDE LYKSGGGSGGGGSGGGGSGGGGSGGGSGDAHVSEDFTWEKPP EDPPDSKNTLVLFGAGFGAVITVVVIVVIIKCFCKHRSCFRRNEA SRETNNSLTFGPEEALAEQTVFLEGRGSLLTCGDVEENPGPMLE QLERAHYNIVTFTEWTS (SEQ ID NO: 72)
S5	mtGFP-T2A-p15E nucleotide sequence	ATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGG GTTCCAGGTTCCACTGGTTCGCGAatggtgagcaagggcgaggagctgttca ccggggtggtgcccatcctggtcgagctggacggcgacgtaaacggccacaagttcagcgtgtcc ggcgagggcgagggcgatgccacctacggcaagctgaccctgaagttcatctgcaccaccggca agctgcccgtgccctggcccaccctcgtgaccaccctgacctacggcgtgcagtgcttcagccgct accccgaccacatgaagcagcacgacttcttcaagtccgccatgcccgaaggctacgtccaggag cgcaccatcttcttcaaggacgacggcaactacaagacccgcgccgaggtgaagttcgagggcga caccctggtgaaccgcatcgagctgaagggcatcgacttcaaggaggacggcaacatcctggggc

		acaagctggagtacaactacaacagccacaacgtctatatcatggccgacaagcagaagaacggc atcaaggtgaacttcaagatccgccacaacatcgaggacggcagcgtgcagctcgccgaccacta ccagcagaacaccccatcggcgacggccccgtgctgctgcccgacaaccactacctgagcacc cagtccgccctgagcaaagaccccaacgagaagcgcgatcacatggtcctgctggagttcgtgac cgccgccgggatcactctcggcatggacgagctgtacaagTCCGGAGGAGGCTCCG GAGGAGGAGGCAGCGGAGGAGGAGGATCCGGCGGAGGAGG CTCTGGCGGAGGAAGCGGCGATGCCCACGTGAGCGAGGATT TCACATGGGAGAAGCCCCCCGAGGACCCTCCCGACAGCAAG AACACACTGGTGCTGTTTGGCGCCGGCTTCGGCGCCGTGATC ACAGTGGTGGTCATTGTGGTCATTATCAAGTGCTTCTGCAAG CACAGATCCTGTTTCAGGAGAAATGAGGCCAGCAGAGAGAC AAACAACAGCCTGACATTTGGCCCTGAGGAGGCCCTGGCCG AGCAGACAGTGTTTCTGGAGGGCAGGGGCAGCCTGCTGACC TGCGGAGACGTGGAGGAGAACCCTGGCCCCATGCTGGAGCA GCTGGAGaaaagcccgtggtttaccaccctgACCGAGTGGACCTCCTGA (SEQ ID NO: 73)
S6	mtGFP-T2A-p15E amino acid sequence	METDTLLLWVLLLWVPGSTGSRMVSKGEELFTGVVPILVELDG DVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTL TYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNY KTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHN VYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGP VLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDE LYKSGGGSGGGGSGGGGSGGGGSGGGSGDAHVSEDFTWEKPP EDPPDSKNTLVLFGAGFGAVITVVVIVVIIKCFCKHRSCFRRNEA SRETNNSLTFGPEEALAEQTVFLEGRGSLLTCGDVEENPGPMLE QLEKSPWFTTLTEWTS (SEQ ID NO: 74)
S7	Human IL12B-(G4S)3-IL12A nucleotide sequence	ATGTGTCATCAACAGCTTGTTATTTCATGGTTTTCACTCGTATT CCTCGCTTCCCCTCTCGTAGCTATTTGGGAACTCAAAAAAGAT GTGTATGTTGTAGAATTAGACTGGTATCCTGACGCCCCCGGAG AAATGGTGGTATTGACCTGCGACACACCAGAAGAAGATGGA ATTACCTGGACTCTGGACCAAAGTTCAGAAGTATTAGGTTCT GGAAAGACTCTAACCATACAGGTTAAAGAGTTCGGGGATGCG GGACAGTACACCTGTCATAAAGGGGGCGAAGTGTTAAGTCA CTCCCTGCTCCTGCTTCACAAGAAGGAGGACGGCATCTGGTC GACCGATATTCTGAAAGATCAGAAGGAACCTAAAAATAAGAC GTTTCTGAGATGTGAAGCTAAGAATTATTCAGGACGGTTTACA TGCTGGTGGCTTACAACAATTAGCACAGATCTTACGTTCTCGG TGAAGAGTTCTAGGGGGTCTTCAGATCCCCAGGGCGTGACTT GCGGTGCCGCAACCCTCAGCGCTGAAAGGGTTCGTGGGGAT AACAAAGAATATGAATACAGCGTGGAATGCCAAGAGGATTCT GCATGTCCTGCAGCTGAAGAGTCCCTACCGATTGAGGTCATG GTCGATGCAGTGCATAAGCTAAAATATGAGAATTACACTTCCT CGTTCTTTATACGCGACATCATCAAACCAGACCCTCCTAAGAA TTTGCAGCTCAAGCCACTAAAGAACTCGCGGCAGGTCGAAG TTTCATGGGAGTACCCTGACACTTGGAGCACTCCGCACTCATA TTTCTCACTGACTTTTTGTGTCCAAGTTCAGGGAAAGAGTAA GCGGGAGAAAAAAGATCGCGTCTTCACTGACAAAACGAGTG CCACAGTAATTTGTAGGAAAAATGCCTCCATCTCAGTGCGTG CGCAGGACCGCTACTATAGCAGTTCCTGGAGCGAGTGGGCCT CTGTGCCCTGCTCAGGTGGTGGGGGTAGCGGCGGTGGCGGC AGCGGAGGAGGAGGCAGTCGAAACCTGCCCGTGGCTACCCC AGATCCTGGCATGTTCCCATGCCTTCATCACTCCCAGAACCTC CTTCGAGCAGTATCTAACATGCTCCAGAAAGCTAGACAGACG TTGGAGTTTTACCCCTGTACCAGTGAGGAGATCGATCATGAG GACATCACTAAGGATAAGACATCTACAGTGGAGGCGTGTCTG CCTCTGGAGCTGACAAAAAACGAGAGCTGTCTCAATAGCAG AGAGACCAGCTTCATTACTAATGGGTCCTGTCTTGCCAGTAG AAAGACCTCCTTCATGATGGCCTTATGCTTGTCCTCAATTTAT GAAGACCTGAAGATGTACCAGGTGGAGTTTAAAACAATGAA

		CGCTAAGTTGCTTATGGACCCAAAAAGGCAGATCTTTTTGGA CCAAAACATGCTGGCTGTCATAGACGAGCTGATGCAGGCCCT AAACTTTAATTCTGAAACAGTTCCCCAAAAATCTAGCCTCGA AGAACCCGATTTCTACAAGACAAAAATTAAATTATGCATCCTA TTACACGCCTTTCGTATCCGAGCAGTAACAATCGACAGAGTA ATGAGTTATCTAAACGCCAGCTAA (SEQ ID NO: 75)
S8	Human IL12B-(G4S)3-IL12A amino acid sequence	MCHQQLVISWFSLVFLASPLVAIWELKKDVYVVELDWYPDAPG EMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLTIQVKEFGDAG QYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFL RCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAA TLSAERVRGDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVH KLKYENYTSSFFIRDIIKPDPPKNLQLKPLKNSRQVEVSWEYPDT WSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSATVICRKNAS ISVRAQDRYYSSSWSEWASVPCS GGGGS GGGGS GGGGS RNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCT SEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCL ASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIF LDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCIL LHAFRIRAVTIDRVMSYLNAS (SEQ ID NO: 76)
S9	murineIL12-TM-T2A-P15E nucleotide sequence	atgtgtcctcagaagctaaccatctcctggtttgccatcgttttgctggtgtctccactcatggccatgtg ggagctggagaaagacgtttatgttgtagaggtggactggactcccgatgcccctggagaaacagt gaacctcacctgtgacacgcctgaagaagatgacatcacctggacctcagaccagagacatggagt cataggctctggaaagaccctgaccatcactgtcaaagagtttctagatgctggccagtacacctgcc acaaaggaggcgagactctgagccactcacatctgctgctccacaagaaggaaaatggaatttggt ccactgaaatttaaaaaatttcaaaaacaagactttcctgaagtgtgaagcaccaaattactccggac ggttcacgtgctcatggctggtgcaaagaaacatggacttgaagttcaacatcaagagcagtagcag ttcccctgactctcgggcagtgacatgtggaatggcgtctctgtctgcagagaaggtcacactggac caaagggactatgagaagtattcagtgtcctgccaggaggatgtcacctgcccaactgccgaggag accctgcccattgaactggcgttggaagcacggcagcagaataaatatgagaactacagcaccagc ttcttcatcagggacatcatcaaaccagacccgcccaagaacttgcagatgaagcctttgaagaactc acaggtggaggtcagctgggagtaccctgactcctggagcactccccattcctacttctccctcaagt tctttgttcgaatccagcgcaagaaagaaaagatgaaggagacagaggaggggtgtaaccagaaaa ggtgcgttcctcgtagagaagacatctaccgaagtccaatgcaaaggcgggaatgtctgcgtgcaa gctcaggatcgctattacaattcctcatgcagcaagtgggcatgtgttccctgcagggtccgatccgg cggaggcggatcaggcggcggtggatctgggggtggcggatcttcgcgaagggtcattccagtct ctggacctgccaggtgtcttagccagtcccgaaacctgctgaagaccacagatgacatggtgaaga cggccagagaaaaactgaaacattattcctgcactgctgaagacatcgatcatgaagacatcacacg ggaccaaaccagcacattgaagacctgtttaccactggaactacacaagaacgagagttgcctggct actagagagacttcttccacaacaagagggagctgcctgcccccacagaagacgtctttgatgatga ccctgtgccttggtagcatctatgaggacttgaagatgtaccagacagagttccaggccatcaacgc agcacttcagaatcacaaccatcagcagatcattctagacaagggcatgctggtggccatcgatgag ctgatgcagtctctgaatcataatggcgagactctgcgccagaaacctcctgtgggagaagcagacc cttacagagtgaaaatgaagctctgcatcctgcttcacgccttcagcacccgcgtcgtgaccatcaac agggtgatgggctatctgagctccgccTCCGGAGGAGGCTCCGGAGGAGGA GGCAGCGGAGGAGGAGGATCCGGCGGAGGAGGCTCTGGCG GAGGAAGCGGCGATGCCCACGTGAGCGAGGATTTCACATGG GAGAAGCCCCCCGAGGACCCTCCCGACAGCAAGAACACACT GGTGCTGTTTGGCGCCGGCTTCGGCGCCGTGATCACAGTGGT GGTCATTGTGGTCATTATCAAGTGCTTCTGCAAGCACAGATCC TGTTTCAGGAGAAATGAGGCCAGCAGAGAGACAAACAACAG CCTGACATTTGGCCCTGAGGAGGCCCTGGCCGAGCAGACAG TGTTTCTGGAGGGCAGGGGCAGCCTGCTGACCTGCGGAGAC GTGGAGGAGAACCCTGGCCCCATGCTGGAGCAGCTGGAGaaa agcccgtggtttaccaccctgACCGAGTGGACCTCCTGA (SEQ ID NO: 77)

S10	murineIL12-TM-T2A-P15E amino acid sequence	MCPQKLTISWFAIVLLVSPLMAMWELEKDVYVVEVDWTPDAP GETVNLTCDTPEEDDITWTSDQRHGVIGSGKTLTITVKEFLDAG QYTCHKGGETLSHSHLLLHKKENGIWSTEILKNFKNKTFLKCE APNYSGRFTCSWLVQRNMDLKFNIKSSSSSPDSRAVTCGMASLS AEKVTLDQRDYEKYSVSCQEDVTCPTAEETLPIELALEARQQN KYENYSTSFFIRDIIKPDPPKNLQMKPLKNSQVEVSWEYPDSWS TPHSYFSLKFFVRIQRKKEKMKETEEGCNQKGAFLVEKTSTEVQ CKGGNVCVQAQDRYYNSSCSKWACVPCRVRSGGGGSGGGGS GGGGSSRRVIPVSGPARCLSQSRNLLKTTDDMVKTAREKLKHY SCTAEDIDHEDITRDQTSTLKTCLPLELHKNESCLATRETSSTTR GSCLPPQKTSLMMTLCLGSIYEDLKMYQTEFQAINAALQNHNH QQIILDKGMLVAIDELMQSLNHNGETLRQKPPVGEADPYRVKM KLCILLHAFSTRVVTINRVMGYLSSASGGGSGGGGSGGGGSGG GGSGGGSGDAHVSEDFTWEKPPEDPPDSKNTLVLFGAGFGAVI TVVVIVVIIKCFCKHRSCFRRNEASRETNNSLTFGPEEALAEQTV FLEGRGSLLTCGDVEENPGPMLEQLEKSPWFTTLTEWTS (SEQ ID NO: 78)
S11	E6E7-PEST nucleotide sequence	ATGCACGGCGATACGCCTACCCTGCACGAGTACATGCTCGAC CTGCAGCCAGAGACCACGGACCTCTACTGCTATGAGCAGTTG AATGACAGCAGCGAAGAGGAGGATGAGATAGATGGGCCCGC CGGTCAGGCTGAGCCTGACCGGGCCCACTATAACATCGTCAC TTTTTGCTGCAAGTGTGATTCAACGCTCAGGCTGTGCGTGCA GAGCACGCACGTGGACATACGAACCCTAGAGGATCTCCTGAT GGGGACCCTAGGGATCGTATGTCCAATATGTAGTCAGAAGCC CATGCACCAGAAGCGGACAGCTATGTTCCAGGACCCGCAGG AGCGGCCCCGGAAGCTGCCGCAGCTGTGCACGGAGCTTCAG ACTACAATACACGACATCATCCTTGAGTGCGTGTATTGTAAGC AGCAGCTCCTGAGACGCGAAGTCTATGACTTCGCGTTTCGGG ACCTCTGCATCGTATACCGCGACGGTAATCCGTACGCGGTATG CGATAAGTGTTTGAAGTTCTATTCAAAGATCAGCGAGTACAG GCACTACTGTTACTCGCTGTATGGTACAACGTTGGAGCAGCA GTACAACAAGCCGTTGTGCGACCTGCTCATACGTTGTATCAAT TGCCAGAAGCCGCTGTGTCCTGAGGAGAAGCAGCGGCATCT GGACAAAAAGCAGAGGTTCCACAACATCCGTGGTCGCTGGA CAGGTCGATGCATGTCTTGTTGCAGGAGTAGCCGTACTCGCC GTGAGACTCAGTTAAAGCTTAGCCATGGCTTCCCGCCGGAGG TGGAGGAGCAGGATGATGGCACGCTGCCCATGTCTTGTGCCC AGGAGAGCGGGATGGACCGTCACCCTGCAGCCTGTGCTTCT GCTAGGATCAATGTGTGA (SEQ ID NO: 79)
S12	E6E7-PEST amino acid sequence	MHGDTPTLHEYMLDLQPETTDLYCYEQLNDSSEEEDEIDGPAG QAEPDRAHYNIVTFCCKCDSTLRLCVQSTHVDIRTLEDLLMGTL GIVCPICSQKPMHQKRTAMFQDPQERPRKLPQLCTELQTTIHDII LECVYCKQQLLRREVYDFAFRDLCIVYRDGNPYAVCDKCLKFY SKISEYRHYCYSLYGTTLEQQYNKPLCDLLIRCINCQKPLCPEEK QRHLDKKQRFHNIRGRWTGRCMSCCRSSRTRRETQLKLSHGFP PEVEEQDDGTLPMSCAQESGMDRHPAACASARINV* (SEQ ID NO: 80)
S13	Human IL12-TM-T2A-E6/E7-PEST nucleotide sequence	ATGTGTCATCAACAGCTTGTTATTTCATGGTTTTCACTCGTATT CCTCGCTTCCCCTCTCGTAGCTATTTGGGAACTCAAAAAAGAT GTGTATGTTGTAGAATTAGACTGGTATCCTGACGCCCCCGGAG AAATGGTGGTATTGACCTGCGACACACCAGAAGAAGATGGA ATTACCTGGACTCTGGACCAAAGTTCAGAAGTATTAGGTTCT GGAAAGACTCTAACCATACAGGTTAAAGAGTTCGGGGATGCG GGACAGTACACCTGTCATAAAGGGGGCGAAGTGTTAAGTCA CTCCCTGCTCCTGCTTCACAAGAAGGAGGACGGCATCTGGTC GACCGATATTCTGAAAGATCAGAAGGAACCTAAAAATAAGAC GTTTCTGAGATGTGAAGCTAAGAATTATTCAGGACGGTTTACA TGCTGGTGGCTTACAACAATTAGCACAGATCTTACGTTCTCGG

		TGAAGAGTTCTAGGGGGTCTTCAGATCCCCAGGGCGTGACTT GCGGTGCCGCAACCCTCAGCGCTGAAAGGGTTCGTGGGGAT AACAAAGAATATGAATACAGCGTGGAATGCCAAGAGGATTCT GCATGTCCTGCAGCTGAAGAGTCCCTACCGATTGAGGTCATG GTCGATGCAGTGCATAAGCTAAAATATGAGAATTACACTTCCT CGTTCTTTATACGCGACATCATCAAACCAGACCCTCCTAAGAA TTTGCAGCTCAAGCCACTAAAGAACTCGCGGCAGGTCGAAG TTTCATGGGAGTACCCTGACACTTGGAGCACTCCGCACTCATA TTTCTCACTGACTTTTTGTGTCCAAGTTCAGGGAAAGAGTAA GCGGGAGAAAAAAGATCGCGTCTTCACTGACAAAACGAGTG CCACAGTAATTTGTAGGAAAAATGCCTCCATCTCAGTGCGTG CGCAGGACCGCTACTATAGCAGTTCCTGGAGCGAGTGGGCCT CTGTGCCCTGCTCAGGTGGTGGGGGTAGCGGCGGTGGCGGC AGCGGAGGAGGAGGCAGTCGAAACCTGCCCGTGGCTACCCC AGATCCTGGCATGTTCCCATGCCTTCATCACTCCCAGAACCTC CTTCGAGCAGTATCTAACATGCTCCAGAAAGCTAGACAGACG TTGGAGTTTTACCCCTGTACCAGTGAGGAGATCGATCATGAG GACATCACTAAGGATAAGACATCTACAGTGGAGGCGTGTCTG CCTCTGGAGCTGACAAAAAACGAGAGCTGTCTCAATAGCAG AGAGACCAGCTTCATTACTAATGGGTCCTGTCTTGCCAGTAG AAAGACCTCCTTCATGATGGCCTTATGCTTGTCCTCAATTTAT GAAGACCTGAAGATGTACCAGGTGGAGTTTAAAACAATGAA CGCTAAGTTGCTTATGGACCCAAAAAGGCAGATCTTTTTGGA CCAAAACATGCTGGCTGTCATAGACGAGCTGATGCAGGCCCT AAACTTTAATTCTGAAACAGTTCCCCAAAAATCTAGCCTCGA AGAACCCGATTTCTACAAGACAAAAATTAAATTATGCATCCTA TTACACGCCTTTCGTATCCGAGCAGTAACAATCGACAGAGTA ATGAGTTATCTAAACGCCAGCTCCGGAGGAGGCTCCGGAGGA GGAGGCAGCGGAGGAGGAGGATCCGGCGGAGGAGGCTCTG GCGGAGGAAGCGGCGATGCCCACGTGAGCGAGGATTTCACA TGGGAGAAGCCCCCCGAGGACCCTCCCGACAGCAAGAACAC ACTGGTGCTGTTTGGCGCCGGCTTCGGCGCCGTGATCACAGT GGTGGTCATTGTGGTCATTATCAAGTGCTTCTGCAAGCACAG ATCCTGTTTCAGGAGAAATGAGGCCAGCAGAGAGACAAACA ACAGCCTGACATTTGGCCCTGAGGAGGCCCTGGCCGAGCAG ACAGTGTTTCTGGAGGGCAGGGGCAGCCTGCTGACCTGCGG AGACGTGGAGGAGAACCCTGGCCCCATGCACGGCGATACGC CTACCCTGCACGAGTACATGCTCGACCTGCAGCCAGAGACCA CGGACCTCTACTGCTATGAGCAGTTGAATGACAGCAGCGAAG AGGAGGATGAGATAGATGGGCCCGCCGGTCAGGCTGAGCCT GACCGGGCCCACTATAACATCGTCACTTTTTGCTGCAAGTGT GATTCAACGCTCAGGCTGTGCGTGCAGAGCACGCACGTGGA CATACGAACCCTAGAGGATCTCCTGATGGGGACCCTAGGGAT CGTATGTCCAATATGTAGTCAGAAGCCCATGCACCAGAAGCG GACAGCTATGTTCCAGGACCCGCAGGAGCGGCCCCGGAAGC TGCCGCAGCTGTGCACGGAGCTTCAGACTACAATACACGACA TCATCCTTGAGTGCGTGTATTGTAAGCAGCAGCTCCTGAGAC GCGAAGTCTATGACTTCGCGTTTCGGGACCTCTGCATCGTATA CCGCGACGGTAATCCGTACGCGGTATGCGATAAGTGTTTGAA GTTCTATTCAAAGATCAGCGAGTACAGGCACTACTGTTACTCG CTGTATGGTACAACGTTGGAGCAGCAGTACAACAAGCCGTTG TGCGACCTGCTCATACGTTGTATCAATTGCCAGAAGCCGCTGT GTCCTGAGGAGAAGCAGCGGCATCTGGACAAAAAGCAGAGG TTCCACAACATCCGTGGTCGCTGGACAGGTCGATGCATGTCT TGTTGCAGGAGTAGCCGTACTCGCCGTGAGACTCAGTTAAAG CTTAGCCATGGCTTCCCGCCGGAGGTGGAGGAGCAGGATGAT GGCACGCTGCCCATGTCTTGTGCCCAGGAGAGCGGGATGGA CCGTCACCCTGCAGCCTGTGCTTCTGCTAGGATCAATGTGTG A (SEQ ID NO: 81)

S14	Human IL12-TM-T2A-E6/E7-PEST amino acid sequence	MCHQQLVISWFSLVFLASPLVAIWELKKDVYVVELDWYPDAPG EMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLTIQVKEFGDAG QYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFL RCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAA TLSAERVRGDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVH KLKYENYTSSFFIRDIIKPDPPKNLQLKPLKNSRQVEVSWEYPDT WSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSATVICRKNAS ISVRAQDRYYSSSWSEWASVPCSGGGGSGGGGSGGGGSRNLPV ATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDH EDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKT SFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQN MLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAF RIRAVTIDRVMSYLNASSGGGSGGGGSGGGGSGGGGSGGGSGD AHVSEDFTWEKPPEDPPDSKNTLVLFGAGFGAVITVVVIVVIIKC FCKHRSCFRRNEASRETNNSLTFGPEEALAEQTVFLEGRGSLLT CGDVEENPGPMHGDTPTLHEYMLDLQPETTDLYCYEQLNDSSE EEDEIDGPAGQAEPDRAHYNIVTFCCKCDSTLRLCVQSTHVDIR TLEDLLMGTLGIVCPICSQKPMHQKRTAMFQDPQERPRKLPQL CTELQTTIHDIILECVYCKQQLLRREVYDFAFRDLCIVYRDGNPY AVCDKCLKFYSKISEYRHYCYSLYGTTLEQQYNKPLCDLLIRCI NCQKPLCPEEKQRHLDKKQRFHNIRGRWTGRCMSCCRSSRTRR ETQLKLSHGFPPEVEEQDDGTLPMSCAQESGMDRHPAACASAR INV (SEQ ID NO: 82)
S15	mIL12-CD80TM nucleotide sequence	atgtgtcctcagaagctaaccatctcctggtttgccatcgttttgctggtgtctccactcatggccatgtg ggagctggagaaagacgtttatgttgtagaggtggactggactcccgatgcccctggagaaacagt gaacctcacctgtgacacgcctgaagaagatgacatcacctggacctcagaccagagacatggagt cataggctctggaaagaccctgaccatcactgtcaaagagtttctagatgctggccagtacacctgcc acaaaggaggcgagactctgagccactcacatctgctgctccacaagaaggaaaatggaatttggt ccactgaaattttaaaaaatttcaaaaacaagactttcctgaagtgtgaagcaccaaattactccggac ggttcacgtgctcatggctggtgcaaagaaacatggacttgaagttcaacatcaagagcagtagcag ttcccctgactctcgggcagtgacatgtggaatggcgtctctgtctgcagagaaggtcacactggac caaagggactatgagaagtattcagtgtcctgccaggaggatgtcacctgcccaactgccgaggag accctgcccattgaactggcgttggaagcacggcagcagaataaatatgagaactacagcaccagc ttcttcatcagggacatcatcaaaccagacccgcccaagaacttgcagatgaagcctttgaagaactc acaggtggaggtcagctgggagtaccctgactcctggagcactccccattcctacttctccctcaagt tctttgttcgaatccagcgcaagaaagaaaagatgaaggagacagaggaggggtgtaaccagaaa ggtgcgttcctcgtagagaagacatctaccgaagtccaatgcaaaggcgggaatgtctgcgtgcaa gctcaggatcgctattacaattcctcatgcagcaagtgggcatgtgttccctgcagggtccgatccgg cggaggcggatcaggcggcggtggatctgggggtggcggatcttcgcgaagggtcattccagtct ctggacctgccaggtgtcttagccagtcccgaaacctgctgaagaccacagatgacatggtgaaga cggccagagaaaaactgaaacattattcctgcactgctgaagacatcgatcatgaagacatcacacg ggaccaaaccagcacattgaagacctgtttaccactggaactacacaagaacgagagttgcctggct actagagagacttcttccacaacaagagggagctgcctgcccccacagaagacgtctttgatgatga ccctgtgccttggtagcatctatgaggacttgaagatgtaccagacagagttccaggccatcaacgc agcacttcagaatcacaaccatcagcagatcattctagacaagggcatgctggtggccatcgatgag ctgatgcagtctctgaatcataatggcgagactctgcgccagaaacctcctgtgggagaagcagacc cttacagagtgaaaatgaagctctgcatcctgcttcacgccttcagcacccgcgtcgtgaccatcaac agggtgatgggctatctgagctccgccTCCGGAGGAGGCTCCGGAGGAGGA GGCAGCGGAGGAGGAGGATCCGGCGGAGGAGGCTCTGGCG GAGGAAGCGGCGATGCCCACGTGAGCGAGGATTTCACATGG GAGAAGCCCCCCGAGGACCCTCCCGACAGCAAGAACACACT GGTGCTGTTTGGCGCCGGCTTCGGCGCCGTGATCACAGTGGT GGTCATTGTGGTCATTATCAAGTGCTTCTGCAAGCACAGATCC TGTTTCAGGAGAAATGAGGCCAGCAGAGAGACAAACAACAG CCTGACATTTGGCCCTGAGGAGGCCCTGGCCGAGCAGACAG TGTTTCTGTGA (SEQ ID NO: 83)

S16	mIL12-CD80TM amino acid sequence	MCPQKLTISWFAIVLLVSPLMAMWELEKDVYVVEVDWTPDAP GETVNLTCDTPEEDDITWTSDQRHGVIGSGKTLTITVKEFLDAG QYTCHKGGETLSHSHLLLHKKENGIWSTEILKNFKNKTFLKCE APNYSGRFTCSWLVQRNMDLKFNIKSSSSSPDSRAVTCGMASLS AEKVTLDQRDYEKYSVSCQEDVTCPTAEETLPIELALEARQQN KYENYSTSFFIRDIIKPDPPKNLQMKPLKNSQVEVSWEYPDSWS TPHSYFSLKFFVRIQRKKEKMKETEEGCNQKGAFLVEKTSTEVQ CKGGNVCVQAQDRYYNSSCSKWACVPCRVRSGGGGSGGGGS GGGGSSRRVIPVSGPARCLSQSRNLLKTTDDMVKTAREKLKHY SCTAEDIDHEDITRDQTSTLKTCLPLELHKNESCLATRETSSTTR GSCLPPQKTSLMMTLCLGSIYEDLKMYQTEFQAINAALQNHNH QQIILDKGMLVAIDELMQSLNHNGETLRQKPPVGEADPYRVKM KLCILLHAFSTRVVTINRVMGYLSSASGGGSGGGGSGGGGSGG GGSGGGSGDAHVSEDFTWEKPPEDPPDSKNTLVLFGAGFGAVI TVVVIVVIIKCFCKHRSCFRRNEASRETNNSLTFGPEEALAEQTV FL* (SEQ ID NO: 84)
	human CD8A transmembrane domain	IYIWAPLAGTCGVLLLSLVITLYCY (SEQ ID NO: 85)

CLAIMS

1. An mRNA tumor vaccine encoding a membrane-tethered IL-12 cytokine adjuvant, wherein the mRNA comprises three components: an IL-12-encoding sequence (IL12), a membrane-domain-encoding sequence (MD), and a tumor-antigen-encoding sequence (Ag).

2. The mRNA tumor vaccine according to claim 1, wherein

the IL-12-encoding sequence (IL12) and the membrane-domain-encoding sequence (MD) are located on the same mRNA, and the tumor-antigen-encoding sequence (Ag) is located on another mRNA; or

the IL-12-encoding sequence (IL12), the membrane-domain-encoding sequence (MD), and the tumor-antigen-encoding sequence (Ag) are on the same mRNA, arranged in the order of IL12-L1-MD-L2-Ag or Ag-L2-IL12-L1-MD;

wherein L1 is a glycine/serine-based polypeptide linker; and L2 is an IRES sequence, a glycine/serine-based polypeptide linker, or a 2A polypeptide linker, preferably a 2A polypeptide linker.

3. The mRNA tumor vaccine according to claim 1 or 2, wherein the tumor antigen Ag comprises a mutation-derived tumor neoantigen, a virus-derived tumor-specific antigen, or a tumor-associated antigen highly expressed in tumors.

4. The mRNA tumor vaccine according to claim 3, wherein the mutation-derived tumor neoantigen comprises KRAS G12C, KRAS G12D, EGFRvIII, or $BRAF^{V600E}$; the virus-derived tumor-specific antigen comprises HPV E6/E7, the endogenous retroviral antigen hERT, LMP1, or LMP2; and the tumor-associated antigen highly expressed in tumors comprises WT1, MAGE-A3, GP100, NY-ESO-1, HER2/Neu, Claudin18.2, Mesothelin, or MUC.

5. The mRNA tumor vaccine according to claim 1 or 2, wherein the IL-12-encoding sequence comprises ORFs encoding an IL-12B polypeptide and an IL-12A polypeptide, wherein the IL-12B and IL-12A are linked via a peptide linker; the linker comprises a Gly/Ser-based linker including $(GnS)_m$, wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20, and m is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20; preferably, the linker is a $(G4S)_3$ linker.

6. The mRNA tumor vaccine according to claim 1 or 2, wherein the membrane domain comprises a transmembrane domain, preferably comprises a transmembrane domain and an

intracellular domain;

the transmembrane domain comprises a CD80 transmembrane domain, a CD86 transmembrane domain, a CD4 transmembrane domain, a CD8 transmembrane domain, a VEGFR transmembrane domain, or a PDGF-RB transmembrane domain, preferably a CD80 transmembrane domain; and

the intracellular domain comprises a CD80 intracellular domain, a CD86 intracellular domain, a CD4 intracellular domain, a CD8 intracellular domain, a VEGFR intracellular domain, a PDGF-RB intracellular domain, or a truncated PDGF-RB intracellular domain;

preferably, wherein the membrane domain comprises a CD80 transmembrane domain in combination with a CD80 intracellular domain.

7. The mRNA tumor vaccine according to claim 1 or 2, further comprising a nucleic acid carrier loaded with the mRNA, wherein the nucleic acid carrier comprises a liposome, LPP, or LPX, preferably an LNP.

8. A pharmaceutical composition comprising the mRNA tumor vaccine according to any one of claims 1-7 and a second anticancer agent, wherein the second anticancer agent comprises an immune checkpoint inhibitor and/or a bispecific T-cell engager (BiTE).

9. Use of the mRNA tumor vaccine according to any one of claims 1-7 or the pharmaceutical composition according to claim 8 in the manufacture of a medicament for the treatment or prevention of a tumor.

10. The use according to claim 9, wherein the tumor comprises B-cell lymphoma, bronchial carcinoma, prostate cancer, bladder cancer, cancer of the brain or central nervous system, cancer of the peripheral nervous system, esophageal cancer, cervical cancer, uterine or endometrial cancer, head and neck cancer, oral cancer, laryngeal cancer, salivary gland cancer, thymic cancer, adrenal cancer, osteosarcoma, chondrosarcoma, liposarcoma, testicular cancer, malignant fibrous histiocytoma, colorectal cancer, melanoma, gastric cancer, pancreatic cancer, lung cancer, liver cancer, kidney cancer, cholangiocarcinoma, small-intestinal or appendiceal cancer, squamous cell carcinoma, breast cancer, or ovarian cancer.