

embodiments, the nanoparticle compositions are administered after cancer immunotherapy, such as checkpoint inhibitor therapy and/or after adoptive T cell immunotherapy, and thereby provide enhanced and/or sustained immunological attack on a variety of cancers.

5 The invention further provides polynucleotides encoding the amino acid sequences described herein, as well as host cells expressing the same.

The details of the invention are set forth in the accompanying description and claims below. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, illustrative methods and materials are now described. Other features, objects, and advantages of the invention will be apparent
10 from the description and from the claims. In the specification and the appended claims, the singular forms also include the plural unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

15 BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1-3 show three humanized variable heavy sequences for anti-CD28.

FIGS. 4-6 show three humanized variable light sequences for anti-CD28.

FIG. 7 shows a modified constant heavy sequence.

FIG. 8 shows a constant κ Light sequence.

20 **FIG. 9** shows a humanized non-CD28-binding variable region for constructing an HLA fusion.

FIG. 10 shows the amino acid sequence for humanized HLA-IgG4HC(SEQ ID NO. 19).

FIG. 11 shows the amino acid sequence for Light Chain 3 (LC3, or $V\kappa 3$)(SEQ ID
25 NO. 20).

FIG. 12 shows the amino acid sequence for Heavy Chain 1 (HC1)(SEQ ID NO. 21).

FIG. 13 shows the amino acid sequence for Heavy Chain 2 (HC2)(SEQ ID NO. 22).

may contain a human IGHV4 heavy chain amino acid sequence, which may be modified with from one to fifteen amino acid modifications. The modifications may comprise murine framework residues to support the integrity of the antigen binding site(s).

The complementarity determining region (CDR) in some embodiments is based on a murine antibody amino acid sequence, which may optionally have from one to ten, such as from one to five, amino acid modifications. In some embodiments, one, two, three, or more CDRs are based on mouse 9.3 mAb (Tan *et al.* J. Exp. Med. 1993 177:165), which is publicly available. Exemplary CDRs are shown in **FIGURES 1-6**. In some embodiments, the antibody has the full set of heavy chain and/or full set of light chain CDRs of 9.3 mAb. For example, in some embodiments the heavy chain variable region contains one, two or three of the following CDRs, which optionally may each be modified by one, two, or three amino acid substitutions, deletions, or additions: CDR1 (DYGVH(SEQ ID NO. 23)), CDR2 (VIWAGGGTNYNSALMS(SEQ ID NO. 24)), and CDR3 (DKGYSYYYSMDY(SEQ ID NO. 25)). In some embodiments, the light chain variable region contains one, two, or three of the following CDRs, which each may be modified by one, two, or three amino acid substitutions, deletions, or additions: CDR1 (RASESVEYYVTSLMQ(SEQ ID NO. 26)), CDR2 (AASNVES(SEQ ID NO. 27)), and CDR3 (QQSRKVPYT(SEQ ID NO. 28)).

In some embodiments, the anti-CD28 ligand binds to the same or overlapping epitope as 9.3 mAb, or binds the same or overlapping epitope as an antibody having CDR1, CDR2, and CDR3 of 9.3 mAb. Antibodies with the same or overlapping epitope can be selected by any suitable technique, including competitive immunoassays, using, for example, Surface Plasmon Resonance (Biacore).

Alternative CDR sequences, variable regions, or CD28-binding ligands may be employed in various embodiments. Alternative ligands, CD28 epitopes, and anti-CD28 antibodies are described in US Patent 7,612,170, US Patent 6,987,171, and US Patent 6,887,466, for example, and these disclosures are hereby incorporated by reference in their entireties.

In some embodiments, the antibody heavy chain comprises a variant of a human IGHV4-59 germline framework (FW), which is modified to include from 5 to 15 murine FW residues. In some embodiments, the antibody comprises light chain amino acid sequences, and the light chain sequences may be a variant of human IGKV4-01 FW sequences, and which may be modified to include from 3 to 15 murine FW residues.

ligands per particle. In some embodiments, the polymeric nanoparticles have less than about 150 ligands per particle, or less than about 100 ligands per particle. For example, the particles may have from about 5 to about 90 ligands per particle. In various embodiments, the nanoparticles comprise less than about 90 conjugated ligands per particle, or less than about 80, or less than about 70, or less than about 60, or less than about 50, or less than about 40, or less than about 30, or less than about 20 conjugated ligands per particle. In some embodiments, the particle contains from 10 to about 80 conjugated ligands on its surface, or from 10 to about 70, or about 10 to about 50 conjugated ligands per particle.

In still other embodiments, the antigen presenting complexes (e.g., HLA sequences) do not contain Ig fusion partners, and are monomeric. For example, in some embodiments, the C-terminal end of the antigen presenting complex or HLA molecule (e.g. HLA-A2, etc.) contains a peptide tether sequence suitable for site-directed binding to a functional group (e.g. a maleimide moiety) on a solid/semi-solid substrate such as a synthetic nanoparticle (e.g. containing PLGA-PEG-maleimide or PLA-PEG-maleimide block polymers). The tether sequence may contain any suitable sequence, which may be predominately composed of hydrophilic residues such as Gly, Ser, Ala, and Thr, such as two, three, four, or five repeats of GGSG (SEQ ID NO. 29) or AAAGG (SEQ ID NO. 30), with cysteine residue incorporated somewhere within the about 5 to about 15 (or about 5 to about 10 amino acid) tether. The cysteine residue should be incorporated at a site predicted not to form intramolecular disulfide bonds.

In some embodiments, the HLA-Ig fusion or other HLA construct further comprises an antigenic peptide bound to the HLA for presentation to T cells. The antigenic peptide can comprise an antigenic portion of one or more of tyrosinase, hTERT, MAGE-1, MAGE-3, gp-100, NY-ESO-1, Melan A/Mart-1, HPV 16-E7, gp75/brown, BAGE, and S-100 and/or any of the antigenic peptides as described in WO 2004/006951 for presentation by Class I or Class II complexes, the contents of which are hereby incorporated by reference in their entirety.

Other signals that can be provided with the antigen presenting complex include: CD80 (B7-1), CD86 (B7-2), B7-H3, 4-1BBL, CD27, CD30, CD134 (OX-40L), B7h (B7RP-1), CD40, LIGHT, (or Ig fusions, optionally humanized as described herein, of the such molecules or active portions thereof), antibodies that specifically bind to HVEM, antibodies that specifically bind to CD40L, antibodies that specifically bind to OX40, antibodies that specifically bind Fas, antibodies that specifically bind PD1, antibodies that specifically bind to GITR, and antibodies that specifically bind to 4-1BB. Where the co-stimulatory signal is

found to contain two promiscuous MHC class II binding sequences, one high affinity (with p1 anchor at position 2) and one moderate affinity (with p1 anchor at position 4). The C-terminal linker was found to contain one promiscuous moderate affinity MHC class II binding peptide with p1 anchor at position 11.

5 A BLAST search of Antitope's T cell epitope database (TCED™) was carried out using the same sequences as used in the iTope™ analysis to determine any homology with previously identified epitopes. The TCED™ is used to search any test sequence against a large (>10,000 peptides) database of peptides derived from unrelated sequences which have been tested in EpiScreen™ T cell epitope mapping assays. Neither of the linker sequences
10 was found to contain any 'hits' in the TCED™.

iTope™ was further used to assess sequence changes to the linkers in order to reduce their propensity for binding to MHC class II. It was noted that the N-terminal linker could be removed entirely such that the N-terminus of HLA-A*02:01 is fused directly either to the signal sequence provided in the pBFKsr vector or to its natural signal sequence. This would
15 ensure that the N-terminus of the fusion protein would contain only human germline sequence and avoid the risk of T cell epitopes. The recommended linker sequences below were found to reduce MHC class II binding to background residual levels (<5 of the alleles bound by any 9mer), and to provide suitable restriction sites for cloning (although both sequences will require modification of the vector):

N-terminal linker:

Q V Q L T R E G S G S H S M R Y F (SEQ ID NO. 32)
CAGGTCCAACTGacgcgtGAGGGGTCCGGCTCTCACTCCATGAGGTATTTTC (SEQ ID NO. 31)
Vector | Linker | HLA-A*02:01

C-terminal linker:

E G L P K P L T W A R E V S E V K L Q (SEQ ID NO. 34)
GAGGGTTTGCCCAAGCCCTCACCTGGGctcgagAGGTGAGCGAGGTCAAGCTGCAG (SEQ ID NO. 33)
HLA-A*02:01 | Linker | Anti-CD28

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Example 3: Codon optimization of sequences and expression cloning

Codons were optimized using GeneOptimizer®, and optimized sequences were cloned for expression as shown below.