scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1-3 show three humanized variable heavy sequences for anti-CD28; SEQ ID NOs: 1, 2, 3, 4, 5 and 6.

FIGS. 4-6 show three humanized variable light sequences for anti-CD28; SEQ ID NOs: 7, 8, 9, 10, 11 and 12.

FIG. 7 shows a modified constant heavy sequence; SEQ ID NOs: 13 and 14.

FIG. 8 shows a constant κ Light sequence; SEQ ID NOs: 15 and 16.

FIG. 9 shows a humanized non-CD28-binding variable region for constructing an HLA fusion; SEQ ID NOs: 17 and 18.

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 Vκ3); SEQ ID 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.

FIGS. 14-16 show expression constructs for expression in STABLEFAST-NS0 Cell Line.

FIG. 17 shows that the humanized anti-CD28 mAb is not a super-agonist.

FIG. 18 shows that the humanized anti-CD28 clones specifically stain CD28 on a human T-cell line. FIG 18(A): staining with murine anti-human CD8 mAb (clone 9.3, Isotype IgG2a); FIG18(B): staining with humanized anti-CD28 (isotype IgG4).

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).

Alternative CDR sequences, variable regions, or CD28-binding ligands may be employed in various embodiments. Alternative ligands and 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.

The anti-CD28 human heavy chain sequence may be modified, for example, to comprise one or more (e.g., 2 or more, 3 or more, 4 or more, 5 or more, or all) murine Fw residues at positions 1, 3, 6, 37, 48, 67, 71, 73, 76, 78, 82, 82a, and 82c (based on Kabat numbering). The murine Fw residues at these positions can be as in 9.3 mAb. The light chain may be modified to comprise one or more (e.g., 2 or more, 3 or more, 4 or more, 5 or more, or all) murine Fw residues at positions 3, 4, 49, 70, 85, 87, and 80. Selected murine Fw residues may support the integrity of the antigen-binding sites. The

one or more of the murine framework residues described with respect to anti-CD28. In some embodiments, the heavy chain of the anti-CD28 antibody described above (with or without murine framework residues) is fused to HLA in accordance with this aspect, and in such embodiments, the variable region is modified to reduce or eliminate CD28 binding.

In some embodiments, the HLA fusion construct contains no variable chain sequences. For example, the HLA or antigen presenting complex can be fused to an Ig constant region sequence above the hinge region to provide a dimeric HLA. For example, an HLA or antigen presenting portion thereof may be conjugated to a CH1 portion of each IgG heavy chain. All IgG molecules consist of two identical heavy chains (constant and variable regions) joined together by disulfide bonds in the hinge region (upper and lower). For example, in some embodiments, an HLA molecule or antigen presenting complex is fused to the CH1 (N-terminal end of the IgH chain above the hinge region), thereby creating a dimeric fusion protein that is smaller due to lack of any VH and VL light chain sequences. Such a construct may provide manufacturing advantages, as well as exhibit less potential for immunogenicity.

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. PLGA-PEG-maleimide block polymers, or other particles described herein). 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 GGGSG; 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.

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 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

CAGGTCCAACTGacgcgtGAGGGGTCCGGCTCTCACTCCATGAGGTATTTC — 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

GAGGGTTTGCCCAAGCCCCTCACCTGGGctcgagAGGTGAGCGAGGTCAAGCTGCAG — SEQ ID NO: 33

HLA-A*02:01 | Linker | Anti-CD28

Example 3: Codon optimization of sequences and expression cloning

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

Sequences were engineered with *Pme*I restriction sites, Kozak sequence, and signal peptide for expression in NS0 cells. Translation starts immediately downstream of the Kozak sequence.

The full translated amino acid sequence of the HLA-IgG4HC fusion is shown in **FIGURE 10**.

The translated sequence of LC3 (VK3) is shown in **FIGURE 11**.

The translated sequence for HC1 is shown in **FIGURE 12**.

The translated sequence for HC2 is shown in **FIGURE 13**.