

**Figure 34** (SEQ ID NO: 183) shows the sequence of the extracellular domain (ECD) of wild type huCD27 and a mutated version with substitutions at positions 85, 87, 88 and 89.

**Figure 35** is a graph showing binding of anti-CD27 antibody 2B3 to both wild type huCD27 and mutated huCD27 with sequences as shown in Figure 34.

5 **Figure 36** is a graph showing improved production (expression) of a modified 9H9x2B3 (DD) construct compared to the original (unmodified) 9H9x2B3 construct.

## **I. Detailed Description of the Invention**

CD27 is an important co-stimulatory receptor that can be exploited for  
10 immunotherapy using agonist molecules. CD27 plays a key role in diverse immunological processes, including the survival, activation and effector functions of T cells, as well as the proliferation and cytotoxic activity of natural killer (NK) cells. These events occur in response to appropriate interaction of the ligand (CD70) with CD27 resulting in intracellular signaling events that lead to an activation of NF-kB and expression of relevant genes. As  
15 with most co-stimulatory molecules, effective stimulation of T cells with either the ligand or agonist antibodies also requires simultaneous stimulation through the T cell receptor (TCR).

Most CD27 agonists molecules require multimerization or crosslinking for their activity. For example, anti-CD27 antibodies that are agonists are cross-linked through interaction of their Fc domains with Fc receptors through cis or trans interactions. *In vitro*  
20 this can also be performed artificially using a secondary anti-Ig antibody or by adsorbing the antibody to a solid phase matrix. The requirement for FcR interaction of anti-CD27 agonist antibodies implies that the T cell stimulating activity is at least partially dependent on the number FcR expressing cells.

To eliminate the requirement for FcR interactions, CD27 agonists have been invented  
25 that 1) eliminate the need for interaction with receptors other than CD27 (herein termed CD27 hyper-crosslinking agents), or 2) can provide the crosslinking through interaction of alternate receptors that may be on different specific cell types not expressing FcR, and which alternate receptors may also provide additional function (herein termed CD27 multi-specific agents).

30 In one aspect, the invention provides bispecific constructs (or multispecific constructs) that comprise an anti-CD27 antibody, or antigen-binding fragment thereof, linked to an anti-PD-L1 antibody, or antigen-binding fragment thereof. Such anti-CD27 x anti-PD-L1 bispecific agents of the invention have now been shown to exhibit synergistic effects *in*

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|-------------------------------------------------------|--------------------|
| SEQ ID NO:115<br>3C2 V <sub>H</sub> CDR3<br>(Chothia) | YPLIRGAFDY         |
| SEQ ID NO:116<br>3C2 V <sub>L</sub> CDR1<br>(Chothia) | RSSQNLLHTNGYNYLD   |
| SEQ ID NO:117<br>3C2 V <sub>L</sub> CDR2<br>(Chothia) | LGSNRAS            |
| SEQ ID NO:118<br>3C2 V <sub>L</sub> CDR3<br>(Chothia) | MQALQTPLT          |
| SEQ ID NO:119<br>2B3 V <sub>H</sub> CDR1<br>(Chothia) | GYTFTGY            |
| SEQ ID NO:120<br>2B3 V <sub>H</sub> CDR2<br>(Chothia) | NPNSGG             |
| SEQ ID NO:121<br>2B3 V <sub>H</sub> CDR3<br>(Chothia) | DRLVLPWFGEIFPDAFDI |
| SEQ ID NO:122<br>2B3 V <sub>L</sub> CDR1<br>(Chothia) | RASQSIRSNLA        |
| SEQ ID NO:123<br>2B3 V <sub>L</sub> CDR2<br>(Chothia) | GASTRAT            |
| SEQ ID NO:124<br>2B3 V <sub>L</sub> CDR3<br>(Chothia) | QQYNNWPLT          |
| SEQ ID NO:125<br>7H7 V <sub>H</sub> CDR1<br>(Chothia) | GGTISTS            |
| SEQ ID NO:126<br>7H7 V <sub>H</sub> CDR2<br>(Chothia) | KQDGSE             |
| SEQ ID NO:127<br>7H7 V <sub>H</sub> CDR3<br>(Chothia) | DRPVAGASAL         |
| SEQ ID NO:128<br>7H7 V <sub>L</sub> CDR1<br>(Chothia) | RASQSISGWLA        |
| SEQ ID NO:129<br>7H7 V <sub>L</sub> CDR2<br>(Chothia) | KASSLES            |
| SEQ ID NO:130<br>7H7 V <sub>L</sub> CDR3<br>(Chothia) | QQYYGSSRT          |
| SEQ ID NO:131                                         | GGTISTS            |