ANTIBODIES TO ICOS

RELATED APPLICATIONS

[0001] This application claims the benefit of priority to US Provisional Application Nos. 62/137,034 filed March 23, 2015; 62/147,484 filed April 14, 2015; 62/156,588 filed May 4, 2015; 62/242,489 filed October 16, 2015; and 62/255,635 filed November 16, 2015, each of which is incorporated by reference herein in its entirety for any purpose.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on March 15, 2016, is named 2016-03-22_01140-0001-00PCT_ST25.txt and is 81,925 bytes in size.

FIELD OF THE INVENTION

[0003] Antibodies that bind to Inducible T-Cell Costimulator (ICOS) are provided. Methods of treatment comprising administering anti-ICOS antibodies are also provided.

BACKGROUND

[0004] ICOS is a member of the B7/CD28/CTLA-4 immunoglobulin superfamily and is specifically expressed on T cells. Unlike CD28, which is constitutively expressed on T cells and provides co-stimulatory signals necessary for full activation of resting T cells, ICOS is expressed only after initial T cell activation.

[0005] ICOS has been implicated in diverse aspects of T cell responses (reviewed in Simpson *et al.*, 2010, *Curr. Opin. Immunol.*, 22: 326-332). It plays a role in the formation of germinal centers, T/B cell collaboration, and immunoglobulin class switching. ICOS-deficient mice show impaired germinal center formation and have decreased production of interleukin IL-10. These defects have been specifically linked to deficiencies in T follicular helper cells.

[0006] ICOS also plays a role in the development and function of other T cell subsets, including Th1, Th2, and Th17. Notably, ICOS co-stimulates T cell proliferation and cytokine secretion associated with both Th1 and Th2 cells. Accordingly, ICOS KO mice demonstrate impaired development of autoimmune phenotypes in a variety of disease models, including diabetes (Th1), airway inflammation (Th2) and EAE neuro-inflammatory models (Th17).

[0007] In addition to its role in modulating T effector (Teff) cell function, ICOS also modulates T regulatory cells (Tregs). ICOS is expressed at high levels on Tregs, and has been implicated in Treg homeostasis and function.

[0008] Upon activation, ICOS, a disulfide-linked homodimer, induces a signal through the PI3K and AKT pathways. Subsequent signaling events result in expression of lineage specific transcription factors (e.g., T-bet, GATA-3) and, in turn, effects on T cell proliferation and survival.

[0009] ICOS ligand (ICOSL; B7-H2; B7RP1; CD275; GL50), also a member of the B7 superfamily, is the only ligand for ICOS and is expressed on the cell surface of B cells, macrophages and dendritic cells. ICOSL functions as a non-covalently linked homodimer on the cell surface in its interaction with ICOS. Human ICOSL, although not mouse ICOSL, has been reported to bind to human CD28 and CTLA-4 (Yao *et al.*, 2011, *Immunity*, 34: 729-740).

SUMMARY

[0010] In some embodiments, an isolated antibody that binds ICOS is provided, wherein the antibody is an agonist of CD4 T cells (such as CD4 T effector (Teff) cells).

[0010a] Disclosed is an isolated antibody that binds to ICOS, wherein the antibody comprises:

i) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 62; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 63; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 64; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 65; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 66; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 67; or

ii) (a) HCDR1 comprising an amino acid sequence of SEQ ID NO: 62; (b) HCDR2 comprising an amino acid sequence selected from SEQ ID NOs: 63 and 93; (c) HCDR3 comprising an amino acid sequence of SEQ ID NO: 64; (d) LCDR1 comprising an amino acid sequence of SEQ ID NO: 65; (e) LCDR2 comprising an amino acid sequence selected from SEQ ID NOs: 66, 76, and 86; and (f) LCDR3 comprising an amino acid sequence of SEQ ID NO: 67; or

iii) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 12; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 13; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 14; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 15; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 16; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 17; or

iv) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 42; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 43; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 44; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 45; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 46; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 47; or

v) a) HCDR1 comprising an amino acid sequence of SEQ ID NO: 32; (b) HCDR2 comprising an amino acid sequence selected from SEQ ID NOs: 33 and 173; (c) HCDR3 comprising an amino acid sequence of SEQ ID NO: 34; (d) LCDR1 comprising an amino acid sequence of SEQ ID NO: 35; (e) LCDR2 comprising an amino acid sequence selected from SEQ ID NOs: 36, 166, and 186; and (f) LCDR3 comprising an amino acid sequence of SEQ ID NO: 37; or

vi) (a) HCDR1 comprising an amino acid sequence of SEQ ID NO: 52; (b) HCDR2 comprising an amino acid sequence of SEQ ID NO: 53; (c) HCDR3 comprising an amino acid sequence of SEQ ID NO: 54; (d) LCDR1 comprising an amino acid sequence of SEQ ID NO: 55; (e) LCDR2 comprising an amino acid sequence selected from SEQ ID NOs: 56, 136, 146, and 156; and (f) LCDR3 comprising an amino acid sequence of SEQ ID NO: 57; or

vii) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 32; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 33; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 34; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 35; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 36; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 37; or

viii) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 52; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 53; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 54; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 55; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 56; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 57; or

ix) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 62; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 63; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 64; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 65; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 76; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 67; or

x) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 62; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 63; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 64; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 65; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 86; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 67; or

xi) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 62; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 93; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 64; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 65; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 66; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 67; or

xii) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 62; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 93; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 64; (d) LCDR1 comprising

the amino acid sequence of SEQ ID NO: 65; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 76; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 67; or

xiii) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 62; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 93; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 64; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 65; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 86; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 67; or

xiv) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 52; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 53; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 54; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 55; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 136; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 57; or

xv) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 52; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 53; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 54; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 55; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 146; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 57; or

xvi) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 52; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 53; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 54; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 55; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 156; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 57; or

xvii) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 32; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 33; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 34; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 35; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 166; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 37; or

xviii) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 32; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 173; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 34; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 35; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 36; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 37; or

xix) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 32; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 33; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 34; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 35; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 186; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 37.

[0010b] In one aspect, there is provided an isolated monoclonal antibody that binds to ICOS, wherein the antibody comprises: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 62; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 63; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 64; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 65; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 66; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 67.

[0010c] In some embodiments, an isolated antibody that binds ICOS is provided, wherein the antibody is an agonist of CD4 T cells (such as CD4 Teff cells) and depletes T regulatory (Treg) cells. In some embodiments, an isolated antibody that binds ICOS is provided, wherein the antibody depletes Treg cells, but does not deplete Teff cells. In some embodiments, an isolated antibody that binds ICOS is provided, wherein the antibody induces pAKT signaling on CD4 T cells. In some embodiments, an isolated antibody that binds ICOS is provided, wherein the antibody induces pAKT signaling on CD4 T cells and depletes Treg cells. In some embodiments, an isolated antibody that binds to ICOS is provided, wherein the antibody comprises:

i) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 12; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 13; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 14; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 15; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 16; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 17; or

ii) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 42; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 43; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 44; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 45; (e) LCDR2 comprising the amino

acid sequence of SEQ ID NO: 46; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 47; or

iii) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 62; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 63; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 64; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 65; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 66; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 67; or

iv) (a) HCDR1 comprising an amino acid sequence selected from SEQ ID NOs: 22, 62, 72, 82, 92, 102, and 112; (b) HCDR2 comprising an amino acid sequence selected from SEQ ID NOs: 23, 63, 73, 83, 93, 103, and 113; (c) HCDR3 comprising an amino acid sequence selected from SEQ ID NOs: 24, 64, 74, 84, 94, 104, and 114; (d) LCDR1 comprising an amino acid sequence selected from SEQ ID NOs: 25, 65, 75, 85, 95, 105, and 115; (e) LCDR2 comprising an amino acid sequence selected from SEQ ID NOs: 26, 66, 76, 86, 96, 106, and 116; and (f) LCDR3 comprising an amino acid sequence selected from SEQ ID NOs: 27, 67, 77, 87, 97, 107, and 117; or

v) (a) HCDR1 comprising an amino acid sequence selected from SEQ ID NOs: 32, 162, 172, and 182; (b) HCDR2 comprising an amino acid sequence selected from SEQ ID NOs: 33, 163, 173, and 183; (c) HCDR3 comprising an amino acid sequence selected from SEQ ID NOs: 34, 164, 174, and 184; (d) LCDR1 comprising an amino acid sequence selected from SEQ ID NOs: 35, 165, 175, and 185; (e) LCDR2 comprising an amino acid sequence selected from SEQ ID NOs: 36, 166, 176, and 186; and (f) LCDR3 comprising an amino acid sequence selected from SEQ ID NOs: 37, 167, 177, and 187; or

vi) (a) HCDR1 comprising an amino acid sequence selected from SEQ ID NOs: 52, 122, 132, 142, and 152; (b) HCDR2 comprising an amino acid sequence selected from SEQ ID NOs: 53, 123, 133, 143, and 153; (c) HCDR3 comprising an amino acid sequence selected from SEQ ID NOs: 54, 124, 134, 144, and 154; (d) LCDR1 comprising an amino acid sequence selected from SEQ ID NOs: 55, 125, 135, 145, and 155; (e) LCDR2 comprising an amino acid sequence selected from SEQ ID NOs: 56, 126, 136, 146, and 156; and (f) LCDR3 comprising an amino acid sequence selected from SEQ ID NOs: 57, 127, 137, 147, and 157; or

vii) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 22; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 23; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 24; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 25; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 26; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 27; or

viii) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 32; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 33; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 34; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 35; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 36; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 37; or

ix) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 52; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 53; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 54; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 55; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 56; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 57; or

x) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 72; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 73; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 74; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 75; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 76; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 77; or

xi) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 82; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 83; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 84; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 85; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 86; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 87; or

xii) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 92; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 93; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 94; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 95; (e) LCDR2 comprising the amino

acid sequence of SEQ ID NO: 96; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 97; or

xiii) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 102; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 103; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 104; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 105; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 106; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 107; or

xiv) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 112; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 113; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 114; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 115; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 116; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 117; or

xv) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 122; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 123; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 124; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 125; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 126; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 127; or

xvi) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 132; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 133; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 134; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 135; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 136; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 137; or (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 142; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 143; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 144; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 145; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 146; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 147; or

xvii) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 152; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 153; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 154; (d) LCDR1

comprising the amino acid sequence of SEQ ID NO: 155; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 156; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 157; or

xviii) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 162; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 163; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 164; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 165; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 166; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 167; or

xix) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 172; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 173; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 174; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 175; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 176; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 177; or

xx) (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 182; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 183; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 184; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 185; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 186; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 187.

[0011] In some embodiments, an antibody that binds to ICOS is provided, wherein the antibody comprises a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein:

i) the V_H is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 10 and the V_L is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 11; or

ii) the V_H is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 20 and the V_L is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 21; or

iii) the V_H is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 30 and the V_L is at

least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 31; or

iv) the V_H is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 40 and the V_L is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 41; or

v) the V_H is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 50 and the V_L is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 51; or

vi) the V_H is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 60 and the V_L is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 61; or

vii) the V_H is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 70 and the V_L is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 71; or

viii) the V_H is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 80 and the V_L is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 81; or

ix) the V_H is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 90 and the V_L is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 91; or

x) the V_H is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 100 and the V_L is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 101; or

xi) the V_H is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 110 and the V_L is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 111; or

xii) the V_H is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 120 and the V_L is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 121; or

xiii) the V_H is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 130 and the V_L is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 131; or

xiv) the V_H is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 140 and the V_L is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 141; or

xv) the V_H is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 150 and the V_L is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 151; or

xvi) the V_H is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 160 and the V_L is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 161; or

xvii) the V_H is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 170 and the V_L is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 171; or

xviii) the V_H is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 180 and the V_L is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 181.

[0012] In some embodiments, an antibody that binds to ICOS is provided, wherein the antibody comprises a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein:

i) the V_H comprises the amino acid sequence of SEQ ID NO: 10 and the V_L comprises the amino acid sequence of SEQ ID NO: 11; or

ii) the V_H comprises the amino acid sequence of SEQ ID NO: 20 and the V_L comprises the amino acid sequence of SEQ ID NO: 21; or

iii) the V_H comprises the amino acid sequence of SEQ ID NO: 30 and the V_L comprises the amino acid sequence of SEQ ID NO: 31; or

iv) the V_H comprises the amino acid sequence of SEQ ID NO: 40 and the V_L comprises the amino acid sequence of SEQ ID NO: 41; or

v) the V_H comprises the amino acid sequence of SEQ ID NO: 50 and the V_L comprises the amino acid sequence of SEQ ID NO: 51; or

vi) the V_H comprises the amino acid sequence of SEQ ID NO: 60 and the V_L comprises the amino acid sequence of SEQ ID NO: 61; or

vii) the V_H comprises the amino acid sequence of SEQ ID NO: 70 and the V_L comprises the amino acid sequence of SEQ ID NO: 71; or

viii) the V_H comprises the amino acid sequence of SEQ ID NO: 80 and the V_L comprises the amino acid sequence of SEQ ID NO: 81; or

ix) the V_H comprises the amino acid sequence of SEQ ID NO: 90 and the V_L comprises the amino acid sequence of SEQ ID NO: 91; or

x) the V_H comprises the amino acid sequence of SEQ ID NO: 100 and the V_L comprises the amino acid sequence of SEQ ID NO: 101; or

xi) the V_H comprises the amino acid sequence of SEQ ID NO: 110 and the V_L comprises the amino acid sequence of SEQ ID NO: 111; or

xii) the V_H comprises the amino acid sequence of SEQ ID NO: 120 and the V_L comprises the amino acid sequence of SEQ ID NO: 121; or

xiii) the V_H comprises the amino acid sequence of SEQ ID NO: 130 and the V_L comprises the amino acid sequence of SEQ ID NO: 131; or

xiv) the V_H comprises the amino acid sequence of SEQ ID NO: 140 and the V_L comprises the amino acid sequence of SEQ ID NO: 141; or

xv) the V_H comprises the amino acid sequence of SEQ ID NO: 150 and the V_L comprises the amino acid sequence of SEQ ID NO: 151; or

xvi) the V_H comprises the amino acid sequence of SEQ ID NO: 160 and the V_L comprises the amino acid sequence of SEQ ID NO: 161; or

xvii) the V_H comprises the amino acid sequence of SEQ ID NO: 170 and the V_L comprises the amino acid sequence of SEQ ID NO: 171; or

xviii) the V_H comprises the amino acid sequence of SEQ ID NO: 180 and the V_L comprises the amino acid sequence of SEQ ID NO: 181.

[0013] In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a chimeric antibody or a humanized antibody. In some

embodiments, the antibody is an antibody fragment selected from a Fab, Fab', Fv, scFv or $(Fab')_2$ fragment. In some embodiments, the antibody is a full length antibody.

[0014] In some embodiments, an antibody that binds to ICOS is provided, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 188 and a light chain comprising the amino acid sequence of SEQ ID NO: 189.

[0015] In some embodiments, administration of the antibody to a mammal results in an increase in T effector (Teff) cells in the mammal. In some embodiments, administration of the antibody to a mammal results in activation of T effector (Teff) cells in the mammal. In some embodiments, administration of the antibody to a mammal increases the ratio of Teff cells to Treg cells. In some embodiments, the Teff cells are CD4+ FoxP3- T cells. In some embodiments, the Teff cells are CD4+ FoxP3- T cells and CD8+ T cells. In some embodiments, the Teff cells are CD8+ T cells. In some embodiments, administration of the antibody to a mammal results in a decrease in T regulatory (Treg) cells in the mammal. In some embodiments, the Treg cells are CD4+ FoxP3+ T cells.

[0016] In some embodiments, an isolated antibody is provided that binds to human ICOS, wherein the antibody also binds to mouse ICOS and/or rat ICOS. In some embodiments, the isolated antibody binds to human ICOS with an affinity (K_D) of less than 5 nM. In some embodiments, the isolated antibody binds to rat ICOS with an affinity (K_D) of less than 10 nM. In some embodiments, affinity is determined using biolayer interferometry (*see, e.g.*, Abdiche et al., 2008, *Anal Biochem*, 377: 209-217; and ForteBio Octet® system). In some embodiments, the antibody binds to human ICOS, mouse ICOS, and rat ICOS. In some embodiments, the antibody binds to cynomolgus monkey ICOS. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a chimeric antibody or a humanized antibody. In some embodiments, the antibody is an antibody fragment selected from a Fab, Fab', Fv, scFv or $(Fab')_2$ fragment. In some embodiments, the antibody is a full length antibody. In some embodiments, administration of the antibody to a mammal results in an increase in T effector (Teff) cells in the mammal. In some embodiments, administration of the antibody to a mammal results in activation of T effector (Teff) cells in the mammal. In some embodiments, the Teff cells are CD4+ FoxP3- T cells. In some embodiments, the Teff cells are CD4+ FoxP3- T cells and CD8+ T cells. In some embodiments, the Teff cells are CD8+ T cells. In some embodiments, administration of the antibody to a mammal results in a decrease in T regulatory (Treg) cells in the mammal. In some embodiments, the Treg cells are CD4+ FoxP3+ T cells. In some embodiments, the mammal is selected from a mouse, rat, cynomolgus monkey, and human.

[0017] In some embodiments, following treatment of lung tumor tissue with an antibody provided herein, the level of a chemokine or cytokine selected from GZMa, GZMb, CSF2, IL2, CXCL9, CXCL10, CXCL11, and CXCL13 is at least a 2-fold, or at least 3-fold higher than the level of the chemokine following treatment of the lung tumor tissue with a control antibody. In some embodiments, the level is an mRNA level. In some embodiments, the level is a protein level. In some embodiments, a control antibody is an isotype-matched antibody that binds to an unrelated antigen, and which is not expected to have an effect on the chemokine levels. In some embodiments, the level of the chemokine is measured 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, or 6 hours after treatment. In some embodiments, the chemokine is CXCL11. In some embodiments, the level of the chemokine is measured 6 hours, 8 hours, 10 hours, 12 hours, 14 hours, 16 hours, 18 hours, 20 hours, 22 hours, 24 hours, 36 hours, or 48 hours after treatment. In some embodiments, the level of the chemokine is measured 24 hours after treatment. In some embodiments, the lung tumor tissue is human lung tumor tissue.

[0018] In some embodiments, an antibody provided herein increases the level of at least one chemokine and/or cytokine selected from GZMa, GZMb, CSF2, IL2, CXCL9, CXCL10, CXCL11, and CXCL13 in a mammal that has been administered the antibody by at least a 2-fold. In some embodiments, the level of the chemokine is measured 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, or 6 hours after administration of the antibody. In some embodiments, the level is an mRNA level. In some embodiments, the level is a protein level. In some embodiments, the chemokine is CXCL11. In some embodiments, the level of the chemokine is measured 6 hours, 8 hours, 10 hours, 12 hours, 14 hours, 16 hours, 18 hours, 20 hours, 22 hours, 24 hours, 36 hours, or 48 hours after administration of the antibody. In some embodiments, the level of the chemokine is measured 24 hours after administration of the antibody.

[0019] In some embodiments, an agonist anti-ICOS antibody is provided, wherein the antibody increases the level of at least one chemokine and/or cytokine mRNA selected from GZMa, GZMb, CSF2, IL2, CXCL9, CXCL10, CXCL11, and CXCL13 in a mammal that has been administered the antibody by at least a 2-fold. In some embodiments, an anti-ICOS agonist antibody is provided, wherein the antibody increases the level of at least one chemokine and/or cytokine selected from GZMa, GZMb, CSF2, IL2, CXCL9, CXCL10, and CXCL11 in a mammal that has been administered the antibody by at least a 2-fold. In some embodiments, the level of the chemokine is measured 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, or 6 hours after administration of the antibody. In some embodiments, the level is an mRNA level. In some embodiments, the level is a protein level. In some embodiments, the chemokine is CXCL11. In some embodiments, the level of the chemokine is measured 6 hours, 8 hours, 10 hours, 12 hours, 14 hours, 16 hours, 18 hours, 20 hours, 22 hours, 24 hours, 36 hours, or 48

hours after administration of the antibody. In some embodiments, the level of the chemokine is measured 24 hours after administration of the antibody. In some embodiments, the mammal is a human. In some embodiments, the human has cancer. In some embodiments, the cancer is selected from melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), gastric cancer, bladder cancer, diffuse large B-cell lymphoma (DLBCL), Hodgkin's lymphoma, ovarian cancer, head & neck squamous cell cancer (HNSCC), and triple negative breast cancer (TNBC). In some embodiments, the cancer is selected from melanoma, gastric cancer, head & neck squamous cell cancer (HNSCC), non-small cell lung cancer (NSCLC), and triple negative breast cancer (TNBC).

[0020] In some embodiments, an antibody that binds to ICOS is provided, wherein the antibody increases the level of the ligand for NKp46 (NKp46-L) on T cells. In some embodiments, the increased level of NKp46-L on T cells is determined using a soluble NKp46 extracellular domain in a flow cytometry assay. In some embodiments, the antibody increases the level of NKp46-L on Treg cells more than the antibody increases the level of NKp46-L on Teff cells. In some embodiments, the antibody increases CD16 shedding on NK cells.

[0021] In some aspects, an isolated nucleic acid encoding an antibody described herein is provided. In some aspects, a vector comprising the nucleic acid is provided. In some aspects, a host cell comprising the vector is provided. In some aspects, a host cell that produces an antibody described herein is provided. In some aspects, a method for making an anti-ICOS antibody is provided, comprising culturing the host cell under conditions suitable for expression of the antibody. In some embodiments, the method comprises recovering the antibody produced by the host cell.

[0022] In some aspects, a pharmaceutical composition is provided, which comprises an anti-ICOS antibody described herein and a pharmaceutically acceptable carrier.

[0023] In some embodiments, methods of treating cancer are provided, comprising administering an effective amount of an anti-ICOS antibody described herein or a pharmaceutical composition described herein. In some embodiments, the cancer is selected from melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), gastric cancer, bladder cancer, diffuse large B-cell lymphoma (DLBCL), Hodgkin's lymphoma, ovarian cancer, head & neck squamous cell cancer (HNSCC), and triple negative breast cancer (TNBC). In some embodiments, the cancer is selected from melanoma, gastric cancer, head & neck squamous cell cancer (HNSCC), non-small cell lung cancer (NSCLC), and triple negative breast cancer (TNBC).

[0024] In some embodiments, use of an anti-ICOS antibody described herein or a pharmaceutical composition described herein is provided in the manufacture of a medicament for increasing the number of T effector (Teff) cells in a mammal. In some embodiments, a method of increasing the number of T effector (Teff) cells in a mammal is provided, comprising administering an effective amount of an anti-ICOS

antibody described herein or a pharmaceutical composition described herein. In some embodiments, the method further comprises activating Teff cells. In some embodiments, use of an anti-ICOS antibody described herein or a pharmaceutical composition described herein is provided in the manufacture of a medicament for activating T effector (Teff) cells in a mammal. In some embodiments, a method of activating T effector (Teff) cells in a mammal is provided, comprising administering an effective amount of an anti-ICOS antibody described herein or a pharmaceutical composition described herein. In some embodiments, a method of increasing the ratio of Teff cells to Treg cells in a mammal is provided, comprising administering an effective amount of an anti-ICOS antibody described herein or a pharmaceutical composition described herein. In some embodiments, the Teff cells are CD4+ FoxP3- T cells. In some embodiments, the Teff cells are CD4+ FoxP3- T cells and CD8+ T cells. In some embodiments, the Teff cells are CD8+ T cells. In some embodiments, the method further comprises decreasing the number of T regulatory (Treg) cells.

[0025] In some embodiments, use of an anti-ICOS antibody described herein or a pharmaceutical composition described herein is provided in the manufacture of a medicament for decreasing the number of T regulatory (Treg) cells in a mammal. In some embodiments, a method of decreasing the number of T regulatory (Treg) cells in a mammal is provided, comprising administering an effective amount of an anti-ICOS antibody described herein or a pharmaceutical composition described herein. In some embodiments, the Treg cells are CD4+ FoxP3+ T cells.

[0026] In some embodiments, use of an anti-ICOS antibody described herein is provided in the manufacture of a medicament for increasing the level of at least one chemokine and/or cytokine mRNA selected from GZMa, GZMb, CSF2, IL2, CXCL9, CXCL10, CXCL11, and CXCL13 in a mammal. In some embodiments, a method of increasing the level of at least one chemokine and/or cytokine selected from GZMa, GZMb, CSF2, IL2, CXCL9, CXCL10, CXCL11, and CXCL13 in a mammal is provided, comprising administering to the mammal an antibody provided herein. In some embodiments, the level of at least one chemokine is increased by at least 2-fold or at least 3-fold. In some embodiments, the level of the chemokine is measured 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, or 6 hours after administration of the antibody. In some embodiments, the level is an mRNA level. In some embodiments, the level is a protein level. In some embodiments, the chemokine is CXCL11. In some embodiments, the level of the chemokine is measured 6 hours, 8 hours, 10 hours, 12 hours, 14 hours, 16 hours, 18 hours, 20 hours, 22 hours, 24 hours, 36 hours, or 48 hours after administration of the antibody. In some embodiments, the level of the chemokine is measured 24 hours after administration of the antibody. In some embodiments, the mammal is a human. In some embodiments, the human has cancer. In some embodiments, the cancer is selected from melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), gastric cancer, bladder cancer, diffuse large B-cell lymphoma (DLBCL), Hodgkin's lymphoma, ovarian cancer, head & neck squamous cell cancer (HNSCC), and triple negative breast cancer (TNBC). In some embodiments, the cancer is selected from melanoma, gastric cancer, head & neck squamous cell cancer (HNSCC), non-small cell lung cancer (NSCLC), and triple negative breast cancer (TNBC).

[0027] In some embodiments, the mammal is a human.

[0028] In some embodiments, the mammal is administered at least one additional therapeutic agent. In some embodiments, the additional therapeutic agent is administered concurrently or sequentially with the anti-ICOS antibody. In some embodiments, the additional therapeutic agent is a PD-1 therapy. In some embodiments, the additional therapeutic agent is selected from an anti-PD-1 antibody and an anti-PD-L1 antibody. In some embodiments, an anti-ICOS antibody provided herein is administered with nivolumab. In some embodiments, an anti-ICOS antibody provided herein is administered with pembrolizumab. In some embodiments, an anti-ICOS antibody provided herein is administered with atezolizumab. In some embodiments, an anti-ICOS antibody provided herein is administered with avelumab. In some embodiments, an anti-ICOS antibody provided herein is administered with durvalumab.

[0029] In some embodiments, the additional therapeutic is a cancer vaccine. In some such embodiments, the cancer vaccine is developed using a neoantigen. In some embodiments, the cancer vaccine is a DNA vaccine. In some embodiments, the cancer vaccine is an engineered virus comprising a cancer antigen, such as PROSTVAC (rilimogene galvacirepvec/rilimogene glafolivec). In some embodiments, the cancer vaccine comprises engineered tumor cells, such as GVAX.

[0030] In some embodiments, the anti-ICOS antibody provided herein is administered with an agonist anti-OX40 antibody. In some embodiments, the anti-ICOS antibody provided herein is administered with an anti-CTLA4 antibody. In some embodiments, the anti-ICOS antibody provided herein is administered with ipilimumab.

[0031] In some embodiments, the additional therapeutic is a chemotherapeutic agent. Nonlimiting exemplary chemotherapeutic agents include capectiabine, cyclophosphamide, dacarbazine, temozolomide, cyclophosphamide, docetaxel, doxorubicin, daunorubicin, cisplatin, carboplatin, epirubicin, eribulin, 5-FU, gemcitabine, irinotecan, ixabepilone, methotrexate, mitoxantrone, oxaliplatin, paclitaxel, nab-paclitaxel, ABRAXANE® (protein-bound paclitaxel), pemetrexed, vinorelbine, and vincristine. In some embodiments, the anti-ICOS antibody provided herein is administered with ABRAXANE® (Celgene). In some embodiments, an anti-ICOS antibody provided herein is administered with at least one kinase inhibitor. Nonlimiting exemplary kinase inhibitors include erlotinib, afatinib, gefitinib, crizotinib, dabrafenib, trametinib, vemurafenib, and cobimetanib.

[0032] In some embodiments, the additional therapeutic agent is an IDO inhibitor. Nonlimiting exemplary IDO inhibitors include Indoximod (New Link Genetics), INCB024360 (Incyte Corp), 1-methyl-D-tryptophan (New Link Genetics), and GDC-0919 (Genentech). In some embodiments, the additional therapeutic agent is an immune-modifying drug (IMiD). Nonlimiting exemplary IMiDs include thalidomide, lenalidomide, and pomalidomide.

[0033] In some embodiments, the mammal receives CAR-T therapy in addition to administration of anti-ICOS an antibody described herein.

[0034] In some embodiments, the mammal undergoes surgery and/or radiation therapy in addition to administration of an anti-ICOS antibody described herein, with or without an additional therapeutic agent. In some embodiments, the mammal undergoes radiation therapy in addition to administration of anti-ICOS an antibody described herein, with or without an additional therapeutic agent.

[0035] In some aspects, use of an antibody described herein or a pharmaceutical composition described herein is provided in the manufacture of a medicament for treating cancer in a mammal. In some embodiments, the cancer is selected from melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), gastric cancer, bladder cancer, diffuse large B-cell lymphoma (DLBCL), Hodgkin's lymphoma, ovarian cancer, head & neck squamous cell cancer (HNSCC), and triple negative breast cancer (TNBC). In some embodiments, the cancer is selected from melanoma, gastric cancer, head & neck squamous cell cancer (HNSCC), non-small cell lung cancer (NSCLC), and triple negative breast cancer (TNBC). In some embodiments, the medicament is for administration with at least one additional therapeutic agent. In some embodiments, the additional therapeutic agent is selected from an anti-PD-1 antibody and an anti-PD-L1 antibody.

[0036] In some embodiments, use of an antibody described herein or a pharmaceutical composition described herein is provided for treating cancer. In some embodiments, the cancer is selected from melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), gastric cancer, bladder cancer, diffuse large B-cell lymphoma (DLBCL), Hodgkin's lymphoma, ovarian cancer, head & neck squamous cell cancer (HNSCC), and triple negative breast cancer (TNBC). In some embodiments, the cancer is selected from melanoma, gastric cancer, head & neck squamous cell cancer (HNSCC), non-small cell lung cancer (NSCLC), and triple negative breast cancer (TNBC).

[0037] In some embodiments, use of an antibody described herein or a pharmaceutical composition described herein and at least one additional therapeutic agent is provided for treating cancer. In some aspects, use of an antibody described herein or a pharmaceutical composition described herein and at least one additional therapeutic agent is provided in the manufacture of a medicament for treating cancer. In some embodiments, the additional therapeutic agent is selected from an anti-PD-1 antibody and an anti-PD-L1 antibody. In some embodiments, the cancer is selected from melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), gastric cancer, bladder cancer, diffuse large B-cell lymphoma (DLBCL), Hodgkin's lymphoma, ovarian cancer, head & neck squamous cell cancer (HNSCC), and triple negative breast cancer (TNBC). In some embodiments, the cancer is selected from melanoma, gastric cancer, head & neck squamous cell cancer (HNSCC), non-small cell lung cancer (NSCLC), and triple negative breast cancer (TNBC).

[0038] In some aspects, an antibody described herein or a pharmaceutical composition described herein is provided for use in treating cancer. In some embodiments, the cancer is selected from melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), gastric cancer, bladder cancer, diffuse large B-cell lymphoma (DLBCL), Hodgkin's lymphoma, ovarian cancer, head & neck squamous cell cancer (HNSCC), and triple negative breast cancer (TNBC). In some embodiments, the cancer is selected from melanoma, gastric cancer, head & neck squamous cell cancer (HNSCC), non-small cell lung cancer (NSCLC), and triple negative breast cancer (TNBC).

BRIEF DESCRIPTION OF THE DRAWINGS

[0039] Figure 1A-B. Levels of ICOS mRNA across multiple human tumors. A) Mean intensity and 75% confidence intervals of normalized ICOS mRNA levels across various indications are plotted. Samples with intensities outside of the 75% confidence range are indicated (dot). B) The percentage of each indicated tumor type showing 0, 1+, 2+ or 3+ ICOS staining by immunohistochemistry (IHC).

[0040] Figure 2. Correlation of ICOS expression with T-cell infiltration. ICOS mRNA levels from ~450 HNSCC tumors were compared with a 12 gene T-cell associated chemokine signature score or FoxP3 mRNA levels. Levels of normalized chemokine signature or FoxP3 mRNA for each tumor is plotted on the Y axis, mRNA levels of ICOS are plotted in the X-axis. The Spearman correlation (R) of the association is show on the graph [Corr(S)]. A correlation of >0.75 (Spearman R) is viewed as cut-off for strong correlation.

[0041] Figure 3. ICOS mRNA expression was compared for ~450 HNSCC tumors using TCGA RNA sequencing data (NCI). Levels of normalized CTLA-4 or PD-1 or PD-L1 mRNA for each tumor is plotted on the Y axis, mRNA levels of ICOS are plotted in the X-axis. The Spearman correlation (R) of the association is show on the graph [Corr(S)]. ICOS expression levels significantly correlated with expression of checkpoint molecules CTLA-4, PD-1. A weak correlation between ICOS and PD-L1 was observed. A correlation of >0.75 (Spearman R) is viewed as cut-off for strong correlation.

[0042] Figure 4. Representative images of the varying intensities of ICOS staining in human NSCLC tumor.

[0043] Figure 5. Distribution of ICOS cell density from human tumors. ICOS cell density was determined in each of the human tumors, and mean ICOS density from each tumor type is plotted in Y axis [NSCLC (N=100); HNSCC (N=102); Breast cancer, all major subtypes (N=94); triple negative subtype of breast cancer, TNBC (N=95); ovarian cancer (N=94)]. The statistical analysis was performed using ANOVA.

[0044] Figure 6. Diversity of ICOS cell density in NSCLC samples. A) The density of ICOS expression was evaluated in a panel of lung tumor samples (N=98) and tumors were ranked based on ICOS+ density of positive cells / mm2. B) Diversity of ICOS expression in a second independent cohort of NSCLC clinical samples (N=204).

[0045] Figure 7. Distribution of ICOS cell density in different T cell subsets from human cancer. A) Representative image depicting the ICOS staining in the distinct T cell compartments. The arrows point to ICOS+ FOXP3+ Treg cells or ICOS+ CD8- CD4 effectors. B) ICOS cell density from individual tumors was analyzed in FoxP3 positive CD4 Tregs or CD8 positive T cells or CD8 negative and FoxP3 negative CD4 effs. The mean ICOS density and standard deviation from each of these tumor types are plotted. [Lung cancer (N=100); HNSCC (N=102); Triple negative subtype of breast cancer, TNBC (N=95); Ovarian cancer (N=94)].

[0046] Figure 8A-B. High ICOS expression is observed in PD-L1 high NSCLC tumors. By utilizing the PD-L1/ICOS/PD-1 multiplex IHC we evaluated ICOS and PD-L1 levels in a set of adenocarcinoma of NSCLC (n=150). A) Representative images of a PD-L1 high (left panel) or a PD-L1 low (right panel) tumor stained with ICOS, PD-1 and PD-L1. B) Quantification of ICOS cell density in PD-L1 high (>5% of cells are PD-L1 positive) or PD-L1 low (<5% of the cells are PD-L1 positive) squamous cell carcinoma (left panel) and adenocarcinoma (right panel).

[0047] Figure 9A-C. Human tumor TIL analysis show ICOS expression in the Treg cells and CD4 effectors. A) Representative contour plots of PD-1 and ICOS expression in different T-cells subsets from HNSCC patients (N=4). B) The frequency of ICOS alone positive cells or the ICOS PD-1 double positive cells in the T-cell compartment is shown (HNSCC N=4; NSCLC N=3; Ovarian N=4). C) Comparison of ICOS levels in CD4 Treg cells and CD4 Teff. The staining intensity of ICOS as measured by Mean Fluorescent Intensity (MFI; or ICOS geometric mean) in the CD4 T-cell subsets from patient tumor samples is plotted.

[0048] Figure 10A-C. A) Effect of anti-ICOS antibodies on proliferation of primary human CD4+ T cells in a plate-bound format in the presence of suboptimal anti-CD3 is shown. Percent of cells divided is graphed. B) Effect of anti-ICOS antibodies on proliferation of human CD4+ T cells in soluble format in the presence of suboptimal PMA is shown. Percent of cells divided is graphed. C) Effect of anti-ICOS antibody 37A10S713-hIgG1 on proliferation of primary human CD4+ T cells in a plate-bound format in the presence of suboptimal anti-CD3.

[0049] Figure 11A-B. Evaluation of anti-ICOS antibodies in an NF-kB reporter assay is shown. The graphs show percent GFP+ cells.

[0050] Figure 12. Evaluation of soluble anti-ICOS antibodies in a PBMC assay with super-antigen (SEB) stimulation is shown. The readout is IFNg production.

[0051] Figure 13. Anti-ICOS antibodies were evaluated for potential superagonism in a human T cell proliferation assay in the absence anti-CD3. The readout in this assay is percent proliferation.

[0052] Figure 14A-B. Evaluation of an anti-ICOS antibody in a phospho-AKT (pAKT) assay in the presence or absence of a secondary cross-linker. The readout is percent of CD4 T cells that are pAKT-positive. A) Results in the absence of secondary cross-linker. B) Results in the presence of secondary cross-linker.

[0053] Figure 15A-B. Anti-ICOS antibodies were evaluated in the Sa1/N fibrosarcoma syngeneic tumor model. Tumor growth is plotted on the y-axis. A) Dashed lines indicate tumor growth of individual mice; solid line indicates average growth curve for the group. Number of tumor-free mice per group is indicated. B) Average tumor volume in each treatment group.

[0054] Figure 16. Tumor-free mice previously treated with anti-ICOS 7F12 were re-challenged with Sa1/N tumors Tumor growth is plotted on the y-axis.

[0055] Figure 17. Effect of hamster anti-ICOS antibody 37A10 with mG1 and mG2a Fc on Sa1/N tumor growth. Dashed lines indicate individual tumor growth of individual mice; solid line indicates average tumor growth curve for the group. Number of tumor-free mice per group is indicated.

[0056] Figure 18. Evaluation of anti-ICOS antibodies as single agents or in combination with anti-PD1 in the CT26 syngeneic tumor model. Dashed lines indicate individual mice; solid line indicates average growth curve for the group. Number of tumor-free mice per group is indicated.

[0057] Figure 19. Depletion of FoxP3+ Tregs in Sa1/N tumors upon anti-ICOS antibody treatment. Frequency of CD8, CD4 Teff and Treg cells in spleen and tumor, and number of Tregs per mg of tumor is shown. Each shape indicates an individual mouse.

[0058] Figure 20. Depletion of Tregs and activation of Teff cells in Sa1/N tumors upon anti-ICOS antibody treatment. Top row: Frequency of CD8, CD4 Teff and Treg cells, CD8 to Treg ratio and frequency of PD-1+ CD8 T cells in tumors. Bottom row: Frequency of dividing Ki-67+ CD8 and CD4 Teff cells, and Tbet+ CD4 Teff cells among CD3+ cells in tumors. Each shape indicates an individual mouse.

[0059] Figure 21. Evaluation of an anti-ICOS antibody in the Sa1/N tumor model following depletion of T cells. Tumor growth over time is plotted. Number of tumor free mice is indicated.

[0060] Figure 22A-B. A) Reduction of Tegs cells in a PBMC assay upon treatment with a humanized anti-ICOS antibody. B) Treg and Teff cells express similar levels of ICOS following five days of IL-2 treatment.

[0061] Figure 23. Tumor re-challenge following treatment with anti-ICOS antibody. Left panel shows tumor volume in mice following administration of one or two doses of anti-ICOS antibody. Right panel shows tumor volume in control mice or mice that were tumor free following administration of anti-ICOS antibody, following tumor re-challenge.

[0062] Figure 24A-B. Increase in ICOSL expression in Sa1/N tumor bearing mice (A) and cynomolgus monkeys (B) administered anti-ICOS antibody.

[0063] Figure 25. Change in Th-1 chemokine and cytokine expression following treatment of lung tumor tissue with anti-ICOS antibody (right panels) or anti-PD-1 antibody (left panels), at 6 hours (top panels) or 24 hours (bottom panels).

[0064] Figure 26. NKp46 ligand levels on Teff cells (A, C, E) and Treg cells (B, D, F) from three different donors, following treatment with agonist and antagonist anti-ICOS antibodies.

[0065] Figure 27. Loss of CD16 (CD16 shedding) from NK cells treated with agonist anti-ICOS antibody.

DETAILED DESCRIPTION OF SOME EMBODIMENTS

[0066] Antibodies that bind ICOS are provided. Antibody heavy chains and light chains that are capable of forming antibodies that bind ICOS are also provided. In addition, antibodies, heavy chains, and light chains comprising one or more particular complementarity determining regions (CDRs) are provided. Polynucleotides encoding antibodies to ICOS are provided. Polynucleotides encoding antibody heavy chains or lights chains are also provided. Methods of producing and/or purifying antibodies to ICOS are provided. Methods of treatment using antibodies to ICOS are provided. Such methods include, but are not limited to, methods of treating cancer. Methods of detecting ICOS are provided. Such methods include methods to identify an individual who may benefit from treatment with an anti-ICOS antibody, to monitor treatment of an individual with an anti-ICOS antibody and to improve therapeutic efficacy of an anti-ICOS antibody in an individual.

[0067] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0068] All references cited herein, including patent applications, patent publications, and Genbank Accession numbers are herein incorporated by reference, as if each individual reference were specifically and individually indicated to be incorporated by reference in its entirety.

[0069] The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in Sambrook *et al.*, Molecular Cloning: A Laboratory Manual 3rd. edition (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (F. M. Ausubel, *et al. eds.*, (2003)); the series METHODS IN ENZYMOLOGY (Academic Press, Inc.): PCR 2: A PRACTICAL APPROACH (M. J. MacPherson, B. D. Hames and G. R. Taylor *eds.* (1995)), Harlow and Lane, *eds.* (1988) ANTIBODIES, A LABORATORY MANUAL, and ANIMAL CELL CULTURE (R. I. Freshney, *ed.* (1987)); Oligonucleotide Synthesis (M. J. Gait, ed., 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook (J. E. Cellis, *ed.*, 1998) Academic Press; Animal Cell Culture (R. I. Freshney), *ed.*, 1987); Introduction to Cell and Tissue Culture (J. P. Mather and P. E. Roberts, 1998) Plenum Press; Cell and Tissue Culture Laboratory Procedures (A. Doyle, J. B. Griffiths, and D. G. Newell, eds., 1993-8) J. Wiley and Sons; Handbook of Experimental Immunology (D. M. Weir and C. C. Blackwell, *eds.*); Gene Transfer Vectors for Mammalian Cells (J. M. Miller and M. P. Calos, *eds.*, 1987); PCR: The Polymerase Chain Reaction, (Mullis *et al.*, *eds.*, 1994); Current Protocols in Immunology (J. E. Coligan *et al.*, *eds.*, 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C. A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: A Practical Approach (D. Catty., *ed.*, IRL Press, 1988-1989); Monoclonal Antibodies: A Practical Approach (P. Shepherd and C. Dean, *eds.*, Oxford University Press, 2000); Using Antibodies: A Laboratory Manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanetti and J. D. Capra, *eds.*, Harwood Academic Publishers, 1995); and Cancer: Principles and Practice of Oncology (V. T. DeVita *et al.*, *eds.*, J.B. Lippincott Company, 1993); and updated versions thereof.

I. Definitions

[0070] Unless otherwise defined, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context or expressly indicated, singular terms shall include pluralities and plural terms shall include the singular. For any conflict in definitions between various sources or references, the definition provided herein will control.

[0071] It is understood that embodiments of the invention described herein include "consisting" and/or "consisting essentially of" embodiments. As used herein, the singular form

"a", "an", and "the" includes plural references unless indicated otherwise. Use of the term "or" herein is not meant to imply that alternatives are mutually exclusive.

[0072] In this application, the use of "or" means "and/or" unless expressly stated or understood by one skilled in the art. In the context of a multiple dependent claim, the use of "or" refers back to more than one preceding independent or dependent claim.

[0073] As is understood by one skilled in the art, reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X".

[0074] The terms "nucleic acid molecule", "nucleic acid" and "polynucleotide" may be used interchangeably, and refer to a polymer of nucleotides. Such polymers of nucleotides may contain natural and/or non-natural nucleotides, and include, but are not limited to, DNA, RNA, and PNA. "Nucleic acid sequence" refers to the linear sequence of nucleotides that comprise the nucleic acid molecule or polynucleotide.

[0075] The terms "polypeptide" and "protein" are used interchangeably to refer to a polymer of amino acid residues, and are not limited to a minimum length. Such polymers of amino acid residues may contain natural or non-natural amino acid residues, and include, but are not limited to, peptides, oligopeptides, dimers, trimers, and multimers of amino acid residues. Both full-length proteins and fragments thereof are encompassed by the definition. The terms also include post-expression modifications of the polypeptide, for example, glycosylation, sialylation, acetylation, phosphorylation, and the like. Furthermore, for purposes of the present disclosure, a "polypeptide" refers to a protein which includes modifications, such as deletions, additions, and substitutions (generally conservative in nature), to the native sequence, as long as the protein maintains the desired activity. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts which produce the proteins or errors due to PCR amplification.

[0076] "ICOS" and "inducible T-cell costimulatory" as used herein refer to any native ICOS that results from expression and processing of ICOS in a cell. The term includes ICOS from any vertebrate source, including mammals such as primates (e.g., humans and cynomolgus monkeys) and rodents (e.g., mice and rats), unless otherwise indicated. The term also includes naturally occurring variants of ICOS, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human ICOS precursor protein, with signal sequence (with signal sequence, amino acids 1-20) is shown in SEQ ID NO: 1. The amino acid sequence of an exemplary mature human ICOS is shown in SEQ ID NO: 2. The amino acid sequence of an exemplary mouse ICOS precursor protein, with signal sequence (with signal sequence, amino acids 1-20) is shown in SEQ ID NO: 3. The amino acid sequence of an exemplary mature

mouse ICOS is shown in SEQ ID NO: 4. The amino acid sequence of an exemplary rat ICOS precursor protein, with signal sequence (with signal sequence, amino acids 1-20) is shown in SEQ ID NO: 190. The amino acid sequence of an exemplary mature rat ICOS is shown in SEQ ID NO: 191. The amino acid sequence of an exemplary cynomolgus monkey ICOS precursor protein, with signal sequence (with signal sequence, amino acids 1-20) is shown in SEQ ID NO: 5. The amino acid sequence of an exemplary mature cynomolgus monkey ICOS is shown in SEQ ID NO: 6.

[0077] The term "specifically binds" to an antigen or epitope is a term that is well understood in the art, and methods to determine such specific binding are also well known in the art. A molecule is said to exhibit "specific binding" or "preferential binding" if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular cell or substance than it does with alternative cells or substances. An antibody "specifically binds" or "preferentially binds" to a target if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. For example, an antibody that specifically or preferentially binds to an ICOS epitope is an antibody that binds this epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other ICOS epitopes or non-ICOS epitopes. It is also understood by reading this definition that, for example, an antibody (or moiety or epitope) that specifically or preferentially binds to a first target may or may not specifically or preferentially bind to a second target. As such, "specific binding" or "preferential binding" does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means preferential binding. "Specificity" refers to the ability of a binding protein to selectively bind an antigen.

[0078] As used herein, "substantially pure" refers to material which is at least 50% pure (that is, free from contaminants), more preferably, at least 90% pure, more preferably, at least 95% pure, yet more preferably, at least 98% pure, and most preferably, at least 99% pure.

[0079] As used herein, the term "epitope" refers to a site on a target molecule (for example, an antigen, such as a protein, nucleic acid, carbohydrate or lipid) to which an antigen-binding molecule (for example, an antibody, antibody fragment, or scaffold protein containing antibody binding regions) binds. Epitopes often include a chemically active surface grouping of molecules such as amino acids, polypeptides or sugar side chains and have specific three-dimensional structural characteristics as well as specific charge characteristics. Epitopes can be formed both from contiguous and/or juxtaposed noncontiguous residues (for example, amino acids, nucleotides, sugars, lipid moiety) of the target molecule. Epitopes formed from contiguous residues (for example, amino acids, nucleotides, sugars, lipid moiety) typically are

retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding typically are lost on treatment with denaturing solvents. An epitope may include but is not limited to at least 3, at least 5 or 8-10 residues (for example, amino acids or nucleotides). In some examples an epitope is less than 20 residues (for example, amino acids or nucleotides) in length, less than 15 residues or less than 12 residues. Two antibodies may bind the same epitope within an antigen if they exhibit competitive binding for the antigen. In some embodiments, an epitope can be identified by a certain minimal distance to a CDR residue on the antigen-binding molecule. In some embodiments, an epitope can be identified by the above distance, and further limited to those residues involved in a bond (for example, a hydrogen bond) between an antibody residue and an antigen residue. An epitope can be identified by various scans as well, for example an alanine or arginine scan can indicate one or more residues that the antigen-binding molecule can interact with. Unless explicitly denoted, a set of residues as an epitope does not exclude other residues from being part of the epitope for a particular antibody. Rather, the presence of such a set designates a minimal series (or set of species) of epitopes. Thus, in some embodiments, a set of residues identified as an epitope designates a minimal epitope of relevance for the antigen, rather than an exclusive list of residues for an epitope on an antigen.

[0080] A "nonlinear epitope" or "conformational epitope" comprises noncontiguous polypeptides, amino acids and/or sugars within the antigenic protein to which an antibody specific to the epitope binds. In some embodiments, at least one of the residues will be noncontiguous with the other noted residues of the epitope; however, one or more of the residues can also be contiguous with the other residues.

[0081] A "linear epitope" comprises contiguous polypeptides, amino acids and/or sugars within the antigenic protein to which an antibody specific to the epitope binds. It is noted that, in some embodiments, not every one of the residues within the linear epitope need be directly bound (or involved in a bond) with the antibody. In some embodiments, linear epitopes can be from immunizations with a peptide that effectively consisted of the sequence of the linear epitope, or from structural sections of a protein that are relatively isolated from the remainder of the protein (such that the antibody can interact, at least primarily), just with that sequence section.

[0082] The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (for example, bispecific(such as Bi-specific T-cell engagers) and trispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.

[0083] The term antibody includes, but is not limited to, fragments that are capable of binding to an antigen, such as Fv, single-chain Fv (scFv), Fab, Fab', di-scFv, sdAb (single domain antibody) and $(Fab')_2$ (including a chemically linked $F(ab')_2$). Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen. The term antibody also includes, but is not limited to, chimeric antibodies, humanized antibodies, and antibodies of various species such as mouse, human, cynomolgus monkey, *etc*. Furthermore, for all antibody constructs provided herein, variants having the sequences from other organisms are also contemplated. Thus, if a human version of an antibody is disclosed, one of skill in the art will appreciate how to transform the human sequence based antibody into a mouse, rat, cat, dog, horse, etc. sequence. Antibody fragments also include either orientation of single chain scFvs, tandem di-scFv, diabodies, tandem tri-sdcFv, minibodies, etc. Antibody fragments also include nanobodies (sdAb, an antibody having a single, monomeric domain, such as a pair of variable domains of heavy chains, without a light chain). An antibody fragment can be referred to as being a specific species in some embodiments (for example, human scFv or a mouse scFv). This denotes the sequences of at least part of the non-CDR regions, rather than the source of the construct.

[0084] The term "monoclonal antibody" refers to an antibody of a substantially homogeneous population of antibodies, that is, the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. Thus, a sample of monoclonal antibodies can bind to the same epitope on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies may be made by the hybridoma method first described by Kohler and Milstein, 1975, Nature 256:495, or may be made by recombinant DNA methods such as described in U.S. Pat. No. 4,816,567. The monoclonal antibodies may also be isolated from phage libraries generated using the techniques described in McCafferty et al., 1990, Nature 348:552-554, for example.

[0085] The term "CDR" denotes a complementarity determining region as defined by at least one manner of identification to one of skill in the art. In some embodiments, CDRs can be defined in accordance with any of the Chothia numbering schemes, the Kabat numbering scheme, a combination of Kabat and Chothia, the AbM definition, the contact definition, and/or a combination of the Kabat, Chothia, AbM, and/or contact definitions. Exemplary CDRs (CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3) occur at amino acid residues 24-34 of L1, 50-56 of L2, 89-97 of L3, 31-35B of H1, 50-65 of H2, and 95-102 of H3. (Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991)). The AbM definition can include, for example, CDRs (CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3) at amino acid residues 24-34 of L1, 50-56 of L2, 89-97 of L3, H26-H35B of H1, 50-58 of H2, and 95-102 of H3. The Contact definition can include, for example, CDRs (CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3) at amino acid residues 30-36 of L1, 46-55 of L2, 89-96 of L3, 30-35 of H1, 47-58 of H2, and 93-101 of H3. The Chothia definition can include, for example, CDRs (CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3) at amino acid residues 24-34 of L1, 50-56 of L2, 89-97 of L3, 26-32…34 of H1, 52-56 of H2, and 95-102 of H3. CDRs can also be provided as shown in any one or more of the accompanying figures. With the exception of CDR1 in V_H, CDRs generally comprise the amino acid residues that form the hypervariable loops. The various CDRs within an antibody can be designated by their appropriate number and chain type, including, without limitation as: a) CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3; b) CDRL1, CDRL2, CDRL3, CDRH1, CDRH2, and CDRH3; c) LCDR-1, LCDR-2, LCDR-3, HCDR-1, HCDR-2, and HCDR-3; or d) LCDR1, LCDR2, LCDR3, HCDR1, HCDR2, and HCDR3; etc. The term "CDR" is used herein to also encompass HVR or a "hyper variable region", including hypervariable loops. Exemplary hypervariable loops occur at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3). (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987).)

[0086] The term "heavy chain variable region" as used herein refers to a region comprising at least three heavy chain CDRs. In some embodiments, the heavy chain variable region includes the three CDRs and at least FR2 and FR3. In some embodiments, the heavy chain variable region includes at least heavy chain HCDR1, framework (FR) 2, HCDR2, FR3, and HCDR3. In some embodiments, a heavy chain variable region also comprises at least a portion of an FR1 and/or at least a portion of an FR4.

[0087] The term "heavy chain constant region" as used herein refers to a region comprising at least three heavy chain constant domains, C_H1, C_H2, and C_H3. Of course, non-function-

altering deletions and alterations within the domains are encompassed within the scope of the term "heavy chain constant region," unless designated otherwise. Nonlimiting exemplary heavy chain constant regions include γ, δ, and α. Nonlimiting exemplary heavy chain constant regions also include ε and μ. Each heavy constant region corresponds to an antibody isotype. For example, an antibody comprising a γ constant region is an IgG antibody, an antibody comprising a δ constant region is an IgD antibody, and an antibody comprising an α constant region is an IgA antibody. Further, an antibody comprising a μ constant region is an IgM antibody, and an antibody comprising an ε constant region is an IgE antibody. Certain isotypes can be further subdivided into subclasses. For example, IgG antibodies include, but are not limited to, IgG1 (comprising a γ_1 constant region), IgG2 (comprising a γ_2 constant region), IgG3 (comprising a γ_3 constant region), and IgG4 (comprising a γ_4 constant region) antibodies; IgA antibodies include, but are not limited to, IgA1 (comprising an α_1 constant region) and IgA2 (comprising an α_2 constant region) antibodies; and IgM antibodies include, but are not limited to, IgM1 and IgM2.

[0088] The term "heavy chain" as used herein refers to a polypeptide comprising at least a heavy chain variable region, with or without a leader sequence. In some embodiments, a heavy chain comprises at least a portion of a heavy chain constant region. The term "full-length heavy chain" as used herein refers to a polypeptide comprising a heavy chain variable region and a heavy chain constant region, with or without a leader sequence.

[0089] The term "light chain variable region" as used herein refers to a region comprising at least three light chain CDRs. In some embodiments, the light chain variable region includes the three CDRs and at least FR2 and FR3. In some embodiments, the light chain variable region includes at least light chain LCDR1, framework (FR) 2, LCDR2, FR3, and LCDR3. For example, a light chain variable region may comprise light chain CDR1, framework (FR) 2, CDR2, FR3, and CDR3. In some embodiments, a light chain variable region also comprises at least a portion of an FR1 and/or at least a portion of an FR4.

[0090] The term "light chain constant region" as used herein refers to a region comprising a light chain constant domain, C_L. Nonlimiting exemplary light chain constant regions include λ and κ. Of course, non-function-altering deletions and alterations within the domains are encompassed within the scope of the term "light chain constant region," unless designated otherwise.

[0091] The term "light chain" as used herein refers to a polypeptide comprising at least a light chain variable region, with or without a leader sequence. In some embodiments, a light chain comprises at least a portion of a light chain constant region. The term "full-length light

chain" as used herein refers to a polypeptide comprising a light chain variable region and a light chain constant region, with or without a leader sequence.

[0092] An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (V_L) framework or a heavy chain variable domain (V_H) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework derived from a human immunoglobulin framework or a human consensus framework can comprise the same amino acid sequence thereof, or it can contain amino acid sequence changes. In some embodiments, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some embodiments, the V_L acceptor human framework is identical in sequence to the V_L human immunoglobulin framework sequence or human consensus framework sequence.

[0093] "Affinity" refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (for example, an antibody) and its binding partner (for example, an antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_D). Affinity can be measured by common methods known in the art (such as, for example, ELISA K_D, KinExA, bio-layer interferometry (BLI), and/or surface plasmon resonance devices (such as a BIAcore® device), including those described herein).

[0094] The term "K_D", as used herein, refers to the equilibrium dissociation constant of an antibody-antigen interaction.

[0095] In some embodiments, the "K_D," "K_d," "Kd" or "Kd value" of the antibody is measured by using surface plasmon resonance assays using a BIACORE®-2000 or a BIACORE®-3000 (BIAcore, Inc., Piscataway, N.J.) at 25 °C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIACORE, Inc.) are activated with N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 μg/ml (~0.2 μM) before injection at a flow rate of 5 μL/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, serial dilutions of polypeptide, for example, full length antibody, are injected in PBS with 0.05% TWEEN-20™ surfactant (PBST) at 25 °C at a flow rate of approximately 25 μL/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams.

The equilibrium dissociation constant (K_d) is calculated as the ratio k_{off}/k_{on}. See, for example, Chen *et al.*, *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds 10^6 $M^{-1}s^{-1}$ by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation=295 nm; emission=340 nm, 16 nm band-pass) at 25 °C of a 20 nM anti-antigen antibody in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

[0096] In some embodiments, the difference between said two values (for example, K_d values) is substantially the same, for example, less than about 50%, less than about 40%, less than about 30%, less than about 20%, and/or less than about 10% as a function of the reference/comparator value.

[0097] In some embodiments, the difference between said two values (for example, K_d values) is substantially different, for example, greater than about 10%, greater than about 20%, greater than about 30%, greater than about 40%, and/or greater than about 50% as a function of the value for the reference/comparator molecule.

[0098] "Surface plasmon resonance" denotes an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore™ system (BIAcore International AB, a GE Healthcare company, Uppsala, Sweden and Piscataway, N.J.). For further descriptions, see Jonsson et al. (1993) *Ann. Biol. Clin.* 51:19-26.

[0099] "Biolayer interferometry" refers to an optical analytical technique that analyzes the interference pattern of light reflected from a layer of immobilized protein on a biosensor tip and an internal reference layer. Changes in the number of molecules bound to the biosensor tip cause shifts in the interference pattern that can be measured in real-time. A nonlimiting exemplary device for biolayer interferometry is ForteBio Octet® RED96 system (Pall Corporation). *See, e.g.*, Abdiche et al., 2008, *Anal. Biochem.* 377: 209-277.

[00100] The term "k_{on}", as used herein, refers to the rate constant for association of an antibody to an antigen. Specifically, the rate constants (k_{on} and k_{off}) and equilibrium dissociation constants are measured using IgGs (bivalent) with monovalent ICOS antigen. "K_{on}", "k_{on}", "association rate constant", or "ka", are used interchangeably herein. The value indicates the binding rate of a binding protein to its target antigen or the rate of complex formation between an antibody and antigen, shown by the equation:

Antibody("Ab")+Antigen("Ag")→Ab-Ag.

The term "k_{off}", as used herein, refers to the rate constant for dissociation of an antibody from

the antibody/antigen complex. k_{off} is also denoted as "K_{off}" or the "dissociation rate constant". This value indicates the dissociation rate of an antibody from its target antigen or separation of Ab-Ag complex over time into free antibody and antigen as shown by the equation:

$$Ab+Ag \leftarrow Ab\text{-}Ag.$$

[00101] The term "biological activity" refers to any one or more biological properties of a molecule (whether present naturally as found *in vivo*, or provided or enabled by recombinant means). Biological properties include, but are not limited to, binding a receptor, inducing cell proliferation, inhibiting cell growth, inducing other cytokines, inducing apoptosis, and enzymatic activity. In some embodiments, biological activity of an ICOS protein includes, for example, costimulation of T cell proliferation and cytokine secretion associated with Th1 and Th2 cells; modulation of Treg cells; effects on T cell differentiation including modulation of transcription factor gene expression; induction of signaling through PI3K and AKT pathways; and mediating ADCC.

[00102] An "affinity matured" antibody refers to an antibody with one or more alterations in one or more CDRs compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen.

[00103] A "chimeric antibody" as used herein refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while at least a part of the remainder of the heavy and/or light chain is derived from a different source or species. In some embodiments, a chimeric antibody refers to an antibody comprising at least one variable region from a first species (such as mouse, rat, cynomolgus monkey, *etc.*) and at least one constant region from a second species (such as human, cynomolgus monkey, *etc.*). In some embodiments, a chimeric antibody comprises at least one mouse variable region and at least one human constant region. In some embodiments, a chimeric antibody comprises at least one cynomolgus variable region and at least one human constant region. In some embodiments, all of the variable regions of a chimeric antibody are from a first species and all of the constant regions of the chimeric antibody are from a second species. The chimeric construct can also be a functional fragment, as noted above.

[00104] A "humanized antibody" as used herein refers to an antibody in which at least one amino acid in a framework region of a non-human variable region has been replaced with the corresponding amino acid from a human variable region. In some embodiments, a humanized antibody comprises at least one human constant region or fragment thereof. In some embodiments, a humanized antibody is an antibody fragment, such as Fab, an scFv, a $(Fab')_2$, *etc.* The term humanized also denotes forms of non-human (for example, murine) antibodies that are chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv,

Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) that contain minimal sequence of non-human immunoglobulin. Humanized antibodies can include human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are substituted by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, the humanized antibody can comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences, but are included to further refine and optimize antibody performance. In general, the humanized antibody can comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. In some embodiments, the humanized antibody can also comprise at least a portion of an immunoglobulin constant region or domain (Fc), typically that of a human immunoglobulin. Other forms of humanized antibodies have one or more CDRs (CDR L1, CDR L2, CDR L3, CDR H1, CDR H2, and/or CDR H3) which are altered with respect to the original antibody, which are also termed one or more CDRs "derived from" one or more CDRs from the original antibody. As will be appreciated, a humanized sequence can be identified by its primary sequence and does not necessarily denote the process by which the antibody was created.

[00105] An "CDR-grafted antibody" as used herein refers to a humanized antibody in which one or more complementarity determining regions (CDRs) of a first (non-human) species have been grafted onto the framework regions (FRs) of a second (human) species.

[00106] A "human antibody" as used herein encompasses antibodies produced in humans, antibodies produced in non-human animals that comprise human immunoglobulin genes, such as XenoMouse® mice, and antibodies selected using in vitro methods, such as phage display (Vaughan et al., 1996, Nature Biotechnology, 14:309-314; Sheets et al., 1998, Proc. Natl. Acad. Sci. (USA) 95:6157-6162; Hoogenboom and Winter, 1991, J. Mol. Biol., 227:381; Marks et al., 1991, J. Mol. Biol., 222:581), wherein the antibody repertoire is based on a human immunoglobulin sequence. The term "human antibody" denotes the genus of sequences that are human sequences. Thus, the term is not designating the process by which the antibody was created, but the genus of sequences that are relevant.

[00107] A "functional Fc region" possesses an "effector function" of a native sequence Fc region. Exemplary "effector functions" include Fc receptor binding; C1q binding; CDC; ADCC; phagocytosis; down regulation of cell surface receptors (for example B cell receptor;

BCR), *etc.* Such effector functions generally require the Fc region to be combined with a binding domain (for example, an antibody variable domain) and can be assessed using various assays.

[00108] A "native sequence Fc region" comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. Native sequence human Fc regions include a native sequence human IgG1 Fc region (non-A and A allotypes); native sequence human IgG2 Fc region; native sequence human IgG3 Fc region; and native sequence human IgG4 Fc region as well as naturally occurring variants thereof.

[00109] A "variant Fc region" comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification. In some embodiments, a "variant Fc region" comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification, yet retains at least one effector function of the native sequence Fc region. In some embodiments, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, for example, from about one to about ten amino acid substitutions, and preferably, from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. In some embodiments, the variant Fc region herein will possess at least about 80% sequence identity with a native sequence Fc region and/or with an Fc region of a parent polypeptide, at least about 90% sequence identity therewith, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity therewith.

[00110] "Fc receptor" or "FcR" describes a receptor that binds to the Fc region of an antibody. In some embodiments, an FcγR is a native human FcR. In some embodiments, an FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of those receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (see, for example, Daeron, *Annu. Rev. Immunol.* 15:203-234 (1997)). FcRs are reviewed, for example, in Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991); Capel *et al.*, *Immunomethods* 4:25-34 (1994); and de Haas *et al.*, *J. Lab. Clin. Med.* 126:330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein.

[00111] The term "Fc receptor" or "FcR" also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer *et al.*, *J. Immunol.* 117:587 (1976) and Kim *et al.*, *J. Immunol.* 24:249 (1994)) and regulation of homeostasis of immunoglobulins. Methods of measuring binding to FcRn are known (see, for example, Ghetie and Ward., *Immunol. Today* 18(12):592-598 (1997); Ghetie *et al.*, *Nature Biotechnology*, 15(7):637-640 (1997); Hinton *et al.*, *J. Biol. Chem.* 279(8):6213-6216 (2004); WO 2004/92219 (Hinton *et al.*).

[00112] "Effector functions" refer to biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: Clq binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (for example B cell receptor); and B cell activation.

[00113] "Human effector cells" are leukocytes which express one or more FcRs and perform effector functions. In some embodiments, the cells express at least FcγRIII and perform ADCC effector function(s). Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells, and neutrophils. The effector cells may be isolated from a native source, for example, from blood.

[00114] "Antibody-dependent cell-mediated cytotoxicity" or "ADCC" refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (for example NK cells, neutrophils, and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII, and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an in vitro ADCC assay, such as that described in US Pat. Nos. 5,500,362 or 5,821,337 or U.S. Pat. No. 6,737,056 (Presta), may be performed. Useful effector cells for such assays include PBMC and NK cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, for example, in an animal model such as that disclosed in Clynes *et al. Proc. Natl. Acad. Sci. (USA)* 95:652-656 (1998). Additional polypeptide variants with altered Fc region amino acid sequences (polypeptides with a variant Fc region) and increased or decreased ADCC activity are described, for example, in U.S. Pat. No. 7,923,538, and U.S. Pat. No. 7,994,290.

[00115] "Complement dependent cytotoxicity" or "CDC" refers to the lysis of a target cell in the presence of complement. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system (C1q) to antibodies (of the

appropriate subclass), which are bound to their cognate antigen. To assess complement activation, a CDC assay, for example, as described in Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996), may be performed. Polypeptide variants with altered Fc region amino acid sequences (polypeptides with a variant Fc region) and increased or decreased C1q binding capability are described, for example, in U.S. Pat. No. 6,194,551 B1, U.S. Pat. No. 7,923,538, U.S. Pat. No. 7,994,290 and WO 1999/51642. See also, for example, Idusogie *et al.*, *J. Immunol.* 164: 4178-4184 (2000).

[00116] A polypeptide variant with "altered" FcR binding affinity or ADCC activity is one which has either enhanced or diminished FcR binding activity and/or ADCC activity compared to a parent polypeptide or to a polypeptide comprising a native sequence Fc region. The polypeptide variant which "displays increased binding" to an FcR binds at least one FcR with better affinity than the parent polypeptide. The polypeptide variant which "displays decreased binding" to an FcR, binds at least one FcR with lower affinity than a parent polypeptide. Such variants which display decreased binding to an FcR may possess little or no appreciable binding to an FcR, for example, 0-20% binding to the FcR compared to a native sequence IgG Fc region.

[00117] The polypeptide variant which "mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of human effector cells more effectively" than a parent antibody is one which in vitro or in vivo is more effective at mediating ADCC, when the amounts of polypeptide variant and parent antibody used in the assay are essentially the same. Generally, such variants will be identified using the in vitro ADCC assay as herein disclosed, but other assays or methods for determining ADCC activity, for example in an animal model *etc.*, are contemplated.

[00118] The term "substantially similar" or "substantially the same," as used herein, denotes a sufficiently high degree of similarity between two or more numeric values such that one of skill in the art would consider the difference between the two or more values to be of little or no biological and/or statistical significance within the context of the biological characteristic measured by said value. In some embodiments the two or more substantially similar values differ by no more than about any one of 5%, 10%, 15%, 20%, 25%, or 50%.

[00119] The phrase "substantially different," as used herein, denotes a sufficiently high degree of difference between two numeric values such that one of skill in the art would consider the difference between the two values to be of statistical significance within the context of the biological characteristic measured by said values. In some embodiments, the two substantially different numeric values differ by greater than about any one of 10%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, 90%, or 100%.

[00120] The phrase "substantially reduced," as used herein, denotes a sufficiently high degree of reduction between a numeric value and a reference numeric value such that one of skill in the art would consider the difference between the two values to be of statistical significance within the context of the biological characteristic measured by said values. In some embodiments, the substantially reduced numeric values is reduced by greater than about any one of 10%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, 90%, or 100% compared to the reference value.

[00121] The term "leader sequence" refers to a sequence of amino acid residues located at the N-terminus of a polypeptide that facilitates secretion of a polypeptide from a mammalian cell. A leader sequence can be cleaved upon export of the polypeptide from the mammalian cell, forming a mature protein. Leader sequences can be natural or synthetic, and they can be heterologous or homologous to the protein to which they are attached.

[00122] A "native sequence" polypeptide comprises a polypeptide having the same amino acid sequence as a polypeptide found in nature. Thus, a native sequence polypeptide can have the amino acid sequence of naturally occurring polypeptide from any mammal. Such native sequence polypeptide can be isolated from nature or can be produced by recombinant or synthetic means. The term "native sequence" polypeptide specifically encompasses naturally occurring truncated or secreted forms of the polypeptide (for example, an extracellular domain sequence), naturally occurring variant forms (for example, alternatively spliced forms) and naturally occurring allelic variants of the polypeptide.

[00123] A polypeptide "variant" means a biologically active polypeptide having at least about 80% amino acid sequence identity with the native sequence polypeptide after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Such variants include, for instance, polypeptides wherein one or more amino acid residues are added, or deleted, at the N- or C-terminus of the polypeptide. In some embodiments, a variant will have at least about 80% amino acid sequence identity. In some embodiments, a variant will have at least about 90% amino acid sequence identity. In some embodiments, a variant will have at least about 95% amino acid sequence identity with the native sequence polypeptide.

[00124] As used herein, "Percent (%) amino acid sequence identity" and "homology" with respect to a peptide, polypeptide or antibody sequence are defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any

conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or MEGALIGN™ (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[00125] An amino acid substitution may include but are not limited to the replacement of one amino acid in a polypeptide with another amino acid. Exemplary substitutions are shown in Table 1. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, for example, retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

TABLE 1

Original Residue	Exemplary Substitutions
Ala (A)	Val; Leu; Ile
Arg (R)	Lys; Gln; Asn
Asn (N)	Gln; His; Asp, Lys; Arg
Asp (D)	Glu; Asn
Cys (C)	Ser; Ala
Gln (Q)	Asn; Glu
Glu (E)	Asp; Gln
Gly (G)	Ala
His (H)	Asn; Gln; Lys; Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe
Lys (K)	Arg; Gln; Asn
Met (M)	Leu; Phe; Ile
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr
Pro (P)	Ala
Ser (S)	Thr
Thr (T)	Val; Ser
Trp (W)	Tyr; Phe
Tyr (Y)	Trp; Phe; Thr; Ser
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine

[00126] Amino acids may be grouped according to common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

[00127] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[00128] The term "vector" is used to describe a polynucleotide that can be engineered to contain a cloned polynucleotide or polynucleotides that can be propagated in a host cell. A

vector can include one or more of the following elements: an origin of replication, one or more regulatory sequences (such as, for example, promoters and/or enhancers) that regulate the expression of the polypeptide of interest, and/or one or more selectable marker genes (such as, for example, antibiotic resistance genes and genes that can be used in colorimetric assays, for example, β-galactosidase). The term "expression vector" refers to a vector that is used to express a polypeptide of interest in a host cell.

[00129] A "host cell" refers to a cell that may be or has been a recipient of a vector or isolated polynucleotide. Host cells may be prokaryotic cells or eukaryotic cells. Exemplary eukaryotic cells include mammalian cells, such as primate or non-primate animal cells; fungal cells, such as yeast; plant cells; and insect cells. Nonlimiting exemplary mammalian cells include, but are not limited to, NSO cells, PER.C6® cells (Crucell), and 293 and CHO cells, and their derivatives, such as 293-6E and DG44 cells, respectively. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in genomic DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected in vivo with a polynucleotide(s) a provided herein.

[00130] The term "isolated" as used herein refers to a molecule that has been separated from at least some of the components with which it is typically found in nature or produced. For example, a polypeptide is referred to as "isolated" when it is separated from at least some of the components of the cell in which it was produced. Where a polypeptide is secreted by a cell after expression, physically separating the supernatant containing the polypeptide from the cell that produced it is considered to be "isolating" the polypeptide. Similarly, a polynucleotide is referred to as "isolated" when it is not part of the larger polynucleotide (such as, for example, genomic DNA or mitochondrial DNA, in the case of a DNA polynucleotide) in which it is typically found in nature, or is separated from at least some of the components of the cell in which it was produced, for example, in the case of an RNA polynucleotide. Thus, a DNA polynucleotide that is contained in a vector inside a host cell may be referred to as "isolated".

[00131] The terms "individual" or "subject" are used interchangeably herein to refer to an animal; for example a mammal. In some embodiments, methods of treating mammals, including, but not limited to, humans, rodents, simians, felines, canines, equines, bovines, porcines, ovines, caprines, mammalian laboratory animals, mammalian farm animals, mammalian sport animals, and mammalian pets, are provided. In some examples, an "individual" or "subject" refers to an individual or subject in need of treatment for a disease or disorder. In some embodiments, the subject to receive the treatment can be a patient,

designating the fact that the subject has been identified as having a disorder of relevance to the treatment, or being at adequate risk of contracting the disorder.

[00132] A "disease" or "disorder" as used herein refers to a condition where treatment is needed and/or desired.

[00133] "Cancer" and "tumor," as used herein, are interchangeable terms that refer to any abnormal cell or tissue growth or proliferation in an animal. As used herein, the terms "cancer" and "tumor" encompass solid and hematological/lymphatic cancers and also encompass malignant, pre-malignant, and benign growth, such as dysplasia. Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular non-limiting examples of such cancers include squamous cell cancer, small-cell lung cancer, pituitary cancer, esophageal cancer, astrocytoma, soft tissue sarcoma, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, renal cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, brain cancer, endometrial cancer, testis cancer, cholangiocarcinoma, gallbladder carcinoma, gastric cancer, melanoma, and various types of head and neck cancer.

[00134] As used herein, "treatment" is an approach for obtaining beneficial or desired clinical results. "Treatment" as used herein, covers any administration or application of a therapeutic for disease in a mammal, including a human. For purposes of this disclosure, beneficial or desired clinical results include, but are not limited to, any one or more of: alleviation of one or more symptoms, diminishment of extent of disease, preventing or delaying spread (for example, metastasis, for example metastasis to the lung or to the lymph node) of disease, preventing or delaying recurrence of disease, delay or slowing of disease progression, amelioration of the disease state, inhibiting the disease or progression of the disease, inhibiting or slowing the disease or its progression, arresting its development, and remission (whether partial or total). Also encompassed by "treatment" is a reduction of pathological consequence of a proliferative disease. The methods provided herein contemplate any one or more of these aspects of treatment. In-line with the above, the term treatment does not require one-hundred percent removal of all aspects of the disorder.

[00135] "Ameliorating" means a lessening or improvement of one or more symptoms as compared to not administering an anti-ICOS antibody. "Ameliorating" also includes shortening or reduction in duration of a symptom.

[00136] In the context of cancer, the term "treating" includes any or all of: inhibiting growth of cancer cells, inhibiting replication of cancer cells, lessening of overall tumor burden and ameliorating one or more symptoms associated with the disease.

[00137] The term "biological sample" means a quantity of a substance from a living thing or formerly living thing. Such substances include, but are not limited to, blood, (for example, whole blood), plasma, serum, urine, amniotic fluid, synovial fluid, endothelial cells, leukocytes, monocytes, other cells, organs, tissues, bone marrow, lymph nodes and spleen.

[00138] A sample that has an "elevated level of ICOS" or "expresses ICOS at an elevated level" or is "$ICOS^{HIGH}$" means that the level of ICOS that is such that one of skill in the art would conclude that the cancer may be treatable with an anti-ICOS therapy, such as an antibody provided herein. In some embodiments, an "elevated level of ICOS" is one in which 1% of the cells within a tumor sample show staining for ICOS. In some embodiments a "high level" in regard to ICOS is 1% or more staining, for example, 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% of the cells within the tumor sample show staining. In some embodiments, the ICOS levels can be measured by chromogenic IHC or immunofluorescence IHC (Aqua scoring).

[00139] A sample that "expresses ICOS" or has "positive staining for ICOS" or is "ICOS positive" means that 1% or more of the cells in a sample show staining for ICOS. In some embodiments, a sample that is ICOS positive displays at least weak, moderate, and/or strong cell staining (based on membrane expression of ICOS). A sample with moderate or strong cell staining for ICOS is also considered to be "$ICOS^{HIGH}$."

[00140] A sample that has a "low level of PD-L1" or expresses "PD-L1 at a low level" or is "$PD\text{-}L1^{LOW}$" means that the level of PD-L1 is below the threshold level of expression for a cancer that is normally indicated for treatment with a PD-1 therapy. In some embodiments, a "low level of PD-L1" is one in which less than 5% of the cells in the tumor show membrane staining for PD-L1. In some embodiments a "low level" in regard to PD-L1 is less than 5% staining, for example, 4%, 3%, 2%, 1%, or 0% of the cells of the tumor show staining. In some embodiments, the PD-L1 levels can be measured by chromogenic IHC or immunofluorescence IHC (Aqua scoring). A sample that expresses no detectable PD-L1 can also be said to "express a low level of PD-L1." Thus, no detectable PD-L1 is encompassed within the term "low."

[00141] A sample that has an "elevated level of PD-L1" or "expresses PD-L1 at an elevated level" or is "$PD\text{-}L1^{HIGH}$" means that the level of PD-L1 that is such that one of skill in the art would conclude that the cancer may be treatable with a PD-1 therapy. In some embodiments, an "elevated level of PD-L1" is one in which 5% of the cells in the tumor or more have

membrane staining of PD-L1. In some embodiments a "high level" in regard to PD-L1 is 5% or more staining, for example, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% of the cells of the tumor show staining. In some embodiments, the PD-L1 levels can be measured by chromogenic IHC or immunofluorescence IHC (Aqua scoring).

[00142] A sample that "expresses PD-L1" or has "positive staining for PD-L1" or is "PD-L1 positive" means that 1% or more of the cells have membrane staining for PD-L1. In some embodiments, a sample that is PD-L1 positive displays at least weak, moderate, and/or strong cell staining (based on membrane expression of PD-L1). A sample with moderate or strong cell staining for PD-L1 is also considered to be "$PD\text{-}L1^{HIGH}$."

[00143] A sample that "lacks PD-L1 expression" or has "negative staining for PD-L1" or is "PD-L1 negative" means that PD-L1 expression on the surface of cells of the sample is undetectable by IHC, such as chromogenic IHC or immunofluorescence IHC (Aqua scoring). A PD-L1 negative sample is also be considered to be "$PD\text{-}L1^{LOW}$."

[00144] In some embodiments, any method for measuring the level of PD-L1 can be employed. In some embodiments, this can include using the PD-L1 IHC 22C3 pharmDx test (Dako Inc., Carpinteria, CA), which is a clinically validated and FDA approved test for evaluation of PD-L1 expression in NSCLC. PD-L1 IHC 22C3 pharmDx is a qualitative immunohistochemical assay using monoclonal mouse anti-PD-L1 antibody, (clone 22C3), that can be used in the detection of PD-L1 protein in formalin-fixed paraffin-embedded (FFPE) Non-Small Cell Lung Cancer (NSCLC) tissues. The assay can be performed on Autostainer Link 48 system and visualized using the EnVision FLEX system. PD-L1 protein expression is qualified using Tumor Proportion Score (TPS), which is the percentage of viable tumor cells showing partial or complete membrane staining. In some embodiments, the specimen is considered PD-L1 positive if TPS $\geq$ 50% of the viable tumor cells exhibit membrane staining at any intensity. PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying NSCLC patients for treatment with KEYTRUDA® (pembrolizumab). Additional details on the scoring system and response to pembrolizumab are described in the article by Garon et al. (N Engl J Med 2015;372:2018-28). In some embodiments, NSCLC patient specimens can be considered positive for PD-L1 expression if Tumor Proportion Score is $\geq$ 50% of the of viable tumor cells exhibit membrane staining (partial or complete) at any intensity (i.e. $\geq$ 1+). In some embodiments, this can be in specific regard to antibody clone 22C3. In some embodiments, if TPS = 5% to 50% of the viable tumor cells exhibit membrane staining at any intensity, the sample and/or patient is considered to be PD-L1 positive. In some embodiments, if TPS $\geq$ 50% of the viable tumor cells exhibit membrane staining at any intensity, the sample and/or patient is considered to be $PD\text{-}L1^{HIGH}$.

[00145] The term "control" refers to a composition known to not contain an analyte ("negative control") or to contain analyte ("positive control"). A positive control can comprise a known concentration of analyte. "Control," "positive control," and "calibrator" may be used interchangeably herein to refer to a composition comprising a known concentration of analyte. A "positive control" can be used to establish assay performance characteristics and is a useful indicator of the integrity of reagents (for example, analytes).

[00146] "Predetermined cutoff" and "predetermined level" refer generally to an assay cutoff value that is used to assess diagnostic/prognostic/therapeutic efficacy results by comparing the assay results against the predetermined cutoff/level, where the predetermined cutoff/level already has been linked or associated with various clinical parameters (for example, severity of disease, progression/nonprogression/improvement, etc.). While the present disclosure may provide exemplary predetermined levels, it is well-known that cutoff values may vary depending on the nature of the immunoassay (for example, antibodies employed, etc.). It further is well within the skill of one of ordinary skill in the art to adapt the disclosure herein for other immunoassays to obtain immunoassay-specific cutoff values for those other immunoassays based on this disclosure. Whereas the precise value of the predetermined cutoff/level may vary between assays, correlations as described herein (if any) may be generally applicable.

[00147] The terms "inhibition" or "inhibit" refer to a decrease or cessation of any phenotypic characteristic or to the decrease or cessation in the incidence, degree, or likelihood of that characteristic. To "reduce" or "inhibit" is to decrease, reduce or arrest an activity, function, and/or amount as compared to a reference. In some embodiments, by "reduce" or "inhibit" is meant the ability to cause an overall decrease of 20% or greater. In some embodiments, by "reduce" or "inhibit" is meant the ability to cause an overall decrease of 50% or greater. In some embodiments, by "reduce" or "inhibit" is meant the ability to cause an overall decrease of 75%, 85%, 90%, 95%, or greater. In some embodiments, the amount noted above is inhibited or decreased over a period of time, relative to a control dose (such as a placebo) over the same period of time. A "reference" as used herein, refers to any sample, standard, or level that is used for comparison purposes. A reference may be obtained from a healthy and/or non-diseased sample. In some examples, a reference may be obtained from an untreated sample. In some examples, a reference is obtained from a non-diseased on non-treated sample of a subject individual. In some examples, a reference is obtained from one or more healthy individuals who are not the subject or patient.

[00148] As used herein, "delaying development of a disease" means to defer, hinder, slow, retard, stabilize, suppress and/or postpone development of the disease (such as cancer). This

delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease. For example, a late stage cancer, such as development of metastasis, may be delayed.

[00149] "Preventing," as used herein, includes providing prophylaxis with respect to the occurrence or recurrence of a disease in a subject that may be predisposed to the disease but has not yet been diagnosed with the disease. Unless otherwise specified, the terms "reduce", "inhibit", or "prevent" do not denote or require complete prevention over all time.

[00150] As used herein, to "suppress" a function or activity is to reduce the function or activity when compared to otherwise same conditions except for a condition or parameter of interest, or alternatively, as compared to another condition. For example, an antibody which suppresses tumor growth reduces the rate of growth of the tumor compared to the rate of growth of the tumor in the absence of the antibody.

[00151] A "therapeutically effective amount" of a substance/molecule, agonist or antagonist may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the substance/molecule, agonist or antagonist to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the substance/molecule, agonist or antagonist are outweighed by the therapeutically beneficial effects. A therapeutically effective amount may be delivered in one or more administrations. A therapeutically effective amount refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic and/or prophylactic result.

[00152] A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically but not necessarily, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

[00153] The terms "pharmaceutical formulation" and "pharmaceutical composition" refer to a preparation which is in such form as to permit the biological activity of the active ingredient(s) to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered. Such formulations may be sterile.

[00154] A "pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid, or liquid filler, diluent, encapsulating material, formulation auxiliary, or carrier conventional in the art for use with a therapeutic agent that together comprise a "pharmaceutical composition" for administration to a subject. A pharmaceutically acceptable carrier is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the

formulation. The pharmaceutically acceptable carrier is appropriate for the formulation employed.

[00155] A "sterile" formulation is aseptic or essentially free from living microorganisms and their spores.

[00156] A "PD-1 therapy" encompasses any therapy that modulates PD-1 binding to PD-L1 and/or PD-L2. PD-1 therapies may, for example, directly interact with PD-1 and/or PD-L1. In some embodiments, a PD-1 therapy includes a molecule that directly binds to and/or influences the activity of PD-1. In some embodiments, a PD-1 therapy includes a molecule that directly binds to and/or influences the activity of PD-L1. Thus, an antibody that binds to PD-1 or PD-L1 and blocks the interaction of PD-1 to PD-L1 is a PD-1 therapeutic. When a desired subtype of PD-1 therapy is intended, it will be designated by the phrase "PD-1 specific" for a therapy involving a molecule that interacts directly with PD-1, or "PD-L1 specific" for a molecule that interacts directly with PD-L1, as appropriate. Unless designated otherwise, all disclosure contained herein regarding PD-1 therapy applies to PD-1 therapy generally, as well as PD-1 specific and/or PD-L1 specific therapies. Nonlimiting exemplary PD-1 therapies include nivolumab (anti-PD-1 antibody; BMS-936558, MDX-1106, ONO-4538; OPDIVO®; Bristol-Myers Squibb); pidilizumab (anti-PD-1 antibody, CureTech), pembrolizumab (anti-PD-1 antibody; KEYTRUDA®, MK-3475, lambrolizumab); durvalumab (anti-PD-L1 antibody, MEDI-4736; AstraZeneca/MedImmune); RG-7446; MSB-0010718C; AMP-224; BMS-936559 (an anti-PD-L1 antibody; Bristol-Myers Squibb); AMP-514; MDX-1105; ANB-011; anti-LAG-3/PD-1; anti-PD-1 Ab (CoStim); anti-PD-1 Ab (Kadmon Pharm.); anti-PD-1 Ab (Immunovo); anti-TIM-3/PD-1 Ab (AnaptysBio); anti-PD-L1 Ab (CoStim/Novartis); atezolizumab (an anti-PD-L1 antibody, Genentech/Roche); avelumab (an anti-PD-L1 antibody, MSB0010718C, Pfizer); KD-033, PD-1 antagonist (Agenus); STI-A1010; STI-A1110; TSR-042; and other antibodies that are directed against programmed death-1 (PD-1) or programmed death ligand 1 (PD-L1).

[00157] The term "IDO inhibitor" refers to an agent capable of inhibiting the activity of indoleamine 2,3-dioxygenase (IDO) and thereby reversing IDO-mediated immunosuppression. The IDO inhibitor may inhibit IDO1 and/or IDO2 (INDOL1). An IDO inhibitor may be a reversible or irreversible IDO inhibitor. A "reversible IDO inhibitor" is a compound that reversibly inhibits IDO enzyme activity either at the catalytic site or at a non-catalytic site and an "irreversible IDO inhibitor" is a compound that irreversibly inhibits IDO enzyme activity by forming a covalent bond with the enzyme. Nonlimiting exemplary IDO inhibitors include Indoximod (New Link Genetics), INCB024360 (Incyte Corp.), 1-methyl-D-tryptophan (New Link Genetics), and GDC-0919 (Genentech).

[00158] A "chimeric antigen receptor T cell therapy" or "CAR-T therapy" refers to a therapeutic agent comprising a T cell genetically modified to express a receptor that recognizes an antigen expressed by tumor cell. The antigen may be an antigen specifically expressed by the tumor or an antigen expressed by both cancerous cells and healthy tissue. In some embodiments CAR-T therapy is adoptive CAR-T therapy, in which a patients T cells are removed and modified to express the chimeric antigen receptor, and then returned to the patient. *See, e.g.,* Dai et al., 2016, *J Natl Cancer Inst*, 108 (7): djv439, doi: 10.1093/jnci/djv439; Gill et al., 2015, *Blood Rev*, pii: S0268-960X(15)00080-6, doi: 10.1016/j.blre.2015.10.003; Gill et al., 2015, *Immunol Rev*, 263(1):68-89. doi: 10.1111/imr.12243.

[00159] Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive or sequential administration in any order.

[00160] The term "concurrently" is used herein to refer to administration of two or more therapeutic agents, where at least part of the administration overlaps in time or where the administration of one therapeutic agent falls within a short period of time relative to administration of the other therapeutic agent. For example, the two or more therapeutic agents are administered with a time separation of no more than about a specified number of minutes.

[00161] The term "sequentially" is used herein to refer to administration of two or more therapeutic agents where the administration of one or more agent(s) continues after discontinuing the administration of one or more other agent(s), or wherein administration of one or more agent(s) begins before the administration of one or more other agent(s). For example, administration of the two or more therapeutic agents are administered with a time separation of more than about a specified number of minutes.

[00162] As used herein, "in conjunction with" refers to administration of one treatment modality in addition to another treatment modality. As such, "in conjunction with" refers to administration of one treatment modality before, during or after administration of the other treatment modality to the individual.

[00163] The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

[00164] An "article of manufacture" is any manufacture (for example, a package or container) or kit comprising at least one reagent, for example, a medicament for treatment of a disease or disorder (for example, cancer), or a probe for specifically detecting a biomarker described

herein. In some embodiments, the manufacture or kit is promoted, distributed, or sold as a unit for performing the methods described herein.

[00165] The terms "label" and "detectable label" mean a moiety attached to an antibody or its analyte to render a reaction (for example, binding) between the members of the specific binding pair, detectable. The labeled member of the specific binding pair is referred to as "detectably labeled." Thus, the term "labeled binding protein" refers to a protein with a label incorporated that provides for the identification of the binding protein. In some embodiments, the label is a detectable marker that can produce a signal that is detectable by visual or instrumental means, for example, incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (for example, streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (for example, ^{3}H, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, or ^{153}Sm); chromogens, fluorescent labels (for example, FITC, rhodamine, lanthanide phosphors), enzymatic labels (for example, horseradish peroxidase, luciferase, alkaline phosphatase); chemiluminescent markers; biotinyl groups; predetermined polypeptide epitopes recognized by a secondary reporter (for example, leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags); and magnetic agents, such as gadolinium chelates. Representative examples of labels commonly employed for immunoassays include moieties that produce light, for example, acridinium compounds, and moieties that produce fluorescence, for example, fluorescein. In this regard, the moiety itself may not be detectably labeled but may become detectable upon reaction with yet another moiety.

[00166] The term "conjugate" refers to an antibody that is chemically linked to a second chemical moiety, such as a therapeutic or cytotoxic agent. The term "agent" includes a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials. In some embodiments, the therapeutic or cytotoxic agents include, but are not limited to, pertussis toxin, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. When employed in the context of an immunoassay, the conjugate antibody may be a detectably labeled antibody used as the detection antibody.

II. Anti-ICOS Antibodies

[00167] Novel antibodies directed against ICOS are provided. Anti-ICOS antibodies include, but are not limited to, humanized antibodies, chimeric antibodies, mouse antibodies, human antibodies, and antibodies comprising the heavy chain and/or light chain CDRs discussed herein. In some embodiments, an isolated antibody that binds to ICOS is provided. In some embodiments, a monoclonal antibody that binds to ICOS is provided. In some embodiments, an anti-ICOS antibody is an agonist anti-ICOS antibody. In some embodiments, administration of the anti-ICOS antibodies described herein increases the number of Teff cells; activates Teff cells; depletes Treg cells in a subject; and/or increases the ratio of Teff cells to Treg cells. In some embodiments, the Treg cells are CD4+ FoxP3+ T cells. In some embodiments, the Teff cells are CD8+ T cells. In some embodiments, the Teff cells are CD4+ FoxP3- T cells and CD8+ T cells.

[00168] In some embodiments, an anti-ICOS antibody comprises at least one, two, three, four, five, or six CDRs selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 12; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 13; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 14; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 15; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 16; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 17.

[00169] In some embodiments, an anti-ICOS antibody comprises at least one, two, three, four, five, or six CDRs selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 42; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 43; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 44; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 45; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 46; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 47.

[00170] In some embodiments, the anti-ICOS antibody comprises six CDRs including (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 62; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 63; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 64; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 65; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 66; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 67.

[00171] In some embodiments, an anti-ICOS antibody comprises at least one, two, three, four, five, or six CDRs selected from (a) HCDR1 comprising an amino acid sequence selected from SEQ ID NOs: 22, 62, 72, 82, 92, 102, and 112; (b) HCDR2 comprising an amino acid sequence selected from SEQ ID NOs: 23, 63, 73, 83, 93, 103, and 113; (c) HCDR3 comprising

an amino acid sequence selected from SEQ ID NOs: 24, 64, 74, 84, 94, 104, and 114; (d) LCDR1 comprising an amino acid sequence selected from SEQ ID NOs: 25, 65, 75, 85, 95, 105, and 115; (e) LCDR2 comprising an amino acid sequence selected from SEQ ID NOs: 26, 66, 76, 86, 96, 106, and 116; and (f) LCDR3 comprising an amino acid sequence selected from SEQ ID NOs: 27, 67, 77, 87, 97, 107, and 117.

[00172] In some embodiments, an anti-ICOS antibody comprises at least one, two, three, four, five, or six CDRs selected from (a) HCDR1 comprising an amino acid sequence selected from SEQ ID NOs: 32, 162, 172, and 182; (b) HCDR2 comprising an amino acid sequence selected from SEQ ID NOs: 33, 163, 173, and 183; (c) HCDR3 comprising an amino acid sequence selected from SEQ ID NOs: 34, 164, 174, and 184; (d) LCDR1 comprising an amino acid sequence selected from SEQ ID NOs: 35, 165, 175, and 185; (e) LCDR2 comprising an amino acid sequence selected from SEQ ID NOs: 36, 166, 176, and 186; and (f) LCDR3 comprising an amino acid sequence selected from SEQ ID NOs: 37, 167, 177, and 187.

[00173] In some embodiments, an anti-ICOS antibody comprises at least one, two, three, four, five, or six CDRs selected from (a) HCDR1 comprising an amino acid sequence selected from SEQ ID NOs: 52, 122, 132, 142, and 152; (b) HCDR2 comprising an amino acid sequence selected from SEQ ID NOs: 53, 123, 133, 143, and 153; (c) HCDR3 comprising an amino acid sequence selected from SEQ ID NOs: 54, 124, 134, 144, and 154; (d) LCDR1 comprising an amino acid sequence selected from SEQ ID NOs: 55, 125, 135, 145, and 155; (e) LCDR2 comprising an amino acid sequence selected from SEQ ID NOs: 56, 126, 136, 146, and 156; and (f) LCDR3 comprising an amino acid sequence selected from SEQ ID NOs: 57, 127, 137, 147, and 157.

[00174] In some embodiments, an anti-ICOS antibody comprises a heavy chain variable region and a light chain variable region. In some embodiments, an anti-ICOS antibody comprises at least one heavy chain comprising a heavy chain variable region and at least a portion of a heavy chain constant region, and at least one light chain comprising a light chain variable region and at least a portion of a light chain constant region. In some embodiments, an anti-ICOS antibody comprises two heavy chains, wherein each heavy chain comprises a heavy chain variable region and at least a portion of a heavy chain constant region, and two light chains, wherein each light chain comprises a light chain variable region and at least a portion of a light chain constant region. As used herein, a single-chain Fv (scFv), or any other antibody that comprises, for example, a single polypeptide chain comprising all six CDRs (three heavy chain CDRs and three light chain CDRs) is considered to have a heavy chain and a light chain. In some embodiments, the heavy chain is the region of the anti-ICOS antibody that comprises

the three heavy chain CDRs. In some embodiments, the light chain is the region of the anti-ICOS antibody that comprises the three light chain CDRs.

[00175] In some embodiments, the anti-ICOS antibody comprises six CDRs including (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 12; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 13; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 14; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 15; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 16; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 17.

[00176] In some embodiments, the anti-ICOS antibody comprises six CDRs including (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 22; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 23; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 24; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 25; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 26; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 27.

[00177] In some embodiments, the anti-ICOS antibody comprises six CDRs including (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 32; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 33; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 34; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 35; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 36; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 37.

[00178] In some embodiments, the anti-ICOS antibody comprises six CDRs including (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 42; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 43; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 44; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 45; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 46; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 47.

[00179] In some embodiments, the anti-ICOS antibody comprises six CDRs including (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 52; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 53; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 54; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 55; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 56; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 57.

[00180] In some embodiments, the anti-ICOS antibody comprises six CDRs including (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 72; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 73; (c) HCDR3 comprising the amino acid sequence of

SEQ ID NO: 74; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 75; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 76; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 77.

[00181] In some embodiments, the anti-ICOS antibody comprises six CDRs including (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 82; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 83; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 84; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 85; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 86; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 87.

[00182] In some embodiments, the anti-ICOS antibody comprises six CDRs including (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 92; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 93; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 94; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 95; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 96; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 97.

[00183] In some embodiments, the anti-ICOS antibody comprises six CDRs including (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 102; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 103; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 104; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 105; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 106; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 107.

[00184] In some embodiments, the anti-ICOS antibody comprises six CDRs including (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 112; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 113; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 114; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 115; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 116; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 117.

[00185] In some embodiments, the anti-ICOS antibody comprises six CDRs including (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 122; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 123; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 124; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 125; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 126; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 127.

[00186] In some embodiments, the anti-ICOS antibody comprises six CDRs including (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 132; (b) HCDR2 comprising the

amino acid sequence of SEQ ID NO: 133; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 134; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 135; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 136; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 137.

[00187] In some embodiments, the anti-ICOS antibody comprises six CDRs including (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 142; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 143; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 144; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 145; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 146; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 147.

[00188] In some embodiments, the anti-ICOS antibody comprises six CDRs including (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 152; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 153; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 154; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 155; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 156; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 157.

[00189] In some embodiments, the anti-ICOS antibody comprises six CDRs including (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 162; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 163; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 164; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 165; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 166; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 167.

[00190] In some embodiments, the anti-ICOS antibody comprises six CDRs including (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 172; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 173; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 174; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 175; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 176; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 177.

[00191] In some embodiments, the anti-ICOS antibody comprises six CDRs including (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 182; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 183; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 184; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 185; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 186; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 187.

[00192] In some embodiments, the anti-ICOS antibody comprises the six CDRs as described above and binds to ICOS. In some embodiments, the anti-ICOS antibody comprises the six CDRs as described above, binds to ICOS and increases the number of Teff cells and/or activates Teff cells and/or decreases the number of Treg cells and/or increases the ratio of Teff cells to Treg cells in a mammal, such as a human. In some embodiments, the Treg cells are CD4+ FoxP3+ T cells. In some embodiments, the Teff cells are CD8+ T cells. In some embodiments, the Teff cells are CD4+ FoxP3- T cells and/or CD8+ T cells.

[00193] In some embodiments, an anti-ICOS antibody is provided that competes with an anti-ICOS antibody described herein for binding to ICOS. In some embodiments, an antibody that competes for binding with any of the antibodies provided herein can be made and/or used.

[00194] In some embodiments, the anti-ICOS antibody comprises at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 12; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 13; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 14.

[00195] In some embodiments, the anti-ICOS antibody comprises at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 22; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 23; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 24.

[00196] In some embodiments, the anti-ICOS antibody comprises at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 32; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 33; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 34.

[00197] In some embodiments, the anti-ICOS antibody comprises at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 42; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 43; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 44.

[00198] In some embodiments, the anti-ICOS antibody comprises at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 52; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 53; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 54.

[00199] In some embodiments, the anti-ICOS antibody comprises at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 62; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 63; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 64.

[00200] In some embodiments, the anti-ICOS antibody comprises at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 72; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 73; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 74.

[00201] In some embodiments, the anti-ICOS antibody comprises at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 82; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 83; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 84.

[00202] In some embodiments, the anti-ICOS antibody comprises at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 92; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 93; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 94.

[00203] In some embodiments, the anti-ICOS antibody comprises at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 102; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 103; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 104.

[00204] In some embodiments, the anti-ICOS antibody comprises at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 112; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 113; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 114.

[00205] In some embodiments, the anti-ICOS antibody comprises at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 122; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 123; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 124.

[00206] In some embodiments, the anti-ICOS antibody comprises at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 132; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 133; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 134.

[00207] In some embodiments, the anti-ICOS antibody comprises at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 142; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 143; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 144.

[00208] In some embodiments, the anti-ICOS antibody comprises at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of

SEQ ID NO: 152; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 153; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 154.

[00209] In some embodiments, the anti-ICOS antibody comprises at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 162; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 163; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 164.

[00210] In some embodiments, the anti-ICOS antibody comprises at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 172; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 173; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 174.

[00211] In some embodiments, the anti-ICOS antibody comprises at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 182; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 183; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 184.

[00212] In some embodiments, the antibody comprises at least one, at least two, or all three VL CDR sequences selected from (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 15; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 16; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 17.

[00213] In some embodiments, the antibody comprises at least one, at least two, or all three VL CDR sequences selected from (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 25; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 26; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 27.

[00214] In some embodiments, the antibody comprises at least one, at least two, or all three VL CDR sequences selected from (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 35; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 36; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 37.

[00215] In some embodiments, the antibody comprises at least one, at least two, or all three VL CDR sequences selected from (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 45; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 46; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 47.

[00216] In some embodiments, the antibody comprises at least one, at least two, or all three VL CDR sequences selected from (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 55; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 56; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 57.

[00217] In some embodiments, the antibody comprises at least one, at least two, or all three VL CDR sequences selected from (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 65; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 66; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 67.

[00218] In some embodiments, the antibody comprises at least one, at least two, or all three VL CDR sequences selected from (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 75; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 76; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 77.

[00219] In some embodiments, the antibody comprises at least one, at least two, or all three VL CDR sequences selected from (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 85; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 86; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 87.

[00220] In some embodiments, the antibody comprises at least one, at least two, or all three VL CDR sequences selected from (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 95; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 96; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 97.

[00221] In some embodiments, the antibody comprises at least one, at least two, or all three VL CDR sequences selected from (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 105; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 106; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 107.

[00222] In some embodiments, the antibody comprises at least one, at least two, or all three VL CDR sequences selected from (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 115; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 116; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 117.

[00223] In some embodiments, the antibody comprises at least one, at least two, or all three VL CDR sequences selected from (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 125; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 126; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 127.

[00224] In some embodiments, the antibody comprises at least one, at least two, or all three VL CDR sequences selected from (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 135; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 136; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 137.

[00225] In some embodiments, the antibody comprises at least one, at least two, or all three VL CDR sequences selected from (a) LCDR1 comprising the amino acid sequence of SEQ ID

NO: 145; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 146; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 147.

[00226] In some embodiments, the antibody comprises at least one, at least two, or all three VL CDR sequences selected from (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 155; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 156; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 157.

[00227] In some embodiments, the antibody comprises at least one, at least two, or all three VL CDR sequences selected from (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 165; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 166; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 167.

[00228] In some embodiments, the antibody comprises at least one, at least two, or all three VL CDR sequences selected from (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 175; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 176; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 177.

[00229] In some embodiments, the antibody comprises at least one, at least two, or all three VL CDR sequences selected from (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 185; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 186; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 187.

[00230] In some embodiments, any of the six CDRs provided herein can be combined as subparts with any of the other CDRs provided herein, for a total of six CDRs in a construct. Thus, in some embodiments, two CDRs from a first antibody (for example, HCDR1 and HCDR2) can be combined with four CDRs from a second antibody (HCDR3, LCDR1, LCDR2, and LCDR3). In some embodiments, two or fewer residues in one or more of the CDRs can be replaced to obtain a variant thereof. In some embodiments, two or fewer residues can be replaced in 1, 2, 3, 4, 5, or 6 of the CDRs.

[00231] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 12; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 13; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 14; and (II) a VL domain comprising at least one, at least two, or all three VL CDR sequences selected from (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 15; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 16; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 17.

[00232] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1

comprising the amino acid sequence of SEQ ID NO: 22; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 23; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 24; and (II) a VL domain comprising at least one, at least two, or all three VL CDR sequences selected from (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 25; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 26; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 27.

[00233] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 32; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 33; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 34; and (II) a VL domain comprising at least one, at least two, or all three VL CDR sequences selected from (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 35; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 36; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 37.

[00234] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 42; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 43; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 44; and (II) a VL domain comprising at least one, at least two, or all three VL CDR sequences selected from (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 45; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 46; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 47.

[00235] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 52; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 53; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 54; and (II) a VL domain comprising at least one, at least two, or all three VL CDR sequences selected from (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 55; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 56; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 57.

[00236] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 62; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 63; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 64; and (II) a VL domain comprising at least one, at least two, or all three VL CDR

sequences selected from (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 65; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 66; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 67.

[00237] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 72; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 73; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 74; and (II) a VL domain comprising at least one, at least two, or all three VL CDR sequences selected from (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 75; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 76; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 77.

[00238] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 82; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 83; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 84; and (II) a VL domain comprising at least one, at least two, or all three VL CDR sequences selected from (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 85; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 86; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 87.

[00239] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 92; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 93; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 94; and (II) a VL domain comprising at least one, at least two, or all three VL CDR sequences selected from (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 95; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 96; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 97.

[00240] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 102; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 103; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 104; and (II) a VL domain comprising at least one, at least two, or all three VL CDR sequences selected from (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 105; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 106; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 107.

[00241] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 112; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 113; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 114; and (II) a VL domain comprising at least one, at least two, or all three VL CDR sequences selected from (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 115; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 116; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 117.

[00242] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 122; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 123; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 124; and (II) a VL domain comprising at least one, at least two, or all three VL CDR sequences selected from (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 125; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 126; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 127.

[00243] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 132; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 133; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 134; and (II) a VL domain comprising at least one, at least two, or all three VL CDR sequences selected from (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 135; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 136; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 137.

[00244] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 142; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 143; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 144; and (II) a VL domain comprising at least one, at least two, or all three VL CDR sequences selected from (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 145; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 146; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 147.

[00245] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 152; (b) HCDR2 comprising the amino

acid sequence of SEQ ID NO: 153; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 154; and (II) a VL domain comprising at least one, at least two, or all three VL CDR sequences selected from (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 155; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 156; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 157.

[00246] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 162; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 163; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 164; and (II) a VL domain comprising at least one, at least two, or all three VL CDR sequences selected from (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 165; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 166; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 167.

[00247] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising at least one, at least two, or all three VH CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 172; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 173; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 174; and (II) a VL domain comprising at least one, at least two, or all three VL CDR sequences selected from (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 175; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 176; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 177.

[00248] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising at least one, at least two, or all three V_H CDR sequences selected from (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 182; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 183; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 184; and (II) a V_L domain comprising at least one, at least two, or all three V_L CDR sequences selected from (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 185; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 186; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 187.

[00249] In some embodiments, an anti-ICOS antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, or 180. In some embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (for example, conservative substitutions), insertions, or deletions relative

to the reference sequence, but an anti-ICOS antibody comprising that sequence retains the ability to bind to ICOS. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, or 180. In some embodiments, substitutions, insertions, or deletions occur in regions outside the CDRs (that is, in the FRs). Optionally, the anti-ICOS antibody comprises the VH sequence in SEQ ID NO: 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, or 180, including post-translational modifications of that sequence.

[00250] In some embodiments, the VH comprises: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 12; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 13; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 14.

[00251] In some embodiments, the VH comprises: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 22; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 23; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 24.

[00252] In some embodiments, the VH comprises: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 32; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 33; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 34.

[00253] In some embodiments, the VH comprises: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 42; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 43; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 44.

[00254] In some embodiments, the VH comprises: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 52; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 53; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 54.

[00255] In some embodiments, the VH comprises: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 62; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 63; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 64.

[00256] In some embodiments, the VH comprises: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 72; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 73; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 74.

[00257] In some embodiments, the VH comprises: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 82; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 83; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 84.

[00258] In some embodiments, the VH comprises: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 92; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 93; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 94.

[00259] In some embodiments, the VH comprises: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 102; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 103; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 104.

[00260] In some embodiments, the VH comprises: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 112; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 113; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 114.

[00261] In some embodiments, the VH comprises: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 122; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 123; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 124.

[00262] In some embodiments, the VH comprises: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 132; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 133; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 134.

[00263] In some embodiments, the VH comprises: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 142; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 143; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 144.

[00264] In some embodiments, the VH comprises: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 152; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 153; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 154.

[00265] In some embodiments, the VH comprises: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 162; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 163; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 164.

[00266] In some embodiments, the VH comprises: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 172; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 173; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 174.

[00267] In some embodiments, the VH comprises: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 182; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 183; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 184.

[00268] In some embodiments, an anti-ICOS antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 11, 21, 31, 41, 51, 61, 71, 81, 91, 101, 111, 121, 131, 141, 151, 161, 171, or 181. In some embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (for example, conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-ICOS antibody comprising that sequence retains the ability to bind to ICOS. In some embodiments, a total of

1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 11, 21, 31, 41, 51, 61, 71, 81, 91, 101, 111, 121, 131, 141, 151, 161, 171, or 181. In some embodiments, the substitutions, insertions, or deletions occur in regions outside the CDRs (that is, in the FRs). Optionally, the anti-ICOS antibody comprises the VL sequence in SEQ ID NO: 11, 21, 31, 41, 51, 61, 71, 81, 91, 101, 111, 121, 131, 141, 151, 161, 171, or 181, including post-translational modifications of that sequence.

[00269] In some embodiments, the VL comprises: (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 15; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 16; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 17.

[00270] In some embodiments, the VL comprises: (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 25; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 26; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 27.

[00271] In some embodiments, the VL comprises: (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 35; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 36; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 37.

[00272] In some embodiments, the VL comprises: (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 45; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 46; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 47.

[00273] In some embodiments, the VL comprises: (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 55; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 56; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 57.

[00274] In some embodiments, the VL comprises: (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 65; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 66; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 67.

[00275] In some embodiments, the VL comprises: (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 75; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 76; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 77.

[00276] In some embodiments, the VL comprises: (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 85; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 86; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 87.

[00277] In some embodiments, the VL comprises: (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 95; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 96; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 97.

[00278] In some embodiments, the VL comprises: (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 105; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 106; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 107.

[00279] In some embodiments, the VL comprises: (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 115; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 116; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 117.

[00280] In some embodiments, the VL comprises: (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 125; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 126; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 127.

[00281] In some embodiments, the VL comprises: (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 135; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 136; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 137.

[00282] In some embodiments, the VL comprises: (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 145; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 146; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 147.

[00283] In some embodiments, the VL comprises: (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 155; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 156; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 157.

[00284] In some embodiments, the VL comprises: (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 165; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 166; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 167.

[00285] In some embodiments, the VL comprises: (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 175; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 176; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 177.

[00286] In some embodiments, the VL comprises: (a) LCDR1 comprising the amino acid sequence of SEQ ID NO: 185; (b) LCDR2 comprising the amino acid sequence of SEQ ID NO: 186; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 187.

[00287] In some embodiments, an anti-ICOS antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, or 180 and a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 11, 21, 31, 41, 51, 61, 71, 81, 91, 101, 111, 121, 131, 141, 151, 161, 171, or 181. In some embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains

substitutions (for example, conservative substitutions), insertions, or deletions relative to the reference sequence, and a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (for example, conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-ICOS antibody comprising that sequence retains the ability to bind to ICOS. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, or 180. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 11, 21, 31, 41, 51, 61, 71, 81, 91, 101, 111, 121, 131, 141, 151, 161, 171, or 181. In some embodiments, substitutions, insertions, or deletions occur in regions outside the CDRs (that is, in the FRs). Optionally, the anti-ICOS antibody comprises the VH sequence in SEQ ID NO: 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, or 180 and the VL sequence of SEQ ID NO: 11, 21, 31, 41, 51, 61, 71, 81, 91, 101, 111, 121, 131, 141, 151, 161, 171, or 181, including post-translational modifications of one or both sequence.

[00288] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 12; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 13; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 14; and (II) a VL domain comprising: (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 15; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 16; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 17.

[00289] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 22; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 23; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 24; and (II) a VL domain comprising: (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 25; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 26; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 27.

[00290] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 32; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 33; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 34; and (II) a VL domain comprising: (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 35; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 36; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 37.

[00291] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 42; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 43; and (c) HCDR3 comprising the amino

acid sequence of SEQ ID NO: 44; and (II) a VL domain comprising: (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 45; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 46; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 47.

[00292] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 52; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 53; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 54; and (II) a VL domain comprising: (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 55; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 56; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 57.

[00293] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 62; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 63; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 64; and (II) a VL domain comprising: (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 65; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 66; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 67.

[00294] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 72; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 73; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 74; and (II) a VL domain comprising: (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 75; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 76; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 77.

[00295] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 82; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 83; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 84; and (II) a VL domain comprising: (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 85; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 86; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 87.

[00296] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 92; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 93; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 94; and (II) a VL domain comprising: (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 95; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 96; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 97.

[00297] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 102; (b) HCDR2

comprising the amino acid sequence of SEQ ID NO: 103; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 104; and (II) a VL domain comprising: (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 105; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 106; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 107.

[00298] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 112; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 113; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 114; and (II) a VL domain comprising: (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 115; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 116; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 117.

[00299] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 122; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 123; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 124; and (II) a VL domain comprising: (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 125; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 126; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 127.

[00300] In some embodiments, the anti-ICOS antibody comprises (I) a V_H domain comprising: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 132; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 133; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 134; and (II) a VL domain comprising: (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 135; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 136; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 137.

[00301] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 142; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 143; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 144; and (II) a VL domain comprising: (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 145; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 146; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 147.

[00302] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 152; (b) HCDR2

comprising the amino acid sequence of SEQ ID NO: 153; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 154; and (II) a VL domain comprising: (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 155; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 156; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 157.

[00303] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 162; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 163; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 164; and (II) a VL domain comprising: (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 165; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 166; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 167.

[00304] In some embodiments, the anti-ICOS antibody comprises (I) a V_H domain comprising: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 172; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 173; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 174; and (II) a VL domain comprising: (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 175; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 176; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 177.

[00305] In some embodiments, the anti-ICOS antibody comprises (I) a VH domain comprising: (a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 182; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 183; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 184; and (II) a VL domain comprising: (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 185; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 186; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 187.

[00306] In some embodiments, an anti-ICOS antibody comprises a VH as in any of the embodiments provided herein, and a VL as in any of the embodiments provided herein. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 10 and SEQ ID NO: 11, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 20 and SEQ ID NO: 21, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 30 and SEQ ID NO: 31, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in

SEQ ID NO: 40 and SEQ ID NO: 41, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 50 and SEQ ID NO: 51, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 60 and SEQ ID NO: 61, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 70 and SEQ ID NO: 71, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 80 and SEQ ID NO: 81, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 90 and SEQ ID NO: 91, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 100 and SEQ ID NO: 101, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 110 and SEQ ID NO: 111, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 120 and SEQ ID NO: 121, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 130 and SEQ ID NO: 131, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 140 and SEQ ID NO: 141, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 150 and SEQ ID NO: 151, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 160 and SEQ ID NO: 161, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 170 and SEQ ID NO: 171, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 180 and SEQ ID NO: 181, respectively, including post-translational modifications of those sequences.

[00307] In some embodiments, antibodies which compete with the anti-ICOS antibodies provided herein for binding to ICOS are provided. In some embodiments, antibodies compete with the anti-ICOS antibodies provided herein for binding to an epitope on ICOS.

[00308] In some embodiments, competition assays may be used to identify a monoclonal antibody that competes with an anti-ICOS antibody described herein (such as 7F12, 37A10, 35A9, 36E10, 16G10, 37A10S713, 37A10S714, 37A10S715, 37A10S716, 37A10S717, 37A10S718, 16G10S71, 16G10S72, 16G10S73, 16G10S83, 35A9S79, 35A9S710, or 35A9S89) for binding to ICOS. Competition assays can be used to determine whether two antibodies bind the same epitope by recognizing identical or sterically overlapping epitopes or one antibody competitively inhibits binding of another antibody to the antigen. In some embodiments, such a competing antibody binds to the same epitope that is bound by an antibody described herein. Exemplary competition assays include, but are not limited to, routine assays such as those provided in Harlow and Lane (1988) Antibodies: A Laboratory Manual ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.). Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in Methods in Molecular Biology vol. 66 (Humana Press, Totowa, N.J.). In some embodiments, two antibodies are said to bind to the same epitope if each blocks binding of the other by 50% or more. In some embodiments, the antibody that competes with an anti-ICOS antibody described herein is a chimeric, humanized or human antibody. In some embodiments, an antibody that competes with a chimeric, humanized, or human anti-ICOS antibody as described herein is provided.

[00309] In some embodiments, antibodies that bind to any one or more of the epitopes that the antibodies provided herein are provided. In some embodiments, antibodies that bind and overlap an epitope to which the present antibodies bind to are provided. In some embodiments, an antibody is provided that competes with at least one of the antibodies provided herein. In some embodiments, an antibody is provided that competes with at least two of the antibodies provided herein. In some embodiments, an antibody is provided that competes with at least three of the antibodies provided herein. In some embodiments, the antibody binds to an overlapping epitope as an antibody described in the examples herein. In some embodiments, the entire epitope is bound and/or obstructed by the competing antibody. In some embodiments, a part of the epitope is bound and/or obstructed by the competing antibody. In some embodiments, the competing antibody's paratope binds to at least a part of the epitope of an antibody provided herein. In some embodiments, the competing antibody's paratope binds the target, and a different section of the competing antibody's structure obstruct at least a part of the epitope of an antibody provided herein.

Exemplary chimeric antibodies

[00310] In some embodiments, an antibody provided herein is a chimeric antibody. Certain chimeric antibodies are described, for example, in U.S. Patent No. 4,816,567; and Morrison et al., (1984) Proc. Natl. Acad. Sci. USA, 81 :6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (for example, a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

[00311] Nonlimiting exemplary chimeric antibodies include chimeric antibodies comprising the heavy and/or light chain variable regions of an antibody selected from 7F12, 37A10, 35A9, 36E10, 16G10, 37A10S713, 37A10S714, 37A10S715, 37A10S716, 37A10S717, 37A10S718, 16G10S71, 16G10S72, 16G10S73, 16G10S83, 35A9S79, 35A9S710, or 35A9S89. Additional nonlimiting exemplary chimeric antibodies include chimeric antibodies comprising heavy chain CDR1, CDR2, and CDR3, and/or light chain CDR1, CDR2, and CDR3 of an antibody selected from 7F12, 37A10, 35A9, 36E10, 16G10, 37A10S713, 37A10S714, 37A10S715, 37A10S716, 37A10S717, 37A10S718, 16G10S71, 16G10S72, 16G10S73, 16G10S83, 35A9S79, 35A9S710, or 35A9S89. Further nonlimiting exemplary chimeric antibodies include chimeric antibodies comprising heavy chain CDR1, CDR2, and CDR3, and/or light chain CDR1, CDR2, and CDR3 of an antibody selected from 7F12, 37A10, 35A9, 36E10, 16G10, 37A10S713, 37A10S714, 37A10S715, 37A10S716, 37A10S717, 37A10S718, 16G10S71, 16G10S72, 16G10S73, 16G10S83, 35A9S79, 35A9S710, or 35A9S89. In some embodiments, the chimeric anti-ICOS antibody comprises the variable regions described above and binds to ICOS. In some embodiments, the chimeric anti-ICOS antibody comprises the variable regions described above, binds to ICOS, and increases the number of Teff cells and/or activates Teff cells and/or decreases the number of Treg cells and/or increases the ratio of Teff cells to Treg cells. In some embodiments, the Treg cells are CD4+ FoxP3+ T cells. In some embodiments, the Teff cells are CD4+ FoxP3- T cells. In some embodiments, the Teff cells are CD8+ T cells. In some embodiments, the Teff cells are CD4+ FoxP3- T cells and CD8+ T cells.

[00312] In some embodiments, a chimeric anti-ICOS antibody comprises a heavy chain comprising a variable region sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, or 180, wherein the antibody binds ICOS. In some embodiments,

a chimeric anti-ICOS antibody comprises a light chain comprising a variable region sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 11, 21, 31, 41, 51, 61, 71, 81, 91, 101, 111, 121, 131, 141, 151, 161, 171, or 181, wherein the antibody binds ICOS. In some embodiments, a chimeric anti-ICOS antibody comprises a heavy chain comprising a variable region sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, and 180; and a light chain comprising a variable region sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 11, 21, 31, 41, 51, 61, 71, 81, 91, 101, 111, 121, 131, 141, 151, 161, 171, and 181; wherein the antibody binds ICOS.

[00313] Exemplary chimeric anti-ICOS antibodies also include chimeric antibodies that compete for binding to ICOS with an antibody or fragment thereof described herein. Thus, in some embodiments, a chimeric anti-ICOS antibody is provided that competes for binding to ICOS with an antibody selected from 7F12, 37A10, 35A9, 36E10, 16G10, 37A10S713, 37A10S714, 37A10S715, 37A10S716, 37A10S717, 37A10S718, 16G10S71, 16G10S72, 16G10S73, 16G10S83, 35A9S79, 35A9S710, and 35A9S89, or fragment thereof. In some embodiments, the antibody competes for binding to ICOS and increases the number of Teff cells and/or activates Teff cells and/or decreases the number of Treg cells and/or increases the ratio of Teff cells to Treg cells. In some embodiments, the Treg cells are CD4+ FoxP3+ T cells. In some embodiments, the Teff cells are CD8+ T cells. In some embodiments, the Teff cells are CD4+ FoxP3- T cells and CD8+ T cells.

[00314] In some embodiments, a chimeric antibody described herein comprises one or more human constant regions. In some embodiments, the human heavy chain constant region is of an isotype selected from IgA, IgG, and IgD. In some embodiments, the human light chain constant region is of an isotype selected from κ and λ. In some embodiments, a chimeric antibody described herein comprises a human IgG constant region. In some embodiments, a chimeric antibody described herein comprises a human IgG4 heavy chain constant region. In some embodiments, a chimeric antibody described herein comprises a human IgG4 constant region and a human κ light chain.

[00315] As noted above, whether or not effector function is desirable may depend on the particular method of treatment intended for an antibody. Thus, in some embodiments, when effector function is desirable, a chimeric anti-ICOS antibody comprising a human IgG1 heavy

chain constant region or a human IgG3 heavy chain constant region is selected. In some embodiments, when effector function is not desirable, a chimeric anti-ICOS antibody comprising a human IgG4 or IgG2 heavy chain constant region is selected.

Exemplary humanized antibodies

[00316] In some embodiments, humanized antibodies that bind ICOS are provided. Humanized antibodies are useful as therapeutic molecules because humanized antibodies reduce or eliminate the human immune response as compared to non-human antibodies, which can result in an immune response to an antibody therapeutic (such as the human anti-mouse antibody (HAMA) response), and decreased effectiveness of the therapeutic.

[00317] In some embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (for example, the antibody from which the CDR residues are derived), for example, to restore or improve antibody specificity or affinity.

[00318] Humanized antibodies and methods of making them are reviewed, for example, in Almagro and Fransson, (2008) Front. Biosci. 13: 1619-1633, and are further described, for example, in Riechmann *et al.*, (1988) *Nature* 332:323-329; Queen *et al.*, (1989) *Proc. Natl Acad. Sci. USA* 86: 10029-10033; US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, (2005) *Methods* 36:25-34; Padlan, (1991) *Mol. Immunol.* 28:489-498 (describing "resurfacing"); Dall'Acqua *et al.*, (2005) *Methods* 36:43-60 (describing "FR shuffling"); and Osbourn *et al.*, (2005) *Methods* 36:61-68 and Klimka *et al.*, (2000) *Br. J. Cancer*, 83:252-260 (describing the "guided selection" approach to FR shuffling).

[00319] Human framework regions that can be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, for example, Sims *et al.* (1993) *J. Immunol.* 151 :2296); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, for example, Carter *et al.* (1992) *Proc. Natl. Acad. Sci. USA*, 89:4285; and Presta *et al.* (1993) *J. Immunol*, 151:2623); human mature (somatically mutated) framework regions or human germline framework regions (see, for example, Almagro and Fransson, (2008) *Front. Biosci.*

13:1619-1633); and framework regions derived from screening FR libraries (see, for example, Baca *et al.*, (1997) *J. Biol. Chem.* 272: 10678-10684 and Rosok *et al.*, (1996) *J. Biol. Chem.* 271 :22611-22618).

[00320] Nonlimiting exemplary humanized antibodies include 37A10S713, 37A10S714, 37A10S715, 37A10S716, 37A10S717, 37A10S718, 16G10S71, 16G10S72, 16G10S73, 16G10S83, 35A9S79, 35A9S710, and 35A9S89, described herein. Nonlimiting exemplary humanized antibodies also include antibodies comprising a heavy chain variable region of an antibody selected from 37A10S713, 37A10S714, 37A10S715, 37A10S716, 37A10S717, 37A10S718, 16G10S71, 16G10S72, 16G10S73, 16G10S83, 35A9S79, 35A9S710, and 35A9S89 and/or a light chain variable region of an antibody selected from 37A10S713, 37A10S714, 37A10S715, 37A10S716, 37A10S717, 37A10S718, 16G10S71, 16G10S72, 16G10S73, 16G10S83, 35A9S79, 35A9S710, and 35A9S89. Nonlimiting exemplary humanized antibodies include antibodies comprising a heavy chain variable region selected from SEQ ID NOs: 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, and 180 and/or a light chain variable region selected from SEQ ID NOs: 61, 71, 81, 91, 101, 111, 121, 131, 141, 151, 161, 171, and 181. Exemplary humanized antibodies also include, but are not limited to, humanized antibodies comprising heavy chain CDR1, CDR2, and CDR3, and/or light chain CDR1, CDR2, and CDR3 of an antibody selected from 7F12, 37A10, 35A9, 36E10, 16G10, 37A10S713, 37A10S714, 37A10S715, 37A10S716, 37A10S717, 37A10S718, 16G10S71, 16G10S72, 16G10S73, 16G10S83, 35A9S79, 35A9S710, and 35A9S89. In some embodiments, the humanized anti-ICOS antibody comprises the CDRs described above and binds to ICOS. In some embodiments, the humanized anti-ICOS antibody comprises the CDRs described above, binds to ICOS and increases the number of Teff cells and/or activates Teff cells and/or decreases the number of Treg cells and/or increases the ratio of Teff cells to Treg cells. In some embodiments, the Treg cells are CD4+ FoxP3+ T cells. In some embodiments, the Teff cells are CD8+ T cells. In some embodiments, the Teff cells are CD4+ FoxP3- T cells and CD8+ T cells.

[00321] In some embodiments, a humanized anti-ICOS antibody comprises a heavy chain CDR1, CDR2, and CDR3 and/or a light chain CDR1, CDR2, and CDR3 of an antibody selected from 7F12, 37A10, 35A9, 36E10, 16G10, 37A10S713, 37A10S714, 37A10S715, 37A10S716, 37A10S717, 37A10S718, 16G10S71, 16G10S72, 16G10S73, 16G10S83, 35A9S79, 35A9S710, and 35A9S89.

[00322] In some embodiments, a humanized anti-ICOS antibody comprises a heavy chain comprising a variable region sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%

identical to a sequence selected from SEQ ID NOs: 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, and 180, and wherein the antibody binds ICOS. In some embodiments, a humanized anti-ICOS antibody comprises a light chain comprising a variable region sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 61, 71, 81, 91, 101, 111, 121, 131, 141, 151, 161, 171, and 181, wherein the antibody binds ICOS. In some embodiments, a humanized anti-ICOS antibody comprises a heavy chain comprising a variable region sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, and 180; and a light chain comprising a variable region sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 61, 71, 81, 91, 101, 111, 121, 131, 141, 151, 161, 171, and 181; wherein the antibody binds ICOS.

[00323] In some embodiments, any one or more of the CDR sequences provided herein are maintained, while the remain heavy, light, or heavy and light chain region (that is, FR1, FR2, FR3, and FR4) is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 11, 21, 31, 41, 51, 61, 71, 81, 91, 101, 111, 121, 131, 141, 151, 161, 171, and 181.

[00324] In some embodiments, a humanized anti-ICOS antibody comprises at least one of the CDRs discussed herein. That is, in some embodiments, a humanized anti-ICOS antibody comprises at least one CDR selected from a heavy chain CDR1 discussed herein, a heavy chain CDR2 discussed herein, a heavy chain CDR3 discussed herein, a light chain CDR1 discussed herein, a light chain CDR2 discussed herein, and a light chain CDR3 discussed herein. Further, in some embodiments, a humanized anti-ICOS antibody comprises at least one mutated CDR based on a CDR discussed herein, wherein the mutated CDR comprises 1, 2, 3, or 4 amino acid substitutions relative to the CDR discussed herein. In some embodiments, one or more of the amino acid substitutions are conservative amino acid substitutions. One skilled in the art can select one or more suitable conservative amino acid substitutions for a particular CDR sequence, wherein the suitable conservative amino acid substitutions are not predicted to significantly alter the binding properties of the antibody comprising the mutated CDR.

[00325] Exemplary humanized anti-ICOS antibodies also include antibodies that compete for binding to ICOS with an antibody or fragment thereof described herein. Thus, in some embodiments, a humanized anti-ICOS antibody is provided that competes for binding to ICOS

with an antibody or fragment thereof selected from 7F12, 37A10, 35A9, 36E10, 16G10, 37A10S713, 37A10S714, 37A10S715, 37A10S716, 37A10S717, 37A10S718, 16G10S71, 16G10S72, 16G10S73, 16G10S83, 35A9S79, 35A9S710, and 35A9S89. In some embodiments, a humanized anti-ICOS antibody is provided that competes for binding to ICOS with an antibody or fragment thereof selected from 7F12, 37A10, 35A9, 36E10, 16G10, 37A10S713, 37A10S714, 37A10S715, 37A10S716, 37A10S717, 37A10S718, 16G10S71, 16G10S72, 16G10S73, 16G10S83, 35A9S79, 35A9S710, and 35A9S89 and increases the number of Teff cells and/or activates Teff cells and/or decreases the number of Treg cells and/or increases the ratio of Teff cells to Treg cells. In some embodiments, the Treg cells are CD4+ FoxP3+ T cells. In some embodiments, the Teff cells are CD8+ T cells. In some embodiments, the Teff cells are CD4+ FoxP3- T cells and CD8+ T cells.

[00326] In some embodiments, a humanized anti-ICOS antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 188 and a light chain comprising the amino acid sequence of SEQ ID NO: 189.

Exemplary human antibodies

[00327] In some embodiments, an anti-ICOS antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, (2001) *Curr. Opin. Pharmacol.* 5:368-374 and Lonberg, (2008) *Curr. Opin. Immunol.* 20:450-459. In some embodiments, the human antibody is not a naturally occurring antibody. In some embodiments, the human antibody is a monoclonal antibody; thus, in some embodiments, each of the human antibodies in a set can bind to the same epitope on the antigen.

[00328] Human antibodies can be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, (2005) *Nat. Biotech.* 23: 1117-1125. See also, for example, U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Patent No. 5,770,429 describing HUMAB® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VELOCIMOUSE® technology). Human variable regions from intact antibodies

generated by such animals may be further modified, for example, by combining with a different human constant region.

[00329] Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, for example, Kozbor (1984) *J. Immunol*, 133: 3001; Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner *et al*, (1991) *J. Immunol.*, 147:86). Human antibodies generated via human B-cell hybridoma technology are also described in Li *et al.*, (2006) *Proc. Natl. Acad. Sci. USA*, 103:3557-3562. Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, (2006) *Xiandai Mianyixue*, 26(4):265-268 (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, (2005) *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, (2005) *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3): 185-191.

[00330] Human antibodies can also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

[00331] Antibodies may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, for example, in Hoogenboom *et al.* in Methods in Molecular Biology 178: 1-37 (O'Brien *et al.*, *ed.*, Human Press, Totowa, NJ, 2001) and further described, for example, in the McCafferty *et al*, (1990) *Nature* 348:552-554; Clackson *et al*, (1991) *Nature* 352: 624-628; Marks *et al*, (1992) *J. Mol. Biol* 222: 581-597; Marks and Bradbury, in *Methods in Molecular Biology* 248: 161-175 (Lo, *ed.*, Human Press, Totowa, NJ, 2003); Sidhu *et al*, (2004) *J. Mol. Biol.* 338(2): 299-310; Lee *et al.*, (2004) *J. Mol. Biol.* 340(5): 1073-1093; *Fellouse*, (2004) P*roc. Natl. Acad. Sci. USA* 101(34): 12467-12472; and Lee *et al*, (2004) *J. Immunol. Methods* 284(1-2): 119-132 and PCT publication WO 99/10494.

[00332] In certain phage display methods, repertoires of V_H and V_L genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter *et al.*, (1994) *Ann. Rev. Immunol.*, 12:433-455. Phage typically display antibody fragments, either as single-

chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (for example, from human) to provide a single source of antibodies to a wide range of non-self and also self-antigens without any immunization as described by Griffiths *et al.*, (1993) *EMBO J* 12:725-734. Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement in vitro, as described by Hoogenboom and Winter (1992), *J. Mol. Biol,* 227:381-388. Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

[00333] In some embodiments, a human anti-ICOS antibody binds to a polypeptide having the sequence of SEQ ID NO: 1 or 2. In some embodiments, the human anti-ICOS antibody binds to ICOS and increases the number of Teff cells and/or activates Teff cells and/or decreases the number of Treg cells and/or increases the ratio of Teff cells to Treg cells. In some embodiments, the Treg cells are CD4+ FoxP3+ T cells. In some embodiments, the Teff cells are CD8+ T cells. In some embodiments, the Teff cells are CD4+ FoxP3- T cells and CD8+ T cells.

[00334] Exemplary human anti-ICOS antibodies also include antibodies that compete for binding to ICOS with a human antibody or fragment thereof described herein. Thus, in some embodiments, a human anti-ICOS antibody is provided that competes for binding to ICOS with an antibody or fragment thereof selected from 7F12, 37A10, 35A9, 36E10, 16G10, 37A10S713, 37A10S714, 37A10S715, 37A10S716, 37A10S717, 37A10S718, 16G10S71, 16G10S72, 16G10S73, 16G10S83, 35A9S79, 35A9S710, and 35A9S89. In some embodiments, a human anti-ICOS antibody is provided that competes for binding to ICOS with an antibody or fragment thereof selected from 7F12, 37A10, 35A9, 36E10, 16G10, 37A10S713, 37A10S714, 37A10S715, 37A10S716, 37A10S717, 37A10S718, 16G10S71, 16G10S72, 16G10S73, 16G10S83, 35A9S79, 35A9S710, and 35A9S89 and increases the number of Teff cells and/or activates Teff cells and/or decreases the number of Treg cells and/or increases the ratio of Teff cells to Treg cells. In some embodiments, the Treg cells are CD4+ FoxP3+ T cells. In some embodiments, the Teff cells are CD8+ T cells. In some embodiments, the Teff cells are CD4+ FoxP3- T cells and CD8+ T cells.

[00335] In some embodiments, a chimeric human anti-ICOS antibody is provided, where the antibody comprises the variable region from a human antibody that binds ICOS and the

constant region from a different human antibody. In some embodiments, a chimeric human anti-ICOS antibody, where the antibody comprises the CDRs from a human antibody that binds ICOS and a framework from a different human antibody is provided. In some embodiments, the antibody is not a naturally occurring human antibody.

[00336] In some embodiments, a human anti-ICOS antibody comprises one or more human constant regions. In some embodiments, the human heavy chain constant region is of an isotype selected from IgA, IgG, and IgD. In some embodiments, the human light chain constant region is of an isotype selected from κ and λ. In some embodiments, a human antibody described herein comprises a human IgG constant region. In some embodiments, a human antibody described herein comprises a human IgG4 heavy chain constant region. In some embodiments, a human antibody described herein comprises a human IgG4 constant region and a human κ light chain.

[00337] In some embodiments, when effector function is desirable, a human anti-ICOS antibody comprising a human IgG1 heavy chain constant region or a human IgG3 heavy chain constant region is selected. In some embodiments, when effector function is not desirable, a human anti-ICOS antibody comprising a human IgG4 or IgG2 heavy chain constant region is selected.

[00338] As noted herein, the term "human antibody" denotes the genus of possible sequences for the antibody construct, rather than a source of the antibody.

Exemplary Antibody Constant Regions

[00339] In some embodiments, an antibody described herein comprises one or more human constant regions. In some embodiments, the human heavy chain constant region is of an isotype selected from IgA, IgG, and IgD. In some embodiments, the human light chain constant region is of an isotype selected from κ and λ. In some embodiments, an antibody described herein comprises a human IgG constant region. In some embodiments, an antibody described herein comprises a human IgG4 heavy chain constant region. In some embodiments, an antibody described herein comprises a human IgG4 constant region and a human κ light chain.

[00340] Throughout the present specification and claims unless explicitly stated or known to one skilled in the art, the numbering of the residues in an immunoglobulin heavy chain is that of the EU index as in Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991), expressly incorporated herein by reference. The "EU index as in Kabat" refers to the residue numbering of the human IgG1 EU antibody.

[00341] As noted above, whether or not effector function is desirable may depend on the particular method of treatment intended for an antibody. Thus, in some embodiments, when effector function is desirable, an anti-ICOS antibody comprising a human IgG1 heavy chain constant region or a human IgG3 heavy chain constant region is selected. In some embodiments, when effector function is not desirable, an anti-ICOS antibody comprising a human IgG4 or IgG2 heavy chain constant region is selected.

[00342] In some embodiments, an antibody comprises a variant Fc region has at least one amino acid substitution compared to the Fc region of a wild-type IgG or a wild-type antibody. In some embodiments, the variant Fc region has two or more amino acid substitutions in the Fc region of the wild-type antibody. In some embodiments, the variant Fc region has three or more amino acid substitutions in the Fc region of the wild-type antibody. In some embodiments, the variant Fc region has at least one, two or three or more Fc region amino acid substitutions described herein. In some embodiments, the variant Fc region herein will possess at least about 80% homology with a native sequence Fc region and/or with an Fc region of a parent polypeptide. In some embodiments, the variant Fc region herein will possess at least about 90% homology with a native sequence Fc region and/or with an Fc region of a parent polypeptide. In some embodiments, the variant Fc region herein will possess at least about 95% homology with a native sequence Fc region and/or with an Fc region of a parent polypeptide.

[00343] In some embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[00344] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, for example, Wright *et al. TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, for example, mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody may be made in order to create antibody variants with certain improved properties.

[00345] In some embodiments, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from

20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (for example, complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (EU numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, that is, between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, for example, US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki *et al. J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki *et al. Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka *et al. Arch. Biochem. Biophys.* 249:533-545 (1986); US Patent Application No. US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, FUT8, knockout CHO cells (see, for example, Yamane-Ohnuki *et al. Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. *et al., Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

[00346] Antibody variants are further provided with bisected oligosaccharides, for example, in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, for example, in WO 2003/011878 (Jean-Mairet *et al.*); US Patent No. 6,602,684 (Umana *et al.*); and US 2005/0123546 (Umana *et al.*). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, for example, in WO 1997/30087 (Patel *et al.*); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

[00347] Antibody variants are also provided with amino-terminal leader extensions. For example, one or more amino acid residues of the amino-terminal leader sequence are present at the amino-terminus of any one or more heavy or light chains of an antibody. An exemplary

amino-terminal leader extension comprises or consists of three amino acid residues, VHS, present on one or both light chains of an antibody variant.

[00348] The in vivo or serum half-life of human FcRn high affinity binding polypeptides can be assayed, for example, in transgenic mice, in humans, or in non-human primates to which the polypeptides with a variant Fc region are administered. See also, for example, Petkova *et al.* *International Immunology* 18(12):1759-1769 (2006).

[00349] In some embodiments, the antibody variant mediates ADCC in the presence of human effector cells more effectively than a parent antibody. In some embodiments, the antibody variant is substantially more effective at mediating ADCC in vitro, when the amounts of polypeptide variant and parent antibody used in the assay are essentially the same. In some embodiments, the antibody variant is substantially more effective at mediating ADCC in vivo, when the amounts of polypeptide variant and parent antibody used in the assay are essentially the same. Generally, such variants will be identified using the in vitro ADCC assay as herein disclosed, but other assays or methods for determining ADCC activity, for example in an animal model *etc.*, are contemplated.

Exemplary Antibody Conjugates

[00350] In some embodiments, an anti-ICOS antibody is conjugated to another molecule. In some embodiments, the additional molecule can be a detectable marker, such as a label. In some embodiments, the additional molecule can be a therapeutic molecule, such as a cytotoxic agent. In some embodiments, a label and/or a cytotoxic agent can be conjugated to the antibody. As used herein, a label is a moiety that facilitates detection of the antibody and/or facilitates detection of a molecule to which the antibody binds. Nonlimiting exemplary labels include, but are not limited to, radioisotopes, fluorescent groups, enzymatic groups, chemiluminescent groups, biotin, epitope tags, metal-binding tags, *etc.* One skilled in the art can select a suitable label according to the specific application.

[00351] As used herein, a cytotoxic agent is a moiety that reduces the proliferative capacity of one or more cells. A cell has reduced proliferative capacity when the cell becomes less able to proliferate, for example, because the cell undergoes apoptosis or otherwise dies, the cell fails to proceed through the cell cycle and/or fails to divide, the cell differentiates, *etc.* Nonlimiting exemplary cytotoxic agents include, but are not limited to, radioisotopes, toxins, and chemotherapeutic agents. One skilled in the art can select a suitable cytotoxic according to the intended application. In some embodiments, the cytotoxic agent is at least one of an anti-metabolite, an alkylating agent, an antibiotic, a growth factor, a cytokine, an anti-angiogenic agent, an anti-mitotic agent, an anthracycline, toxin, or an apoptotic agent

[00352] In some embodiments, a label and/or a cytotoxic agent is conjugated to an antibody using chemical methods in vitro. Nonlimiting exemplary chemical methods of conjugation are known in the art, and include services, methods and/or reagents commercially available from, for example, Thermo Scientific Life Science Research Produces (formerly Pierce; Rockford, Ill.), Prozyme (Hayward, Calif.), SACRI Antibody Services (Calgary, Canada), AbD Serotec (Raleigh, N.C.), *etc.* In some embodiments, when a label and/or cytotoxic agent is a polypeptide, the label and/or cytotoxic agent can be expressed from the same expression vector with at least one antibody chain to produce a polypeptide comprising the label and/or cytotoxic agent fused to an antibody chain. One skilled in the art can select a suitable method for conjugating a label and/or cytotoxic agent to an antibody according to the intended application.

[00353] In some embodiments, conjugation can be covalent. In some embodiments, conjugation can be non-covalent. In some embodiments, conjugation can be via a specific binding interaction, for example, through the binding of a secondary antibody.

Exemplary Leader Sequences

[00354] In order for some secreted proteins to express and secrete in large quantities, a leader sequence from a heterologous protein may be desirable. In some embodiments, employing heterologous leader sequences can be advantageous in that a resulting mature polypeptide can remain unaltered as the leader sequence is removed in the ER during the secretion process. The addition of a heterologous leader sequence can be useful to express and secrete some proteins.

[00355] Certain exemplary leader sequence sequences are described, for example, in the online Leader sequence Database maintained by the Department of Biochemistry, National University of Singapore. See Choo *et al.*, *BMC Bioinformatics*, 6: 249 (2005); and PCT Publication No. WO 2006/081430.

IV. Antibody Expression and Production

Nucleic Acid Molecules Encoding Anti-ICOS Antibodies

[00356] Nucleic acid molecules comprising polynucleotides that encode one or more chains of an anti-ICOS antibody are provided herein. In some embodiments, a nucleic acid molecule comprises a polynucleotide that encodes a heavy chain or a light chain of an anti-ICOS antibody. In some embodiments, a nucleic acid molecule comprises both a polynucleotide that encodes a heavy chain and a polynucleotide that encodes a light chain, of an anti-ICOS antibody. In some embodiments, a first nucleic acid molecule comprises a first polynucleotide

that encodes a heavy chain and a second nucleic acid molecule comprises a second polynucleotide that encodes a light chain.

[00357] In some embodiments, the heavy chain and the light chain are expressed from one nucleic acid molecule, or from two separate nucleic acid molecules, as two separate polypeptides. In some embodiments, such as when an antibody is an scFv, a single polynucleotide encodes a single polypeptide comprising both a heavy chain and a light chain linked together.

[00358] In some embodiments, a polynucleotide encoding a heavy chain or light chain of an anti-ICOS antibody comprises a nucleotide sequence that encodes at least one of the CDRs provided herein. In some embodiments, a polynucleotide encoding a heavy chain or light chain of an anti-ICOS antibody comprises a nucleotide sequence that encodes at least 3 of the CDRs provided herein. In some embodiments, a polynucleotide encoding a heavy chain or light chain of an anti-ICOS antibody comprises a nucleotide sequence that encodes at least 6 of the CDRs provided herein. In some embodiments, a polynucleotide encoding a heavy chain or light chain of an anti-ICOS antibody comprises a nucleotide sequence that encodes a leader sequence, which, when translated, is located at the N terminus of the heavy chain or light chain. As discussed above, the leader sequence may be the native heavy or light chain leader sequence, or may be another heterologous leader sequence.

[00359] In some embodiments, the nucleic acid is one that encodes for any of the amino acid sequences for the antibodies in the Sequence Table herein. In some embodiments, the nucleic acid is one that is at least 80% identical to a nucleic acid encoding any of the amino acid sequences for the antibodies in the Sequence Table herein, for example, at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical. In some embodiments, the nucleic acid is one that hybridizes to any one or more of the nucleic acid sequences provided herein. In some of the embodiments, the hybridization is under moderate conditions. In some embodiments, the hybridization is under highly stringent conditions, such as: at least about 6X SSC and 1% SDS at 65°C, with a first wash for 10 minutes at about 42°C with about 20% (v/v) formamide in 0.1X SSC, and with a subsequent wash with 0.2 X SSC and 0.1% SDS at 65°C.

[00360] Nucleic acid molecules can be constructed using recombinant DNA techniques conventional in the art. In some embodiments, a nucleic acid molecule is an expression vector that is suitable for expression in a selected host cell. Vectors

[00361] Vectors comprising polynucleotides that encode anti-ICOS heavy chains and/or anti-ICOS light chains are provided. Vectors comprising polynucleotides that encode anti-ICOS heavy chains and/or anti-ICOS light chains are also provided. Such vectors include, but are not limited to, DNA vectors, phage vectors, viral vectors, retroviral vectors, *etc*. In some

embodiments, a vector comprises a first polynucleotide sequence encoding a heavy chain and a second polynucleotide sequence encoding a light chain. In some embodiments, the heavy chain and light chain are expressed from the vector as two separate polypeptides. In some embodiments, the heavy chain and light chain are expressed as part of a single polypeptide, such as, for example, when the antibody is an scFv.

[00362] In some embodiments, a first vector comprises a polynucleotide that encodes a heavy chain and a second vector comprises a polynucleotide that encodes a light chain. In some embodiments, the first vector and second vector are transfected into host cells in similar amounts (such as similar molar amounts or similar mass amounts). In some embodiments, a mole- or mass-ratio of between 5:1 and 1:5 of the first vector and the second vector is transfected into host cells. In some embodiments, a mass ratio of between 1:1 and 1:5 for the vector encoding the heavy chain and the vector encoding the light chain is used. In some embodiments, a mass ratio of 1:2 for the vector encoding the heavy chain and the vector encoding the light chain is used.

[00363] In some embodiments, a vector is selected that is optimized for expression of polypeptides in CHO or CHO-derived cells, or in NSO cells. Exemplary such vectors are described, for example, in Running Deer *et al.*, *Biotechnol. Prog.* 20:880-889 (2004).

Host Cells

[00364] In some embodiments, anti-ICOS antibody heavy chains and/or anti-ICOS antibody light chains may be expressed in prokaryotic cells, such as bacterial cells; or in eukaryotic cells, such as fungal cells (such as yeast), plant cells, insect cells, and mammalian cells. Such expression may be carried out, for example, according to procedures known in the art. Exemplary eukaryotic cells that may be used to express polypeptides include, but are not limited to, COS cells, including COS 7 cells; 293 cells, including 293-6E cells; CHO cells, including CHO-S, DG44. Lec13 CHO cells, and FUT8 CHO cells; PER.C6® cells (Crucell); and NSO cells. In some embodiments, anti-ICOS antibody heavy chains and/or anti-ICOS antibody light chains may be expressed in yeast. See, for example, U.S. Publication No. US 2006/0270045 A1. In some embodiments, a particular eukaryotic host cell is selected based on its ability to make desired post-translational modifications to the anti-ICOS antibody heavy chains and/or anti-ICOS antibody light chains. For example, in some embodiments, CHO cells produce polypeptides that have a higher level of sialylation than the same polypeptide produced in 293 cells.

[00365] Introduction of one or more nucleic acids into a desired host cell may be accomplished by any method, including but not limited to, calcium phosphate transfection,

DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, *etc.* Nonlimiting exemplary methods are described, for example, in Sambrook *et al.*, Molecular Cloning, A Laboratory Manual, 3rd ed. Cold Spring Harbor Laboratory Press (2001). Nucleic acids may be transiently or stably transfected in the desired host cells, according to any suitable method.

[00366] Host cells comprising any of the polynucleotides or vectors described herein are also provided. In some embodiments, a host cell comprising an anti-ICOS antibody is provided. Any host cells capable of over-expressing heterologous DNAs can be used for the purpose of isolating the genes encoding the antibody, polypeptide or protein of interest. Non-limiting examples of mammalian host cells include but not limited to COS, HeLa, and CHO cells. See also PCT Publication No. WO 87/04462. Suitable non-mammalian host cells include prokaryotes (such as *E. coli* or *B. subtillis*) and yeast (such as *S. cerevisae*, *S. pombe*; or *K. lactis*).

Purification of Antibodies

[00367] Anti-ICOS antibodies can be purified by any suitable method. Such methods include, but are not limited to, the use of affinity matrices or hydrophobic interaction chromatography. Suitable affinity ligands include the ROR1 ECD and ligands that bind antibody constant regions. For example, a Protein A, Protein G, Protein A/G, or an antibody affinity column may be used to bind the constant region and to purify an anti-ICOS antibody. Hydrophobic interactive chromatography, for example, a butyl or phenyl column, may also suitable for purifying some polypeptides such as antibodies. Ion exchange chromatography (for example anion exchange chromatography and/or cation exchange chromatography) may also suitable for purifying some polypeptides such as antibodies. Mixed-mode chromatography (for example reversed phase/anion exchange, reversed phase/cation exchange, hydrophilic interaction/anion exchange, hydrophilic interaction/cation exchange, *etc.*) may also suitable for purifying some polypeptides such as antibodies. Many methods of purifying polypeptides are known in the art.

Cell-Free Production of Antibodies

[00368] In some embodiments, an anti-ICOS antibody is produced in a cell-free system. Nonlimiting exemplary cell-free systems are described, for example, in Sitaraman *et al.*, *Methods Mol. Biol.* 498: 229-44 (2009); Spirin, *Trends Biotechnol.* 22: 538-45 (2004); Endo *et al.*, *Biotechnol. Adv.* 21: 695-713 (2003).

Compositions

[00369] In some embodiments, antibodies prepared by the methods described above are provided. In some embodiments, the antibody is prepared in a host cell. In some embodiments, the antibody is prepared in a cell-free system. In some embodiments, the antibody is purified. In some embodiments, the antibody prepared in a host cell or a cell-free system is a chimeric antibody. In some embodiments, the antibody prepared in a host cell or a cell-free system is a humanized antibody. In some embodiments, the antibody prepared in a host cell or a cell-free system is a human antibody. In some embodiments, a cell culture media comprising an anti-ICOS antibody is provided. In some embodiments, a host cell culture fluid comprising an anti-ICOS antibody is provided.

[00370] In some embodiments, compositions comprising antibodies prepared by the methods described above are provided. In some embodiments, the composition comprises an antibody prepared in a host cell. In some embodiments, the composition comprises an antibody prepared in a cell-free system. In some embodiments, the composition comprises a purified antibody. In some embodiments, the composition comprises a chimeric antibody prepared in a host cell or a cell-free system. In some embodiments, the composition comprises a humanized antibody prepared in a host cell or a cell-free system. In some embodiments, the composition comprises a human antibody prepared in a host cell or a cell-free system.

[00371] In some embodiments, a composition comprising anti-ICOS antibody at a concentration of more than about any one of 10 mg/mL, 20 mg/mL, 30 mg/mL, 40 mg/mL, 50 mg/mL, 60 mg/mL, 70 mg/mL, 80 mg/mL, 90 mg/mL, 100 mg/mL, 125 mg/mL, 150 mg/mL, 175 mg/mL, 200 mg/mL, 225 mg/mL, or 250 mg/mL is provided. In some embodiments, the composition comprises a chimeric antibody prepared in a host cell or a cell-free system. In some embodiments, the composition comprises a humanized antibody prepared in a host cell or a cell-free system. In some embodiments, the composition comprises a human antibody prepared in a host cell or a cell-free system.

V. Therapeutic Compositions and Methods

Methods of Treating Diseases using Anti-ICOS Antibodies

[00372] Antibodies and compositions comprising antibodies are provided for use in methods of treatment for humans or animals. Methods of treating disease comprising administering anti-ICOS antibodies are also provided. Nonlimiting exemplary diseases that can be treated with anti-ICOS antibodies include, but are not limited to cancer.

[00373] In some embodiments, a method of treating a tumor is provided, wherein cells within a sample of the tumor express ICOS. In some such embodiments, the tumor may be considered to be ICOS-positive, or to express ICOS. Expression of ICOS may be determined by IHC, e.g., as discussed herein. In some embodiments, a tumor is considered to express ICOS when a sample from the tumor shows 1+, 2+, or 3+ staining of ICOS by IHC. In some embodiments, the sample from the tumor shows 2+ or 3+ staining of ICOS by IHC. In some embodiments, a tumor sample from a subject is analyzed for ICOS expression and the subject is selected for treatment with an antibody described herein if the tumor sample shows ICOS expression. In some embodiments, the subject is selected if the tumor sample shows elevated expression of ICOS.

[00374] In some embodiments, a subject is selected for treatment with an anti-ICOS antibody provided herein if the subject's tumor is PD-L1^{LOW}. In some embodiments, a subject is selected for treatment with an anti-ICOS antibody provided herein if the subject's tumor is ICOS^{HIGH}/PD-L1^{LOW}. In some embodiments, a subject is selected for treatment with an anti-ICOS antibody provided herein if the subject's tumor is ICOS^{HIGH}/PD-L1^{HIGH}.

[00375] The anti-ICOS antibody can be administered as needed to subjects. Determination of the frequency of administration can be made by persons skilled in the art, such as an attending physician based on considerations of the condition being treated, age of the subject being treated, severity of the condition being treated, general state of health of the subject being treated and the like. In some embodiments, an effective dose of an anti-ICOS antibody is administered to a subject one or more times. In some embodiments, an effective dose of an anti-ICOS antibody is administered to the subject once a month, less than once a month, such as, for example, every two months or every three months. In some embodiments, an effective dose of an anti-ICOS antibody is administered less than once a month, such as, for example, once every three weeks, once every two weeks, or once every week. An effective dose of an anti-ICOS antibody is administered to the subject at least once. In some embodiments, the effective dose of an anti-ICOS antibody may be administered multiple times, including for periods of at least a month, at least six months, or at least a year.

[00376] In some embodiments, pharmaceutical compositions are administered in an amount effective for treatment of (including prophylaxis of) cancer. The therapeutically effective amount is typically dependent on the weight of the subject being treated, his or her physical or health condition, the extensiveness of the condition to be treated, or the age of the subject being treated. In general, anti-ICOS antibodies may be administered in an amount in the range of about 10 μg/kg body weight to about 100 mg/kg body weight per dose. In some embodiments, anti-ICOS antibodies may be administered in an amount in the range of about 50

μg/kg body weight to about 5 mg/kg body weight per dose. In some embodiments, anti-ICOS antibodies may be administered in an amount in the range of about 100 μg/kg body weight to about 10 mg/kg body weight per dose. In some embodiments, anti-ICOS antibodies may be administered in an amount in the range of about 100 μg/kg body weight to about 20 mg/kg body weight per dose. In some embodiments, anti-ICOS antibodies may be administered in an amount in the range of about 0.5 mg/kg body weight to about 20 mg/kg body weight per dose.

[00377] Pharmaceutical compositions are administered in an amount effective for increasing the number of Teff cells; activating Teff cells; depleting Treg cells; and/or increasing the ratio of Teff cells to Treg cells. In some embodiments, the Treg cells are CD4+ FoxP3+ T cells. In some embodiments, the Teff cells are CD8+ T cells. In some embodiments, the Teff cells are CD4+ FoxP3- T cells and CD8+ T cells.

[00378] In some embodiments, treatment with anti-ICOS antibody results in a pharmacodynamics readout, such as up-regulation of ICOS ligand (ICOSL). In some embodiments, up-regulation of ICOSL is observed on the surface of B cells. In some embodiments, up-regulation of ICOSL is observed on the surface of granulocytes. In some embodiments, up-regulation of ICOSL is observed on the surface of neutrophils. Up-regulation of ICOSL may be observed on cells in the tumor; on cells in the spleen; on cells in peripheral blood. Up-regulation of ICOSL on the cell surface can be detected, for example, by flow cytometry. In some embodiments, soluble ICOSL is increased in the serum following treatment with anti-ICOS antibody. Soluble ICOSL can be detected by methods including, but not limited to, ELISA, MSD, and mass spectrometry. In some embodiments, ICOS target engagement, as measured by availability of free-receptor, by anti-ICOS antibodies may also be used as a pharmacodynamics readout. In some such embodiments, upon treatment by an anti-ICOS antibody, the number of ICOS receptors on the surface of T lymphocytes that are free to bind additional antibodies may be quantified. Decrease in observed available receptors may serve as an indication that anti-ICOS antibodies are binding their target molecule.

[00379] The therapeutically effective amount is typically dependent on the weight of the subject being treated, his or her physical or health condition, the extensiveness of the condition to be treated, or the age of the subject being treated. In general, anti-ICOS antibodies may be administered in an amount in the range of about 10 μg/kg body weight to about 100 mg/kg body weight per dose. In some embodiments, anti-ICOS antibodies may be administered in an amount in the range of about 50 μg/kg body weight to about 5 mg/kg body weight per dose. In some embodiments, anti-ICOS antibodies may be administered in an amount in the range of about 100 μg/kg body weight to about 10 mg/kg body weight per dose. In some embodiments, anti-ICOS antibodies may be administered in an amount in the range of about 100 μg/kg body

weight to about 20 mg/kg body weight per dose. In some embodiments, anti-ICOS antibodies may be administered in an amount in the range of about 0.5 mg/kg body weight to about 20 mg/kg body weight per dose.

Pharmaceutical compositions

[00380] In some embodiments, compositions comprising anti-ICOS antibodies are provided in formulations with a wide variety of pharmaceutically acceptable carriers (see, for example, Gennaro, Remington: The Science and Practice of Pharmacy with Facts and Comparisons: Drugfacts Plus, 20th ed. (2003); Ansel *et al.*, Pharmaceutical Dosage Forms and Drug Delivery Systems, 7th ed., Lippencott Williams and Wilkins (2004); Kibbe *et al.*, Handbook of Pharmaceutical Excipients, 3rd ed., Pharmaceutical Press (2000)). Various pharmaceutically acceptable carriers, which include vehicles, adjuvants, and diluents, are available. Moreover, various pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are also available. Non-limiting exemplary carriers include saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof.

[00381] In some embodiments, a pharmaceutical composition comprising an anti-ICOS antibody is provided. In some embodiments, the pharmaceutical composition comprises a chimeric antibody. In some embodiments, the pharmaceutical composition comprises a humanized antibody. In some embodiments, the pharmaceutical composition comprises an antibody prepared in a host cell or cell-free system as described herein. In some embodiments, the pharmaceutical composition comprises pharmaceutically acceptable carrier.

[00382] In some embodiments, pharmaceutical compositions are administered in an amount effective for treatment of (including prophylaxis of) cancer. The therapeutically effective amount is typically dependent on the weight of the subject being treated, his or her physical or health condition, the extensiveness of the condition to be treated, or the age of the subject being treated. In general, anti-ICOS antibodies may be administered in an amount in the range of about 0.05 mg/kg body weight to about 100 mg/kg body weight per dose. In some embodiments, anti-ICOS antibodies may be administered in an amount in the range of about 10 μg/kg body weight to about 100 mg/kg body weight per dose. In some embodiments, anti-ICOS antibodies may be administered in an amount in the range of about 50 μg/kg body weight to about 5 mg/kg body weight per dose. In some embodiments, anti-ICOS antibodies may be administered in an amount in the range of about 100 μg/kg body weight to about 10 mg/kg body weight per dose. In some embodiments, anti-ICOS antibodies may be administered in an amount in the range of about 100 μg/kg body weight to about 20 mg/kg

body weight per dose. In some embodiments, anti-ICOS antibodies may be administered in an amount in the range of about 0.5 mg/kg body weight to about 20 mg/kg body weight per dose. In some embodiments, anti-ICOS antibodies may be administered in an amount in the range of about 0.5 mg/kg body weight to about 10 mg/kg body weight per dose. In some embodiments, anti-ICOS antibodies may be administered in an amount in the range of about 0.05 mg/kg body weight to about 20 mg/kg body weight per dose. In some embodiments, anti-ICOS antibodies may be administered in an amount in the range of about 0.05 mg/kg body weight to about 10 mg/kg body weight per dose. In some embodiments, anti-ICOS antibodies may be administered in an amount in the range of about 5 mg/kg body weight or lower, for example less than 4, less than 3, less than 2, or less than 1 mg/kg of the antibody.

[00383] In some embodiments, anti-ICOS antibodies can be present in an amount in the range of about 50 μg/kg body weight to about 5 mg/kg body weight per dose. For example, in some embodiments, a dose for a 20 kg person can be within a range of about 1 mg to about 100 mg. In some embodiments, the dose can be within a range of 2 mg to 200 mg of the anti-ICOS antibody. In some embodiments, the dose can be within a range of 10 mg to 400 mg of the anti-ICOS antibody.

Routes of Administration

[00384] In some embodiments, anti-ICOS antibodies can be administered *in vivo* by various routes, including, but not limited to, intravenous, intra-arterial, parenteral, intratumoral, intraperitoneal or subcutaneous. The appropriate formulation and route of administration may be selected according to the intended application.

Combination Therapy

[00385] Anti-ICOS antibodies can be administered alone or with other modes of treatment. They can be provided before, substantially contemporaneous with, and/or after other modes of treatment, for example, surgery, chemotherapy, radiation therapy, or the administration of a biologic, such as another therapeutic antibody. In some embodiments, an anti-ICOS antibody is administered in conjunction with another anti-cancer agent.

[00386] In some embodiments, the anti-ICOS antibody is given concurrently with a second therapeutic agent. For example, the two or more therapeutic agents are administered with a time separation of no more than about 60 minutes, such as no more than about any of 30, 15, 10, 5, or 1 minutes. In some embodiments, the anti-ICOS antibody is administered sequentially with a second therapeutic agent. For example, administration of the two or more therapeutic agents are administered with a time separation of more than about 15 minutes, such

as about any of 20, 30, 40, 50, or 60 minutes, 1 day, 2 days, 3 days, 1 week, 2 weeks, or 1 month, or longer.

[00387] In some embodiments, the anti-ICOS antibody is administered with a second therapeutic method for treatment. Thus, the administration of an antibody provided herein can be in combination with another system of treatment.

[00388] In some embodiments, an anti-ICOS antibody provided herein is administered with a PD-1 therapy. Exemplary PD-1 therapies include, but are not limited to, nivolumab (OPDIVO®, BMS-936558, MDX-1106, ONO-4538); pidilizumab, lambrolizumab/pembrolizumab (KEYTRUDA, MK-3475); durvalumab (anti-PD-L1 antibody, MEDI-4736; AstraZeneca/MedImmune); RG-7446; avelumab (anti-PD-L1 antibody; MSB-0010718C; Pfizer); AMP-224; BMS-936559 (anti-PD-L1 antibody); AMP-514; MDX-1105; ANB-011; anti-LAG-3/PD-1; anti-PD-1 antibody (CoStim); anti-PD-1 antibody (Kadmon Pharm.); anti-PD-1 antibody (Immunovo); anti-TIM-3/PD-1 antibody (AnaptysBio); anti-PD-L1 antibody (CoStim/Novartis); RG7446/MPDL3280A (anti-PD-L1 antibody, Genentech/Roche); KD-033, PD-1 antagonist (Agenus); STI-A1010; STI-A1110; TSR-042; and other antibodies that are directed against programmed death-1 (PD-1) or programmed death ligand 1 (PD-L1).

[00389] In some embodiments, a subject is selected for treatment with an anti-ICOS antibody provided herein and a PD-1 therapy if the subject's tumor expresses PD-L1. In some embodiments, a subject is selected for treatment with an anti-ICOS antibody provided herein and a PD-1 therapy if the subject's tumor is PD-L1^{HIGH}. In some embodiments, a subject is selected for treatment with an anti-ICOS antibody provided herein and a PD-1 therapy if the subject's tumor expresses ICOS and PD-L1. In some embodiments, a subject is selected for treatment with an anti-ICOS antibody provided herein and a PD-1 therapy if the subject's tumor is ICOS^{HIGH}/PD-L1^{HIGH}. Determining the level of PD-L1 and/or ICOS may be determined, for example, using IHC. A patient's tumor is considered to express PD-L1, in some embodiments, when 1% or more, or 5% or more, of the tumor cells in a sample show PD-L1 membrane staining by IHC. In some embodiments, more than 50% of the tumor cells in a sample show PD-L1 membrane staining by IHC. In some such embodiments, the subject's tumor is considered to be PD-L1^{HIGH}. A patient's tumor is considered to express ICOS, in some embodiments, when 1% or more of the cells in a tumor sample show ICOS staining by IHC. In some embodiments, a subject is first treated with a PD-1 therapy, and is later treated with an anti-ICOS antibody provided herein, with or without continuing the PD-1 therapy. Thus, methods provided herein include treatment of a subject with an anti-ICOS antibody, wherein the subject has previously been treated with a PD-1 therapy.

[00390] In some embodiments, the anti-ICOS antibody provided herein is administered with an agonist anti-OX40 antibody (such as Medi6469, MedImmune; MOXR0916/RG7888, Roche). In some embodiments, the anti-ICOS antibody provided herein is administered with an anti-CTLA4 antibody (such as ipilimumab, YERVOY®, BMS).

[00391] In some embodiments, an additional therapeutic agent is a chemotherapeutic agent. Exemplary chemotherapeutic agents that may be combined with the anti-ICOS antibodies provided herein include, but are not limited to, capectiabine, cyclophosphamide, dacarbazine, temozolomide, cyclophosphamide, docetaxel, doxorubicin, daunorubicin, cisplatin, carboplatin, epirubicin, eribulin, 5-FU, gemcitabine, irinotecan, ixabepilone, methotrexate, mitoxantrone, oxaliplatin, paclitaxel, nab-paclitaxel, ABRAXANE® (protein-bound paclitaxel), pemetrexed, vinorelbine, and vincristine. In some embodiments, an anti-ICOS antibody provided herein is administered with at least one kinase inhibitor. Nonlimiting exemplary kinase inhibitors include erlotinib, afatinib, gefitinib, crizotinib, dabrafenib, trametinib, vemurafenib, and cobimetanib.

[00392] In some embodiments, the additional therapeutic agent is an IDO inhibitor. Nonlimiting exemplary IDO inhibitors are described, e.g., in US 2016/0060237; and US 2015/0352206. Nonlimiting exemplary IDO inhibitors include Indoximod (New Link Genetics), INCB024360 (Incyte Corp), 1-methyl-D-tryptophan (New Link Genetics), and GDC-0919 (Genentech).

[00393] In some embodiments, an anti-ICOS antibody provided herein is administered in combination with an immune-modifying drug (IMiD). Nonlimiting exemplary IMiDs include thalidomide, lenalidomide, and pomalidomide.

[00394] In some embodiments, an additional therapeutic agent is a cancer vaccine. Cancer vaccines have been investigated as a potential approach for antigen transfer and activation of dendritic cells. In particular, vaccination in combination with immunologic checkpoints or agonists for co-stimulatory pathways have shown evidence of overcoming tolerance and generating increased anti-tumor response. A range of cancer vaccines have been tested that employ different approaches to promoting an immune response against the tumor (*see, e.g.*, Emens LA, Expert Opin Emerg Drugs 13(2): 295–308 (2008)). Approaches have been designed to enhance the response of B cells, T cells, or professional antigen-presenting cells against tumors. Exemplary types of cancer vaccines include, but are not limited to, peptide-based vaccines that employ targeting distinct tumor antigens, which may be delivered as peptides/proteins or as genetically-engineered DNA vectors, viruses, bacteria, or the like; and cell biology approaches, for example, for cancer vaccine development against less well-defined

targets, including, but not limited to, vaccines developed from patient-derived dendritic cells, autologous tumor cells or tumor cell lysates, allogeneic tumor cells, and the like.

[00395] Thus, in certain embodiments, the anti-ICOS antibodies provided herein may be used in combination with a cancer vaccine. Exemplary cancer vaccines include, but are not limited to, dendritic cell vaccines, oncolytic viruses, tumor cell vaccines, etc. In some embodiments, such vaccines augment the anti-tumor response. Examples of cancer vaccines that can be used in combination with anti-ICOS antibodies provided herein include, but are not limited to, MAGE3 vaccine (e.g., for melanoma and bladder cancer), MUC1 vaccine (e.g., for breast cancer), EGFRv3 (such as Rindopepimut, e.g., for brain cancer, including glioblastoma multiforme), or ALVAC-CEA (e.g., for CEA+ cancers).

[00396] Nonlimiting exemplary cancer vaccines also include Sipuleucel-T, which is derived from autologous peripheral-blood mononuclear cells (PBMCs) that include antigen-presenting cells (*see, e.g.*, Kantoff PW et al., *N Engl J Med* 363:411-22 (2010)). In Sipuleucel-T generation, the patient's PBMCs are activated *ex vivo* with PA2024, a recombinant fusion protein of prostatic acid phosphatase (a prostate antigen) and granulocyte–macrophage colony-stimulating factor (an immune-cell activator). Another approach to a candidate cancer vaccine is to generate an immune response against specific peptides mutated in tumor tissue, such as melanoma (*see, e.g.*, Carreno BM et al., Science 348:6236 (2015)). Such mutated peptides may, in some embodiments, be referred to as neoantigens. As a nonlimiting example of the use of neoantigens in tumor vaccines, neoantigens in the tumor predicted to bind the major histocompatibility complex protein HLA-A*02:01 are identified for individual patients with a cancer, such as melanoma. Dendritic cells from the patient are matured *ex vivo*, then incubated with neoantigens. The activated dendritic cells are then administered to the patient. In some embodiments, following administration of the cancer vaccine, robust T-cell immunity against the neoantigen is detectable.

[00397] In some such embodiments, the cancer vaccine is developed using a neoantigen. In some embodiments, the cancer vaccine is a DNA vaccine. In some embodiments, the cancer vaccine is an engineered virus comprising a cancer antigen, such as PROSTVAC (rilimogene galvacirepvec/rilimogene glafolivec). In some embodiments, the cancer vaccine comprises engineered tumor cells, such as GVAX, which is a granulocyte-macrophage colony-stimulating factor (GM-CSF) gene-transfected tumor cell vaccine (see, e.g., Nemunaitis, 2005, *Expert Rev Vaccines*, 4: 259-74).

[00398] In some embodiments, an anti-ICOS antibody described herein is administered before, concurrently, and/or after a cancer vaccine. In some embodiments, cancer vaccines developed using neoantigens are used in combination with the anti-ICOS antibodies described

herein. In some such embodiments, the combination is used to treat a cancer with a high mutational burden, such as melanoma, lung, bladder, or colorectal cancer.

[00399] In some embodiments, an anti-ICOS antibody provided herein is administered in combination with a chimeric antigen receptor T cell therapy (CAR-T therapy).

Diagnostic Uses

[00400] Provided herein are methods of using the anti-ICOS antibodies, polypeptides and polynucleotides for detection, diagnosis and monitoring of a disease, disorder or condition associated with the anti-ICOS antibody epitope expression (either increased or decreased relative to a normal sample, and/or inappropriate expression, such as presence of expression in tissues(s) and/or cell(s) that normally lack the epitope expression). Provided herein are methods of determining whether a patient will respond to anti-ICOS antibody therapy.

[00401] In some embodiments, the method comprises detecting whether the patient has cells that express ICOS using an anti-ICOS antibody. In some embodiments, the method of detection comprises contacting the sample with an antibody, polypeptide, or polynucleotide and determining whether the level of binding differs from that of a reference or comparison sample (such as a control). In some embodiments, the method may be useful to determine whether the antibodies or polypeptides described herein are an appropriate treatment for the subject.

[00402] In some embodiments, the cells or cell/tissue lysate are contacted with an anti-ICOS antibody and the binding between the antibody and the cell is determined. When the test cells are shown binding activity as compared to a reference cell of the same tissue type, it may indicate that the subject would benefit from treatment with an anti-ICOS antibody. In some embodiments, the test cells are from human tissues.

[00403] Various methods known in the art for detecting specific antibody-antigen binding can be used. Exemplary immunoassays which can be conducted include fluorescence polarization immunoassay (FPIA), fluorescence immunoassay (FIA), enzyme immunoassay (EIA), nephelometric inhibition immunoassay (NIA), enzyme linked immunosorbent assay (ELISA), and radioimmunoassay (RIA). An indicator moiety, or label group, can be attached to the subject antibodies and is selected so as to meet the needs of various uses of the method which are often dictated by the availability of assay equipment and compatible immunoassay procedures. Appropriate labels include, without limitation, radionuclides (for example ^{125}I, ^{131}I, ^{35}S, ^{3}H, or ^{32}P), enzymes (for example, alkaline phosphatase, horseradish peroxidase, luciferase, or β-glactosidase), fluorescent moieties or proteins (for example, fluorescein, rhodamine, phycoerythrin, GFP, or BFP), or luminescent moieties (for example, Qdot™

nanoparticles supplied by the Quantum Dot Corporation, Palo Alto, Calif.). General techniques to be used in performing the various immunoassays noted above are known to those of ordinary skill in the art.

[00404] For purposes of diagnosis, the polypeptide including antibodies can be labeled with a detectable moiety including but not limited to radioisotopes, fluorescent labels, and various enzyme-substrate labels know in the art. Methods of conjugating labels to an antibody are known in the art.

[00405] In some embodiments, the anti-ICOS antibodies need not be labeled, and the presence thereof can be detected using a second labeled antibody which binds to the first anti-ICOS antibody.

[00406] In some embodiments, the anti-ICOS antibody can be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Zola, Monoclonal Antibodies: A Manual of Techniques, pp. 147-158 (CRC Press, Inc. 1987).

[00407] The anti-ICOS antibodies and polypeptides can also be used for in vivo diagnostic assays, such as in vivo imaging. Generally, the antibody or the polypeptide is labeled with a radionuclide (such as ^{111}In, ^{99}Tc, ^{14}C, ^{131}I, ^{125}I, ^{3}H, or any other radionuclide label, including those outlined herein) so that the cells or tissue of interest can be localized using immunoscintiography.

[00408] The antibody may also be used as staining reagent in pathology using techniques well known in the art.

[00409] In some embodiments, a first antibody is used for a diagnostic and a second antibody is used as a therapeutic. In some embodiments, the first and second antibodies are different. In some embodiments, the first antibody is from a non-human, while the therapeutic is from a human. In some embodiments, the first and second antibodies can both bind to the antigen at the same time, by binding to separate epitopes.

Kits/Articles of Manufacture

[00410] Provided herein are also kits, medicines, compositions, and unit dosage forms for use in any of the methods described herein.

[00411] Kits can include one or more containers comprising an anti-ICOS antibody (or unit dosage forms and/or articles of manufacture). In some embodiments, a unit dosage is provided wherein the unit dosage contains a predetermined amount of a composition comprising an anti-ICOS antibody, with or without one or more additional agents. In some embodiments, such a unit dosage is supplied in single-use prefilled syringe for injection. In some embodiments, the

composition contained in the unit dosage can comprise saline, sucrose, or the like; a buffer, such as phosphate, or the like; and/or be formulated within a stable and effective pH range. In some embodiments, the composition can be provided as a lyophilized powder that may be reconstituted upon addition of an appropriate liquid, for example, sterile water. In some embodiments, the composition comprises one or more substances that inhibit protein aggregation, including, but not limited to, sucrose and arginine. In some embodiments, a composition comprises heparin and/or a proteoglycan.

[00412] In some embodiments, the amount of the anti-ICOS antibody used in the unit dose can be any of the amounts provided herein for the various methods and/or compositions described.

[00413] In some embodiments, kits further comprise instructions for use in the treatment of cancer in accordance with any of the methods described herein. The kit may further comprise a description of selection an individual suitable or treatment. Instructions supplied in the kits are typically written instructions on a label or package insert (for example, a paper sheet included in the kit), but machine-readable instructions (for example, instructions carried on a magnetic or optical storage disk) are also acceptable. In some embodiments, the kit further comprises another therapeutic agent.

[00414] The kits are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (for example, sealed Mylar or plastic bags), and the like. Kits may optionally provide additional components such as buffers and interpretative information. The present application thus also provides articles of manufacture, which include vials (such as sealed vials), bottles, jars, flexible packaging, and the like.

EXAMPLES

[00415] The examples discussed below are intended to be purely exemplary of the invention and should not be considered to limit the invention in any way. The examples are not intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (for example, amounts, temperature, *etc.*) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1: Bioinformatics Analysis of ICOS mRNA Expression in Human Tumors.

[00416] Utilizing RNA sequencing data collected as part of TCGA, ICOS expression in ~7500 tumors was compared across 24 different indications. High ICOS mRNA levels were

found in subsets of head & neck squamous cell cancer (HNSCC), non-small cell lung cancer (NSCLC) and triple negative breast cancer (TNBC). *See* Figure 1A.

[00417] The association between T cell infiltration and levels of ICOS expression was investigated. Expression of a set of 12 chemokine genes has been associated with high levels of T cell infiltration and formation of lymph node-like structures (Messina *et al.*, 2012, *Sci Reports*. 2:765-771). The chemokine signature score was computed for each sample based on the average expression of these 12 chemokine genes. This signature score was calculated across all of the head and neck squamous cell cancer samples (HNSCC). Levels of the chemokine signature or a Treg cell marker (FoxP3) with ICOS levels in HNSCC tumors were correlated. *See* Figure 2. There was a strong correlation between chemokine signature and ICOS levels (R=0.83; Spearman correlation) or ICOS and FoxP3, a Treg cell marker (R=0.88; Spearman correlation). These data demonstrate that ICOS expression is closely associated with T cell infiltration and Treg cells. Similar data was observed in NSCLC and TNBC.

[00418] In the HNSCC tumors, ICOS expression was significantly correlated (R=0.93 and 0.78 respectively) with expression levels of other check point molecules such as CTLA-4 and PD-1. *See* Figure 3. The correlation of ICOS with PD-L1 was weak (R=0.62). Similar data was observed in other indications such as NSCLC. These data suggest that ICOS may be expressed on the same T cells that are expressing other checkpoint molecules such as CTLA-4 and PD-1. The weaker correlation with PD-L1 and ICOS suggest that there could be a subset of ICOS high patients that could be PD-L1 low or negative. These data are supportive of ICOS as a single agent in PD-L1 negative patients and also a combination strategy with anti-PD-1 or anti-CTLA-4 therapies.

Example 2: IHC Analysis of Human Tumors

[00419] ICOS protein expression levels were determined using an immunohistochemistry (IHC) assay. This assay, using a rabbit anti-ICOS monoclonal antibody (SP98, Spring Biosciences, Pleasanton, CA), was validated for assay specificity, assay precision (intra-run and inter-run, and lot-to-lot reproducibility), and sensitivity. The validation studies were performed using formalin-fixed, paraffin embedded (FFPE) tissue sections and control cell lines (CHO engineered to express human ICOS (positive control) or non-ICOS expressing CHO vector control cell line (negative control)). The assay was performed on a Leica-Bond Rx automated staining platform and specific staining of ICOS was detected in the positive control CHO cells and in T cell subsets in normal human tonsil.

[00420] Slides were scored by a trained pathologist using the following criteria for chromogenic staining:

Frequency of ICOS-positive cells	Score
< 1% cells are ICOS positive	0
> 1% but < 5% cells are ICOS positive	1+
> 5% but < 15% cells are ICOS positive	2+
> 15% cells are ICOS positive	3+

[00421] Tissue microarrays from 11 different tumor types were stained and classified using this scoring system. *See* Figure 1B. The IHC data confirmed the mRNA based data in that HNSCC, NSCLC and TNBC contained the greatest percentage of high ICOS+ immune cell infiltration (i.e., 3+). *See* Figure 1B. In addition to these tumors, subsets of patients from melanoma, colorectal cancer and gastric adenocarcinoma had moderate levels of ICOS-positive cell infiltration. *See* Figure 1B.

[00422] To evaluate the prevalence and nature of T cells that express ICOS, a multiplex immuno-fluorescence IHC assay for detecting ICOS, FOXP3 and CD8 was developed. A DNA marker (DAPI) to count the total number of nuclei in the human tumor sections was utilized.

[00423] ICOS expression was determined using immunohistochemistry analysis with the rabbit monoclonal antibody clone that recognizes human ICOS (Spring Biosciences Inc. Pleasanton, CA). The specificity and sensitivity of the ICOS IHC assay was confirmed using human tonsil and cell lines constitutively over-expressing ICOS. The staining intensity was scored by a trained pathologist using the following criteria. All positive staining was score based on membrane expression in at least two-thirds of the cells.

[00424] A representative image for the scoring schema is shown in Figure 4. The scoring was performed based on the following criteria for immunofluorescence:

0 (negative) = No or less than 0.1% of cells have membrane staining
1+ (mild) = 0.1 to 5% of the cells are positive
2+ (moderate) = 5 to 10 % of the cells are positive
3+ (Strong) = > 10 to 50 % in cells are positive

[00425] Prevalence of ICOS expression in various subsets of NSCLC or adjacent normal lung samples are summarized in Table 2. Strong ICOS expression was not observed in adjacent normal lung tissue in cancer patients. Strong ICOS expression was observed in all major sub-types of lung cancer. About 29-31% of the most common NSCLC sub-types (adenocarcinoma or squamous cell carcinoma) had strong ICOS staining.

Table 2. Distribution of ICOS staining in various subsets of NSCLC samples based on pathological scoring.

Tumor sub-type	N	Strong (3+)	Moderate (2+)	Weak (1+)	Negative (0)
SCLC	2	0 (0%)	0 (0%)	2 (100%)	0 (0%)
Squamous	49	14 (29%)	14 (29%)	17 (35%)	4 (8%)
Adenocarcinoma	16	5 (31%)	2 (13%)	8 (50%)	1 (6%)
Adenosquamous	9	3 (11%)	2 (11%)	3 (11%)	1 (67%)
Brochiolalveolar carcinoma	9	2 (22%)	2 (22%)	5 (56%)	0 (0%)
Undifferentiated	5	1 (20%)	0 (0%)	3 (60%)	1(20%)
Normal	3		1 (33%)	2 (67%)	0 (0%)

[00426] An automated methodology to measure ICOS positive cells was also employed, using image analysis software (Strataquest from Tissuegnostics Inc., Tarzana, CA). The density of ICOS positive cells in a fixed area of tumor tissue was determined by determining the number of ICOS positive cells in the viable region of human tumor tissue as defined by the DAPI staining region. ICOS cell density was determined from a separate set of approximately 500 individual patients across 4 major tumor types [NSCLC (N=100); HNSCC (N=102); breast cancer, all major subtypes (N=94); triple negative subtype of breast cancer, TNBC (N=95); ovarian cancer (N=94)]. A summary of the results of the analysis are shown in Figure 5. Consistent with the ICOS mRNA analysis, the HNSCC and NSCLC tumors had a significantly higher density of ICOS positive tumors as compared to ovarian or breast cancer. Although ICOS expression is low in breast cancer, high levels of ICOS expression was observed in the TNBC subtype, which constitutes about 10% of breast cancer. *See* Figure 5. This TNBC subtype of breast cancer is the most aggressive subtype with limited treatment options and highest unmet medical need.

[00427] The patient to patient variability in ICOS expression in two different cohorts of a high ICOS expressing tumor types (NSCLC) is shown in Figures 6A and 6B. A range of ICOS cell density was observed in NSCLC from 98 lung cancer samples (Figure 6A) available from a commercial vendor. Similar diversity in ICOS expression was observed using an independent clinical cohort (Figure 6B) of NSCLC (N=204).

[00428] A multiplex IHC with ICOS, FoxP3 (a Treg cell marker) and CD8 from the human tumor samples described above was analyzed and quantified using image analysis software

(Strataquest from Tissuegnostics Inc., Tarzana, CA). A representative image of the multiplex ICOS staining with these T cell markers is shown in Figure 7A. The ICOS positive cells that are also FOXP3 positive are called ICOS+ CD4 Treg cells. The ICOS positive and CD8 positive cells are called ICOS+ CD8 cells. The ICOS positive but negative for CD8 and FoxP3 are called ICOS+ CD4 Teffs. The density of the different sub-sets of ICOS positive T cells were quantified using the image analysis software as described above. In lung (N=100) and TNBC (n=95) samples there was a high number of both CD4 effectors and CD4 Treg cell that were ICOS positive (Figure 7B). In contrast, not many ICOS positive cells were observed in ovarian cancer (n=94). HNSCC tumors (N=102) had a large population of ICOS positive CD4 Treg cells. There was only a small population of ICOS positive CD8 T cells observed in all the tumor types examined. These data suggest that ICOS is predominantly expressed in the CD4 compartment as compared to the CD8 T cells, and that variation in the relative proportions of Treg vs Teff can be seen across indications.

[00429] In order to understand if ICOS expression is directly associated with PD-L1 status, the correlation between PD-L1 levels and ICOS expression was evaluated. Bio-informatics analysis suggested that PD-L1 expression and ICOS levels were weakly correlated (R=0.62). PD-L1, ICOS and PD-1 levels were evaluated by a multiplex IHC. A representative image of the multiplex IHC from a PD-L1 high and low lung tumor is shown. *See* Figure 8. PD-L1 and ICOS in 154 adenocarcinoma sub-type of NSCLC tumors were evaluated. The tumors were subdivided into PD-L1 high or low tumors based on 5% of the cells that are PD-L1 positive. Results indicate that PD-L1 positive tumors had a higher density of ICOS expression.

Example 3: Flow Cytometry Analysis

[00430] To confirm the ICOS expression data obtained by IHC and to compare relative intensities of ICOS expressed in different T cell populations, ICOS expression in tumor infiltrating lymphocytes was evaluated using multi-color flow cytometry. Samples from four HNSCC patients, three lung cancer, and four ovarian cancer patients were analyzed. Consistent with the IHC data, ICOS expression was predominantly observed on CD4 T cells. *See* Figure 9 (HNSCC). The frequency of ICOS positive cells in the CD8 population is very low in a majority of these tumors. We also observed that the majority of CD4 effector cells co-express ICOS and PD-1. These data support developing an ICOS therapeutic in the clinic alone or in combination with anti-PD-1 therapies. The mean fluorescence intensity (MFI) of ICOS staining provides a measure of ICOS expression in the different T-cell population. The MFI of ICOS positive cells in the Treg cells was 2-3 fold higher than the CD4 effectors. *See* Figure 9C. It should be noted that there are small populations of CD4 effectors that have high ICOS

MFI. Confirmation of the difference in ICOS receptor densities in Teffs versus Tregs, coupled with data from ongoing ADCC assays to evaluate differential Teff and Treg sensitivity to depletion, would support development of agonistic antibody with an active Fc that could potentially deplete the Treg cells.

[00431] Translational studies show high levels of tumor infiltrating ICOS positive T cells in a subset of human tumors (such as HNSCC, NSCLC etc.). ICOS expression is correlated with expression of other check-point regulators such as CTLA-4 and PD-1. Analysis of the T cell subsets showed that ICOS expression is predominantly restricted to the CD4 T cell compartment. ICOS is expressed in both FoxP3 positive Treg cells as well as CD4 Teff cells. Studies show, consistent with the literature, that the level of ICOS expression is higher in the Treg cells as compared to CD4 Teff cells.

Example 4: Antibody Generation

Reagents

[00432] ICOS proteins representing human, mouse, rat, and cynomolgus species were produced as homodimeric, Fc fusions (IgG1 backbone), and the human and mouse ICOS-hFc were used as antigens for rodent immunizations. Human ICOS-hFc included human ICOS amino acids 1 to 141 (21 to 141 in mature construct); mouse ICOS-hFc included mouse ICOS amino acids 1 to 142 (21 to 142 in mature construct).

[00433] ICOS-Fc was generated as both bivalent and monovalent Fc fusion molecules to assess avid and monovalent binding of antibodies to ICOS, respectively.

[00434] For screening purposes, CHO stable cell lines over-expressing full length human or mouse ICOS (ICOS+ CHO cells, or "CHO-ICOS cells") were generated as were constructs encoding full length cynomolgus monkey or rat ICOS to enable screening following transient transfection.

Rodent Antibody Campaign

[00435] Rodent campaigns were performed at Precision Antibody. Mice (10), rats (6), Syrian hamsters (6), and Armenian hamsters (6) were immunized with hICOS-hFc or mICOS-hFc. Hybridomas were generated, and supernatants screened by ELISA for binding to hICOS and mICOS, as well as multiplex screening by FACS for binding to CHO-hICOS and CHO-mICOS cells. Hybridomas were additionally assessed for the ability to block ICOSL binding to the CHO-ICOS cells. Clones that scored positive for binding to mouse and human ICOS were selected for protein purification. Purified antibodies were subsequently re-screened in the binding and blocking assays, and antibodies that scored positive proceeded to *in vitro*

assessment as outlined below. All antibodies selected for further investigation from the immunization approach originated from Armenian hamster fusions.

Biochemical Characterization of Antibodies

[00436] Affinity measurements were conducted using Bio-Layer Interferometry (BLI) technology (ForteBio Octet® RED96). The monovalent affinities were generated with IgG versions of the antibodies with monovalent, heterodimeric forms of the ICOS receptor. The avid affinities were generated using full-length IgGs against homodimeric forms of the ICOS receptor. The monomeric and bivalent hICOS affinities of the selected hamster antibodies are shown in Table 3.

Table 3. Hamster-derived antibody affinity.

	Monomeric hICOS Affinity			Bivalent hICOS Affinity		
mAb	**K_D**	**K_{on} (1/Ms)**	**K_{dis} (1/s)**	**K_D (M)**	**K_{on} (1/Ms)**	**K_{dis} (1/s)**
7F12	1.32E-08	1.33E+05	1.75E-03	3.42E-11	6.74E+05	2.30E-05
35A9	2.45E-09	1.78E+05	4.38E-04	<1.0E-12	6.25E+05	<1.0E-07
36E10	1.59E-09	1.43E-05	2.28E-04	<1.0E-12	6.26E+05	<1.0E-07
37A10	3.18E-09	1.51E+05	4.79E-04	3.42E-11	6.74E+05	2.30E-05
16G10	4.37E-09	1.63E+05	7.12E-04	<1.0E-12	1.01E+06	<1.0E-07

[00437] Binding affinity of the antibodies for cynomolgus monkey, mouse, and rat ICOS was also determined, and the antibodies were found to bind all species with comparable affinity. Cross-reactivity measurements were conducted using BLI technology with antibody panels being screened for binding to human, mouse, and cynomolgus ICOS-Fc fusions (homodimeric, bivalent forms). Table 4 shows representative binding data for several hamster antibodies for human and cynomolgus monkey ICOS.

Table 4. Bivalent binding affinities of several hamster antibodies to human and cyno ICOS-Fc.

	hICOS-Fc			cynoICOS-Fc		
ligand/mAb	**K_D (M)**	**K_{on} (1/Ms)**	**K_{dis} (1/s)**	**K_D (M)**	**K_{on} (1/Ms)**	**K_{dis} (1/s)**
hICOSL-mG2a	3.62E-10	9.41E+05	3.41E-04			
7F12	3.42E-11	6.74E+05	2.30E-05	<1.0E-12	5.85E+05	<1.0E-07
35A9	<1.0E-12	1.01E+06	<1.0E-07	<1.0E-12	7.40E+05	<1.0E-07
36E10	<1.0E-12	6.17E+05	<1.0E-07	<1.0E-12	6.97E+05	<1.0E-07
37A10	<1.0E-12	6.25E+05	<1.0E-07	<1.0E-12	7.12E+05	<1.0E-07
16G10	<1.0E-12	6.26E+05	<1.0E-07	<1.0E-12	6.47E+05	<1.0E-07

[00438] To assess specificity, binding to human ICOS+ CHO cells and mouse ICOS+ CHO cells was individually analyzed by flow cytometry. As a control to screen out pan-reactive antibodies, staining on CHO cells lacking ICOS receptor expression was also examined. All of the selected antibodies bound to human and mouse ICOS+ CHO cells, and not to CHO cells lacking ICOS expression.

[00439] To further assess the specificity of the anti-ICOS antibodies, the antibodies were screened for binding to additional members of the CD28 protein family (CD28, BTLA, PD-1 and CTLA-4). No cross-reactivity was observed for the selected antibodies to human or mouse CD28, BTLA, PD-1 or CTLA4. Specifically, binding to Fc fusions (of CD28, BTLA, PD-1 and CTLA-4) in dimeric forms was evaluated, and no binding was observed. For a subset of the CD28 family members, mouse or human protein was over-expressed on the surface of HEK293 and CHOK1 cells. No binding of antibody above background relative to untransduced parental cells was observed.

[00440] Antibodies were also found to not bind to abundant serum proteins nor to platelets or red blood cells.

[00441] Epitope binning was conducted using BLI. Antibodies were also binned against an ICOSL-Fc fusion (homodimeric, bivalent form). All of the selected antibodies were found to be in the same epitope bin, and all blocked binding of ICOS to ICOS ligand.

Humanization

[00442] Selected antibodies were humanized by performing homology studies between the antibody variable framework regions of human and hamster origin. A panel of primary designs for analysis was provided and antibodies were then produced for comparison with wild-type antibody (hamster or chimera form). Once humanized panels were produced, leads were characterized and ranked based on affinity and in vitro activity. Additional humanization designs were executed to reduce sequence liabilities and low-scoring immunogenic sites resulting in minor sequence variations. The sequence liabilities considered included the presence of free cysteines and potential sites for chemical degradation (asparagine deamidation, aspartate isomerization, methionine/tryptophan, and non-enzymatic lysine glycation).

[00443] The affinity of a humanized antibody having the variable regions of 37A10S713 for monomeric forms of ICOS from human, cynomolgus monkey and rat was measured by Bio-Layer Interferometry (BLI) technology (ForteBio Octet® RED96) and K_D's are shown in Table 5. The K_D's were deemed comparable across the species as they were within 2 to 5-fold.

Functionality of binding was confirmed by assessing potency of inducing proliferation of primary CD4+ T cells isolated from each species.

Table 5. Monovalent binding affinity of 37A10S713 to human, cynomolgus monkey, and rat ICOS.

	Human	**Cynomolgus Monkey**	**Rat**
Binding Affinity (K_D nM)[A]	1.50±0.39	0.66±0.16	7.20±2.55
Potency in primary CD4+ T cell proliferation (EC50 nM)[B]	4.27-49.75	8.25 – 13.14	10.7-30.0

[A] affinities shown as mean ± SEM from 6 experiments; [B] range of 4 donors is shown

Example 5: In Vitro Functional Characterization of Anti-ICOS Antibodies

[00444] A number of cell-based *in vitro* assays were used to assess the activity of the anti-ICOS mAbs. The main purpose was to screen for antibodies with agonistic/co-stimulatory properties. Since the cell system (primary cell versus transfected cell line) and the method of antibody presentation (soluble versus plate-bound or cross-linked) can influence agonistic activity, a number of different assay formats were employed. In addition, an assay to detect undesired super-agonistic activity (*see* Suntharalingam *et al.*, 2006, *N. Eng. J. Med.*, 355: 1018-28) was also used.

[00445] The assays designed to look for agonistic/co-stimulatory activity of the anti-ICOS mAbs were performed on the cell types outlined below, with the first signal to the T cells (signal 1) provided using either sub-optimal concentrations of anti-CD3 or PMA or in the PBMC assay with stimulation with super-antigen (SEB):

1. Primary human CD4+ T cell assay
    a. Plate-bound/cross-linked antibody format co-stimulated with anti-CD3
    b. Soluble antibody format co-stimulated with PMA
2. Jurkat assay (reporter cell line with transfected human or mouse ICOS constructs)
    a. Plate-bound/cross-linked format with either anti-CD3 or PMA
    b. Soluble antibody format co-stimulated with PMA
3. Human PBMC assay
    a. Soluble antibody format with super-antigen (SEB)

[00446] Panels of hamster anti-ICOS mAbs were screened in the above assays to identify antibodies with agonistic properties. An example of the agonistic activity observed in an assay using primary human CD4+ T cells co-stimulated with suboptimal anti-CD3 with addition of plate-bound anti-ICOS antibody is shown in Figure 10A for a selection of hamster anti-ICOS

antibodies. In this assay, human CD4+ T cell isolated from PBMCs are activated with suboptimal plate-bound anti-CD3 in the presence of plate-bound hamster anti-ICOS antibodies (7F12, 37A10 and 16G10) at four concentrations (μg/ml). Plate-bound hICOSL and soluble anti-CD28 in the presence of anti-CD3 are used as positive controls. % divided cells is graphed. Multiple anti-ICOS antibodies exhibit activity in this assay. Figure 10B shows the results of the assay using soluble antibody and costimulation with sub-optimal PMA. Human CD4+ T cells were isolated from PBMCs by negative selection and labeled with CFSE. Cells were activated in 96-well plates with suboptimal PMA (0.25 ng/ml) alone or in the presence of different Fc versions (hamster, mG1, mG2a, mG1Agly or hG1) of anti-ICOS antibody 37A10 at indicated concentrations (μg/ml). Soluble anti-CD28 antibody was used as a control. Proliferation was analyzed on day 3 by flow cytometry. The mouse IgG1 and mouse IgG1-agly versions showed activity in the assay, along with the parental fully hamster antibody. At least antibody 37A10 showed agonist activity in both soluble and plate-bound formats. *See* Figures 10A and 10B.

[00447] Figure 10C shows the agonist activity of results of 37A10S713 antibody with a human IgG1 in the primary human CD4+ T cell assay. CD4+ T cells were isolated from PBMC from 4 healthy donors, labeled with CFSE dye and then incubated in plates coated with a sub-optimal concentration of anti-CD3 and various concentrations of either 37A10S713-hIgG1 or a negative control human IgG1 antibody (anti-respiratory syncytial virus (RSV)). After 3 days, the percentage of divided cells was determined using flow cytometry. The EC50 values ranged from 4.27- 49.75nM for the 4 donors tested. Proliferation is plotted as the percentage of divided cells (measured by CFSE dilution using flow cytometry) and are means of duplicates. Figure 10C shows data from a representative donor.

[00448] Another example of an assay in which the hamster antibodies demonstrated agonistic activity is the Jurkat reporter assay. A Jurkat reporter assay was developed in-house by transducing a hICOS-hCD28 chimeric expression construct into a Jurkat NFkB reporter cell line. Jurkat-hICOS-hCD28 reporter cells were activated for 5 hours with PMA and soluble hamster anti-ICOS 37A10 antibody with different Fc ends at 11 concentrations (μg/ml). Soluble anti-CD28 and hICOSL-Fc are used as controls. % GFP+ cells is graphed. Representative data from the Jurkat reporter assay using the hamster anti-ICOS antibodies is shown in Figure 11. Figure 11A shows the results for different Fc versions of anti-ICOS antibody 37A10 (hG1, mG1, mG2a, mG1Agly) at the indicated concentrations (μg/ml). All Fc versions of the anti-ICOS antibody, including the mG1-agly version (i.e. minimal Fc effector function) shows activity in this assay. Figure 11B shows the results for humanized antibodies

37A10S713, 37A10S715, 37A10S716, and 37A10S718 at the indicated concentrations (μg/ml). All four humanized antibodies tested showed agonist activity in the assay.

[00449] Another assay format showing agonistic activity is the PBMC assay with the superantigen staphylococcal enterotoxin B (SEB) using cytokine production (e.g., IFNg) as a readout. This assay typically has a small window of a 1.5 – 3 fold effect on cytokine release. Consistent with published anti-PD-1 data, cytokine induction with the anti-ICOS antibodies is ~2 fold but is reproducibly observed in this assay across multiple donors. *See, e.g.*, Korman et al., 2014, *Cancer Res.*, 2: 846-856. A representative assay is shown in Figure 12. Frozen PBMCs from healthy donors were stimulated with SEB and soluble anti-ICOS 37A10 antibody (with mG1, mG1-agly or hG1 Fc) at indicated concentrations (μg/ml) for 3 days. Supernatants were collected and IFNg levels were measured by cytokine bead array using flow cytometry. Anti-CD28 and mouse IgG1 isotype antibodies were used as controls. IFNg is induced by PBMCs following stimulation with SEB in the presence of soluble anti-ICOS antibodies. In this assay format, an mG1-agly version of 37A10 showed reduced activity.

[00450] To screen out any potential super-agonists, an assay in which primary human CD4+ T cells were incubated with soluble or plate-bound anti-ICOS antibodies in the absence of a signal 1 was employed using a known anti-CD28 super-agonist antibody as the positive control. Human CD4 T cells activated in the absence of anti-CD3 will proliferate only when treated with an anti-CD28 superagonist antibody (clone CD28.2/5D10) in soluble form, but not when treated with ICOSL-Fc or anti-ICOS antibody 37A10 (hamster and hG1 Fc versions), or a non-superagonist anti-CD28 (CD28.2) antibody. None of the anti-ICOS mAbs tested exhibited super-agonistic activity in this assay. Representative data is depicted in Figure 13.

[00451] It is well established that ICOS can signal through the AKT signaling pathway (reviewed in Simpson *et al.*, 2010, *Curr. Opin. Immunol.*, 22: 326-332). The ability of the anti-ICOS antibody to induce signaling through AKT was evaluated as an additional means to demonstrate agonistic activity of the antibody.

[00452] CD4 T cells isolated from human PBMCs were stimulated for 24 hours with anti-CD3/anti-CD28, and then rested for 24 hours in culture media. Cells were then incubated with anti-ICOS 37A10-mG2a, hICOSL-mG2a Fc or PBS for 2, 5, 15 or 30 minutes with or without anti-mouse IgG cross-linking antibody. Following incubation, cells were fixed, permeabilized and then stained with an anti-phospho-AKT antibody. The percentage of pAKT-positive cells was analyzed by flow cytometry.

[00453] As shown in Figures 14A-B, pAKT was induced on CD4 T cells following treatment with 37A10-mG2a with similar kinetics as compared to treatment with hICOSL-mG2a.

Induction of pAKT signaling was only observed in the presence of secondary cross-linking agent.

Example 6: *In Vivo* Functional Characterization of Anti-ICOS Antibodies

[00454] Antibodies selected from the screening assays described above were evaluated *in vivo* using syngeneic tumor models.

[00455] The Sa1N fibrosarcoma model (Ostrand-Rosenberg, 2001, *Curr. Protoc. Immunol.*, Chapter 20) may be used for evaluating anti-ICOS antibodies *in vivo*. Immune profiling of the Sa1/N mouse model shows that it is highly infiltrated with CD4 T cells, and that the CD4 T cells express high levels of ICOS. This immune profile is similar to immune profiles of the NSCLC patient samples in which we observed high levels of CD4 infiltration, with ICOS expression largely restricted to the CD4 compartment.

[00456] A second model used for evaluating efficacy of anti-ICOS antibodies is the CT26 colon carcinoma model (Wang *et al.*, 1995, *J. Immunol.*, 9: 4685-4692). Immune profiling of the CT26 mouse model showed high levels of CD8 infiltration. ICOS expression was observed in the CD8 T cell subset in this model. A small proportion of human NSCLC samples similarly show ICOS expression in the CD8 T cells.

Antibody formats for in vivo evaluation

[00457] Human IgG1 (hIgG1) can bind across multiple Fc receptors, including strong binding to the activating Fc receptors which are capable of receptor cross-linking and mediating ADCC and CDC. Given the ability to bind the activating Fc receptors, hIgG1 is typically capable of depleting cells that express a high level of target. The closest mouse equivalent to hIgG1 is mouse IgG2a (mIgG2a). Thus, as an example, *in vivo* experiments to evaluate an ICOS agonist antibody with depleting capacity would utilize mIgG2a to mimic properties of hIgG1.

[00458] Human IgG4 (hIgG4) is utilized in therapeutic situations where depletion is not desired, although hIgG4 is capable of some level of depletion. It is roughly, although not perfectly, aligned to mouse IgG1 (mIgG1), which almost exclusively binds the inhibitory FcγRII receptors, and is thus capable of cross-linking but not particularly competent at depletion.

[00459] Regarding the anti-ICOS antibodies, the hamster antibodies were initially evaluated *in vivo* as fully hamster Abs. The hamster Abs have a hamster IgG1, which has FcR binding characteristics similar to mIgG1. Hamster antibodies of interest were cloned as chimeras with mouse Fc regions, either as mIgG2a or mIgG1.

Sa1N Fibrosarcoma Model

[00460] The primary *in vivo* model used for screening anti-ICOS antibody candidates for efficacy is the Sa1N fibrosarcoma model. Thus, antibodies selected from the screening assays described above were assessed in the Sa1N model. In initial studies, several hamster antibodies (clones 7F12, 36E10, 37A10, 16G10 and 35A9) demonstrated robust anti-tumor activity when administered as single agents at an 8 mg/kg dose in the Sa1N model. *See* Figure 15. Sa1N fibrosarcoma cells ($1x10^6$) were injected s.c. to right flank of naïve A/J mice (6-8 weeks old, female). When tumor volumes reached 50-100 mm^3 on day 7, mice were randomized. Mice received dose of hamster anti-ICOS (7F12, 36E10, 37A10, 16G10 and 35A9) or hamster IgG isotype antibody i.p. on days 7, 10, 14 and 17. Tumor growth was monitored twice weekly. N=10.

[00461] A potentially beneficial feature of a cancer immunotherapeutic is the ability to mount a sustained and durable immune response against the tumor. The ability of a mouse previously treated with an anti-ICOS antibody to subsequently reject a tumor was determined. Mice were treated at 8 mg/kg antibody on day 7, 10, 14, and 17. Subsequently, mice that were tumor-free were then re-implanted with a tumor on day 60. All of the mice pre-treated with anti-ICOS antibody 7F12 (n=7) rejected the newly implanted tumor, in contrast to naïve mice (n=10) in which tumors grew out in all of the mice. *See* Figure 16.

[00462] The hamster antibodies were cloned as chimeric antibodies with mouse Fc regions (mG1 or mG2a) to enable assessment of the contribution of Fc effector function to *in vivo* activity. Mice received a total of 4 doses biweekly of 4 mg/kg antibody starting on day 11. Anti-CTLA-4 antibody was included as a positive control. The initial screening experiment was performed at a dose of 4 mg/kg dose, and efficacy was observed with both the mG1 and mG2a formats. Representative data for one of the hamster antibodies, 37A10, is shown in Figure 17.

Colon CT26 Syngeneic Tumor Model

[00463] The colon CT26 syngeneic tumor model was used both to assess single agent activity, as well as combination therapy with anti-PD-1 antibody.

[00464] In the CT26 model, several of the anti-ICOS hamster antibodies (e.g., 7F12, 35A9, 36E10, 37A10) exhibited single agent activity. *See* Figure 18. The CT26 model was also used to assess potential combination activity with anti-PD-1. When the anti-ICOS antibodies were combined with an anti-PD-1 antibody, anti-tumor efficacy was markedly enhanced. CT26 tumor-bearing mice were treated biweekly (4 doses starting on day 3) with hamster anti-ICOS antibodies (8 mg/kg) alone or in combination with anti-PD-1 antibody (8 mg/kg). Notably,

combination of anti-PD-1 with anti-ICOS antibody 37A10 resulted in 9/10 mice tumor free. *See* Figure 18.

Example 7: Selective Treg Depletion Contributes to Anti-ICOS Antibody Efficacy

[00465] *Ex vivo* studies were performed to characterize the status of immune cell infiltrates following dosing with anti-ICOS antibodies. Studies in the Sa1N model showed a decrease in the Treg population following treatment with 7F12. Mice received two doses of anti-ICOS hamster 7F12, 7F12-mG1 or 7F12-mG2a at 8 mg/kg on days 7 and 10. Tumors and spleens were harvested and analyzed on day 12. There was a marked reduction in Tregs but not in Teff cells, but little impact on T cell populations in lymphoid organs such as the spleen or lymph node. *See* Figure 19.

[00466] Similar results have also been observed with other anti-ICOS antibodies, such as 37A10. *See* Figure 20. Mice received two doses of anti-ICOS antibodies at 8 mg/kg on days 7 and 10. Tumors were harvested and analyzed on day 12. A similar reduction in the Treg population has also been observed in the CT26 model following dosing with the anti-ICOS antibody.

[00467] Taken together, the TIL (tumor-infiltrating lymphocytes) studies support the hypothesis that Treg cells are selectively depleted by the anti-ICOS antibodies described herein, without corresponding depletion of Teff cell populations, and specifically in tumors but not in other organs or in the periphery.

[00468] To formally demonstrate the contribution of the immune system to efficacy of the anti-ICOS antibody, cell depletion experiments were performed in the context of the tumor model, Sa1N. Specifically, mice were depleted of CD8 T cells, CD4 T cells, or a combination of CD4 + CD8 T cells. At days 6 and 13 post-tumor implantation, mice were treated with anti-CD8, anti-CD4, anti-CD4 + anti-CD8 or control Ig antibody (n=10 per group). Anti-ICOS antibody 7F12 was administered at 8 mg/kg antibody on day 7, 10, 14, and 17. Tumor growth was monitored twice weekly.

[00469] A marked reduction in anti-tumor efficacy of 7F12 was observed when mice were depleted of CD4, CD8, or CD4 + CD8 T cells. *See* Figure 21.

Example 8: Selective Treg Reduction by humanized anti-ICOS antibody

[00470] Human PBMCs were incubated at 37°C with 100ng/ml recombinant human IL-2 for 48 hours in a humidified incubator with 5% CO_2. After 48 hours, antibody 37A10S713 was added at the indicated concentrations. Antibody was prepared as 10-fold serial dilutions in culture media containing IL-2. The antibody/cell mixture was allowed to incubate an

additional 72 hours. Following incubation, cells were stained for CD3, CD4, CD8, CD25, and FoxP3 per standard methods and analyzed by flow cytometry. Quantification of Treg (CD4+ CD25+ FoxP3+) and Teff (CD4+ CD25- FoxP3-) cells was carried out for each concentration and treatment. Data were normalized to percent of each subset in a trastuzumab-treated group for each concentration.

[00471] The results of that experiment are shown in Figure 22A. Antibody 37A10S713 caused a dose-dependent reduction of Treg cells. As shown in Figure 22B, Teff and Treg cells expressed similar levels of ICOS after five days of IL-2 treatment.

Example 9: Tumor re-challenge following treatment with anti-ICOS antibody

[00472] Six to eight week old female A/J mice were inoculated subcutaneously on the right flank with $1x10^6$ Sa1/N cells in 100µl PBS using tuberculin syringes with 27-guage needles. Tumor growth was monitored and on day 7, animals were redistributed into new cages after normalizing the average tumor volume to 100-150mm^3 for each treatment group. Ten mice were included in each treatment group. Animals were treated with antibodies via intraperitoneal injections of 0.25 mg/kg anti-ICOS antibody (37A10S713 VH and VL (SEQ ID NOs: 60 and 61) with a mouse IgG2a) or an isotype control. Dosing was performed on day 7 for single dose or days 7 and 14 for 2 doses. Tumor growth and animal body weights were monitored twice weekly. Mice were sacrificed when tumor volumes reached ~2000 mm^3 or if there were signs of clinical distress such as severe ulcerations as pursuant to IACUC protocol.

[00473] Figure 23, left panel, shows tumor volume in mice administered a single dose of anti-ICOS antibody (n=10) or two doses of anti-ICOS antibody (n=10).

[00474] A tumor re-challenge experiment was performed to assess durability of the response. The 7 mice previously challenged with Sa1/N cells whose tumors were eradicated with a single dose or two doses of 0.25mg/kg 37A10S713-mIgG2a antibody were re-challenged on the contralateral flank with Sa1/N cells 10 weeks after the initial tumor challenge. As a control, naïve mice were also challenged with Sa1/N cells (N=10). Animals were assessed for tumor growth on a bi-weekly basis.

[00475] As shown in Figure 23, right panel, none of the mice that had previously had their tumors eradicated with anti-ICOS antibody treatment showed tumor growth in the re-challenge experiment.

Example 10: ICOS ligand (ICOSL) expression in Sa1/N tumor-bearing mice and cynomolgus monkeys administered anti-ICOS antibody

[00476] Eight week old female A/J mice were inoculated with Sa1/N tumor cells at day zero. At day 7, when tumors reached ~100mm^3, mice were administered a single 5 or 100μg i.p. dose of antibody 37A10 with either a mouse IgG1 or IgG2a, or isotype control antibody. Mice were administered a subsequent dose of antibody on day 10, and tissue (blood, spleen, and tumor) were harvested at day 12. Following tissue processing, cells were incubated with 5% Fc block (5% reconstituted normal rat, mouse and human serum, 5% Fetal Calf Serum, 0.1mg/mL Fc blocking Ab 2.4G2, 0.01% sodium azide) for 15min on ice in flow staining buffer (FSB: 5% FBS, 0.01% sodium azide in 1xPBS). Following Fc block, cells were stained with an extracellular staining cocktail (anti-CD45-BV510, anti-CD19-BV605, anti-ICOSL-PE, Fixable Viability Dye eFluor 780) in FSB for 1hr on ice. Cells were washed twice with FSB. Cells were fixed with Fixation/Permeabilization solution for 30min on ice. Cells were washed twice with 1xPermeabilization Buffer, then stained with an intracellular staining cocktail (anti-CD3-BUV496, in Permeabilization Buffer for 1hr on ice. Cells were washed twice with Permeabilization Buffer, then re-suspended in 1.5% PFA FSB solution. Cells were run on the BD Fortessa and data was analyzed using FlowJo software.

[00477] Samples were analyzed on a BD Fortessa flow cytometer. For analysis of ICOSL expression, staining of ICOSL was analyzed on viable CD45+ CD3- CD19+ B cells. ICOSL mean fluorescent intensities (MFIs) are reported.

[00478] Antibody 37A10S713 with a human IgG1 was administered via 1 hour intravenous infusion to three cynolmolgus monkeys per dose group (0.5 mg/kg, 5 mg/kg, 75 mg/kg, and vehicle alone). Blood was obtained pre-first dose (day 1), 48 hours post-first dose (day 3), 7 days post-first dose (pre-second dose; day 8), and 48 hours post-second dose (day 10). 95μL samples of whole blood were first Fc blocked with 5μL Human TruStain for 15min on ice. Following Fc block, 100μL of an antibody mix containing anti-CD3 FITC, anti-CD20 PE, anti-CD14 PE/Cy7, viability dye e780, and cynoICOS-Fc DyLight 650 was added. Blood and antibody mix was incubated on ice for 60min. Following incubation, samples were centrifuged at 500xg for 5min. Supernatant was decanted, and samples were resuspended in 200μL of FACS staining buffer. Wash steps were repeated three times, with final resuspension in 200μL staining buffer + 0.1% paraformaldehyde.

[00479] Samples were analyzed on a BD Fortessa flow cytometer. For analysis of ICOSL expression, staining of ICOSL by DyLight 650 labeled cynoICOS-Fc was analyzed on viable CD3- CD20+ B cells. ICOSL MFIs were normalized to vehicle at each time point.

[00480] The results of those experiments are shown in Figures 24A and 24B. A dose-dependent increase in ICOS-L expression was observed across all antibody treatments and doses and in all tissues relative to isotype control treated mice. *See* Figure 24A. Similarly, a dose-dependent increase in ICOS-L expression was observed across all time points for the 0.5 and 5mg/kg dose groups in cynomolgus monkeys relative to vehicle and pre-study samples. *See* Figure 24B. Induction of ICOSL was also observed in the 75mg/kg group, but the observed expression may be an underrepresentation due to potential drug interference as the anti-ICOS antibody is capable of binding the staining reagent (cynoICOS-Fc).

[00481] ICOS target engagement may also be assessed as measured by receptor availability assay, for example, as follows. Naive mice were i.p. injected with either the isotype control mIgG2a at 2.5mg/kg or 37A10S713 with a mouse IgG2a at 2.5mg/kg. At various time-points post-injection, blood was collected in EDTA coated microtubes via submandibular draw.

[00482] Whole blood was Fc blocked using mouse TruStain (BioLegend) for 5 minutes on ice. Following incubation, 100µl of 2x concentrated extracellular staining antibody mixture was added to each sample for 30 minutes at 4°C. Samples were spun down and fixed and permeabilized in Foxp3 staining buffer (eBioSciences) for 30 minutes at 4°C. Samples were then spun down and resuspended in intracellular antibody stain for 30 minutes at 4°C. Samples were spun down and resuspended in 0.1% PFA. Samples were analyzed on a BD LSRII Fortessa. Tregs were identified as live CD45+ CD3+ CD4+ Foxp3+. Teff cells were identified as live CD45+ CD3+ CD4+ Foxp3-. CD8+ cells were identified as live CD45+ CD3+ CD8+. Fluorescently labeled 37A10S713-mG2a (DyLight 650 conjugated) was used as the staining reagent for ICOS. Receptor availability at each time point was determined using the following formula:

> % Receptor Available at time t =((MFI of 37A10S713-mG2aDy650at time t-MFI of isotypeDy650at time t))/((MFI of 37A10S713-mG2aDy650prestudy-MFI of isotypeDy650prestudy)) x 100

[00483] Results showed that following administration of anti-ICOS antibodies, the levels of free receptor are undetectable, suggesting antibodies saturate all available target ICOS molecules.

Example 11: Induction of Th-1 chemokines and cytokines following anti-ICOS antibody treatment

[00484] Fresh patient lung tumors were obtained 24 hours post-surgery. Soft tissue was manually removed from the tumor, and the remaining solid tumor was embedded in 4% low melting agar in a cast container and allowed to solidify on ice. The gel-embedded tumor was

cut by vibratome (Leica) (speed: 2, frequency: 9) to generate slices with a thickness of 300μm. If the tumor was too soft and unable to be sliced by vibratome, the tissue was manually cut with a blade.

[00485] Tumor slices were placed in a 40μm transwell filter (Millipore) (~1 slice/well), and the unit was moved to the wells of a 6-well plate, which contained 1.5mL of histoculture media (complete RPMI 1640/AIM-V). The appropriate treatment was then added into the medium of the corresponding well. Treatments included 10μg/mL anti-RSV hIgG1 (Lake Pharma, lot# 3086-849598) as an isotype control, 10μg/mL antibody 37A10S713 with a human IgG1 (SEQ ID NOs: 188 and 189), or 10 μg/ml of anti-PD-1 (IgG4) antibody. Replicate plates were prepared for various time points, ranging from 6-72 hours. The plates were placed in an incubator at 37°C, 5% CO_2.

[00486] At the desired time points, tumor slices were collected and immersed in RNALater (Ambion). RNA was extracted using a RNeasy Mini kit (Qiagen, cat#74106) according to the manufacturer's instructions. Following RNA extraction, 1μg of RNA was used for reverse transcription using a Bio-Rad iScript cDNA Synthesis Kit (cat#170-8891). The RT product was diluted 1 to 7, and 3μl was used for each qPCR reaction. The qPCR was carried out by using TaqMan Gene Expression Master Mix from Thermo Fisher Scientific (cat#4369016) using a Bio-Rad Real-Time System. The TaqMan assays used are listed in Table 6.

[00487] Expression was normalized to CD45, with fold change calculated as:

$$fold\ \text{change} = \frac{1/2^{(\text{exp .target Ct} - \text{exp .CD45 Ct})}}{1/2^{(\text{Iso .target Ct} - \text{Iso .CD45 Ct})}}$$

Table 6. TaqMan chemokine and cytokine assays

Target	Assay ID	Source
CD8B	Hs00174762_m1	ThermoFisher Scientific
CSF2	Hs00929873_m1	ThermoFisher Scientific
PRF1	Hs00169473_m1	ThermoFisher Scientific
GZMA	Hs00989184_m1	ThermoFisher Scientific
GZMB	Hs00188051_m1	ThermoFisher Scientific
IL2	Hs00174114_m1	ThermoFisher Scientific
CXCL9	Hs00171065_m1	ThermoFisher Scientific
CXCL10	Hs01124251_g1	ThermoFisher Scientific
CXCL11	Hs04187682_g1	ThermoFisher Scientific
FOXP3	Hs01085834_m1	ThermoFisher Scientific
CTLA4	Hs00175480_m1	ThermoFisher Scientific
CD45	Hs04189704_m1	ThermoFisher Scientific
CXCL13	Hs00757930_m1	ThermoFisher Scientific

[00488] The results of that experiment are shown in Figure 25. At the 6 hour time point with lung tumor 1, the anti-ICOS antibody resulted in increased expression of GZMa, GZMb, CSF2, IL2, CXCL9, CXCL10, CXCL11, and CXCL13. Anti-PD-1 antibody also increased expression of GZMa, GZMb, CSF2, CXCL9, and CXCL10, although to a lesser extent, and showed a similar increase in CXCL11. For lung tumor 2 at the 24 hour time point, anti-ICOS antibody treatment showed a sustained increase in CXCL11, and some continued elevation of IL2, CXCL9, and CXCL10. The anti-PD-1 antibody showed only a slight elevation in CXCL11 at 24 hours.

Example 12: Induction of NKp46 ligand on Treg cells following agonist anti-ICOS antibody treatment

[00489] Peripheral blood mononuclear cells were isolated from healthy human donors (Research Blood Components) using Ficoll (GE Life Sciences) centrifugation, frozen in BamBanker (Wako-Chem) and stored at -150°C until use. PBMCs were incubated with soluble anti-ICOS antibody and plate bound anti-human CD3 (1μg/ml coating, Biolegend, OKT3) at 37°C in RPMI (Gibco) supplemented with 10% fetal bovine serum (Sigma-Aldrich) and 1% penicillin/streptomycin (Gibco). Three antibodies were tested in the assay: the strong agonist antibody 37A10S713, a weak agonist antibody, and a weak antagonist antibody. After four days, PBMCs were gently scraped from plates and washed with DPBS (Gibco) containing 1% fetal bovine serum, 0.05% sodium azide (Ricco) and 2mM EDTA (Ambion). Cells were then blocked with Human TruStain FcX (Biolegend). To detect NKp46 ligand, cells were incubated with 2μg/mL NKp46-hIgG1 Fc (R&D Systems, 1850-NK). NKp46-hIgG1 Fc bound to cells was detected using a PE conjugated anti-human IgG (Biolegned, polyclonal). Cells were again blocked with Human TruStain FcX and then stained with anti-human CD56 (Biolgend, Brilliant Violet 711, HCD56), anti-human CD16 (Biolegend, Brilliant Violet 785, 3G8), anti-human CD4 (Biolegend, Brilliant Violet 510, OKT4), anti-human CD8 (BD Biosciences, BUV395, RPA-T8), anti-human CD25 (Biolegend, Brilliant Violet 605, BC96), and fixable viability dye (eBioscience, eFluor 780). Following staining, cells were fixed and permeabilized with Foxp3 / Transcription Factor Staining Buffer Set (eBioscience). Following permeabilization, cells were stained intracellularly with anti-human CD3 (BD Biosceinces, PE-CF594, UCHT1) and anti-human Foxp3 (eBioscience, APC, PCH101). Cells were then fixed in paraformaldehyde (Alfa Aesar). Data was acquired on a BD LSRII Fortessa and analyzed on FlowJo v10.1 software.

[00490] The results of that experiment are shown in Figure 26 and Figure 27. Treatment with the agonist anti-ICOS antibody 37A10S713 resulted in strong induction of NKp46 ligand on

Treg cells from three different donors. *See* Figures 26A-F (Figures 26A-B show data from donor 1, Figures 26C-D show data from donor 2, and Figures 26E-F show data from donor 3). Induction of NKp46 ligand on Teff cells was not as strong as on Treg cells. *See* Figures 26A-F. In addition, treatment with agonist anti-ICOS antibody 37A10S713 leads to loss of CD16 (CD16 shedding) on NK cells, suggesting activation of the NK cells. *See* Figure 27.

[00491] Without intending to be bound by any particular theory, it is postulated that the agonist anti-ICOS antibody 37A10S713 significantly increases NKp46 ligand levels on Treg cells and also activates NK cells, leading to selective Treg depletion.

[00492] The disclosure may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting of the disclosure. Scope of the disclosure is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are therefore intended to be embraced herein.

Table of Sequences

SEQ ID NO	Description	Sequence
1	Human ICOS precursor (with signal sequence); UniProtKB/Swiss-Prot: Q9Y6W8.1; 07-JAN-2015	MKSGLWYFFL FCLRIKVLTG EINGSANYEM FIFHNGGVQI LCKYPDIVQQ FKMQLLKGGQ ILCDLTKTKG SGNTVSIKSL KFCHSQLSNN SVSFFLYNLD HSHANYYFCN LSIFDPPPFK VTLTGGYLHI YESQLCCQLK FWLPIGCAAF VVVCILGCIL ICWLTKKKYS SSVHDPNGEY MFMRAVNTAK KSRLTDVTL
2	Human mature ICOS (without signal sequence)	EINGSANYEM FIFHNGGVQI LCKYPDIVQQ FKMQLLKGGQ ILCDLTKTKG SGNTVSIKSL KFCHSQLSNN SVSFFLYNLD HSHANYYFCN LSIFDPPPFK VTLTGGYLHI YESQLCCQLK FWLPIGCAAF VVVCILGCIL ICWLTKKKYS SSVHDPNGEY MFMRAVNTAK KSRLTDVTL
3	Mouse ICOS precursor (with signal sequence); UniProtKB/Swiss-Prot: Q9WVS0.2; 07-JAN-2015	MKPYFCRVFV FCFLIRLLTG EINGSADHRM FSFHNGGVQI SCKYPETVQQ LKMRLFRERE VLCELTKTKG SGNAVSIKNP MLCLYHLSNN SVSFFLNNPD SSQGSYYFCS LSIFDPPPFQ ERNLSGGYLH IYESQLCCQL KLWLPVGCAA FVVVLLFGCI LIIWFSKKKY GSSVHDPNSE YMFMAAVNTN KKSRLAGVTS
4	Mouse mature ICOS (without signal sequence)	EINGSADHRM FSFHNGGVQI SCKYPETVQQ LKMRLFRERE VLCELTKTKG SGNAVSIKNP MLCLYHLSNN SVSFFLNNPD SSQGSYYFCS LSIFDPPPFQ ERNLSGGYLH IYESQLCCQL KLWLPVGCAA FVVVLLFGCI LIIWFSKKKY GSSVHDPNSE YMFMAAVNTN KKSRLAGVTS
5	Cynomolgus monkey ICOS precursor (with signal sequence)	MKSGLWYFFL FCLHMKVLTG EINGSANYEM FIFHNGGVQI LCKYPDIVQQ FKMQLLKGGQ ILCDLTKTKG SGNKVSIKSL KFCHSQLSNN SVSFFLYNLD RSHANYYFCN LSIFDPPPFK VTLTGGYLHI YESQLCCQLK FWLPIGCATF VVVCIFGCIL ICWLTKKKYS STVHDPNGEY MFMRAVNTAK KSRLTGTTP
6	Cynomolgus mature ICOS (without signal sequence)	EINGSANYEM FIFHNGGVQI LCKYPDIVQQ FKMQLLKGGQ ILCDLTKTKG SGNKVSIKSL KFCHSQLSNN SVSFFLYNLD RSHANYYFCN LSIFDPPPFK VTLTGGYLHI YESQLCCQLK FWLPIGCATF VVVCIFGCIL ICWLTKKKYS STVHDPNGEY MFMRAVNTAK KSRLTGTTP
10	7F12 heavy chain variable region	E V Q L V E S G G G L V K P G G S L T L S C A A S G F T F S D Y W M D W V R Q G P G K G L E W V G N I D E D G S T T Y Y A P F V K G R F T I S R D N A K K T L Y L Q M N S V K S E D T A T Y Y C T R W G R Y A F D S W G Q G T L V T V S S
11	7F12 light chain variable region	D I V M T Q S P S S L A V S P G D K V T I N C K S S Q S L L S G N Y N Y L A W Y Q Q K T G Q A P K L L I F Y A S T R H T G V P D R F M G S G S G T D F S L T I N S F Q T E D L G D Y Y C Q H H Y S T P P T F G P G T K L E I K
12	7F12 VH CDR1	G F T F S D Y W M D
13	7F12 VH CDR2	N I D E D G S T T Y Y A P F V K G
14	7F12 VH CDR3	W G R Y A F D S
15	7F12 VL CDR1	K S S Q S L L S G N Y N Y L A
16	7F12 VL CDR2	Y A S T R H T
17	7F12 VL CDR3	Q H H Y S T P P T
20	37A10 heavy chain variable region	E V Q L V E S G G G L V K P G G S L K L S C A A S G F T F S D Y W M D W V R Q A P G K G L E W V G N I D E D G S I T E Y S P F V K G R F T I S R D N V K N T L Y L Q M N S V K S E D T A T Y Y C T R W G R F G F D S W G Q G T L V T V S S
21	37A10 light chain variable region	D I V M T Q S P S S L A V S A G D R V T I N C K S S Q S L L S G S F N Y L T W Y Q Q K T G Q A P K L L I F Y A S T R H T G V P D R F M G S

		G S G T D F T L T I N S F Q T E D L G D Y Y C H H H Y N A P P T F G P G T K L E L R
22	37A10 VH CDR1	G F T F S D Y W M D
23	37A10 VH CDR2	N I D E D G S I T E Y S P F V K G
24	37A10 VH CDR3	W G R F G F D S
25	37A10 VL CDR1	K S S Q S L L S G S F N Y L T
26	37A10 VL CDR2	Y A S T R H T
27	37A10 VL CDR3	H H H Y N A P P T
30	35A9 heavy chain variable region	E V Q L V E S G G G L V K P G G S L K L S C A A S G F T F S D Y W M D W V R Q A P G K G L E W V G N I D E D G S I A E Y S P F V K G R F T I S R D N V K N T L Y L Q M N S V K S E D T A T Y Y C S R W G R F A F D S W G Q G T L V T V S S
31	35A9 light chain variable region	D I V M T Q S P S S L A V S A G D R V T I N C K S S Q S L L S G S F N Y L T W Y Q Q K T G Q A P K L L I F Y A S T R H T G V P D R F M G S G S G T D F T L T I N S F Q T E D L G D Y Y C H H H Y N A P P T F G P G T K L E L R
32	35A9 VH CDR1	G F T F S D Y W M D
33	35A9 VH CDR2	N I D E D G S I A E Y S P F V K G
34	35A9 VH CDR3	W G R F A F D S
35	35A9 VL CDR1	K S S Q S L L S G S F N Y L T
36	35A9 VL CDR2	Y A S T R H T
37	35A9 VL CDR3	H H H Y N A P P T
40	36E10 heavy chain variable region	E V Q L V E S G G G L V K P G G S L K L S C A A S G F T F S D Y W M D W V R Q A P G K G L E W V G N I D E D G S I T E Y S P FV K G R F T I S R D N V K N I L Y L Q M N S V K S E D T A T Y Y C T R W G R F A F D S W G Q G T L V T V S S
41	36E10 light chain variable region	D I V M T Q S P S S L A V S P G D R V T I N C K S S Q S L L S G S F H Y L T W Y Q Q K T G Q A P K L L I F Y A S T R H T G V P D R F M G S G S G T D F T L T I N S F Q T E D L G D Y Y C H H H Y N A P P T F G P G T K L E L R
42	36E10 VH CDR1	G F T F S D Y W M D
43	36E10 VH CDR2	N I D E D G S I T E Y S P F V K G
44	36E10 VH CDR3	W G R F A F D S
45	36E10 VL CDR1	K S S Q S L L S G S F H Y L T
46	36E10 VL CDR2	Y A S T R H T
47	36E10 VL CDR3	H H H Y N A P P T
50	16G10 heavy chain variable region	E V Q L V E S G G G L V K P G G S L K L S C A A S G F T F S D Y W M D W V R Q A P G K G L E W V G N I D H D G N I I N F A P S V K G R F T I S R D N A K N T L Y L Q M N S V K S E D T A T Y Y C A R W G H Y A F D S W G Q G T L V T V S S
51	16G10 light chain variable region	D I V M T Q S P S S L A V S A G D K V T I N C K S S Q S L L S S G Y N Y L I W Y Q Q K T G Q A P K L L I F Y A S T R H T G V P D R F I G S G S G T D F T L T I T S F Q T E D L G D Y Y C Q H H Y S S P P T F G P G T K L E I K
52	16G10 VH CDR1	G F T F S D Y W M D
53	16G10 VH CDR2	N I D H D G N I I N F A P S V K G
54	16G10 VH CDR3	W G H Y A F D S
55	16G10 VL CDR1	K S S Q S L L S S G Y N Y L I
56	16G10 VL CDR2	Y A S T R H T
57	16G10 VL CDR3	Q H H Y S S P P T

60	37A10S713 heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFS DYWMDWVRQA PGKGLVWVSN IDEDGSITEY SPFVKGRFTI SRDNAKNTLY LQMNSLRAED TAVYYCTRWG RFGFDSWGQG TLVTVSS
61	37A10S713 light chain variable region	DIVMTQSPDS LAVSLGERAT INCKSSQSLL SGSFNYLTWY QQKPGQPPKL LIFYASTRHT GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCHHHYNAPP TFGPGTKVDI K
62	37A10S713 VH CDR1	GFTFSDYWMD
63	37A10S713 VH CDR2	NIDEDGSITEYSPFVKG
64	37A10S713 VH CDR3	WGRFGFDS
65	37A10S713 VL CDR1	KSSQSLLSGSFNYLT
66	37A10S713 VL CDR2	YASTRHT
67	37A10S713 VL CDR3	HHHYNAPPT
70	37A10S714 heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFS DYWMDWVRQA PGKGLVWVSN IDEDGSITEY SPFVKGRFTI SRDNAKNTLY LQMNSLRAED TAVYYCTRWG RFGFDSWGQG TLVTVSS
71	37A10S714 light chain variable region	DIVMTQSPDS LAVSLGERAT INCKSSQSLL SGSFNYLTWY QQKPGQPPKL LIFYASTRET GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCHHHYNAPP TFGPGTKVDI K
72	37A10S714 VH CDR1	GFTFSDYWMD
73	37A10S714 VH CDR2	NIDEDGSITEYSPFVKG
74	37A10S714 VH CDR3	WGRFGFDS
75	37A10S714 VL CDR1	KSSQSLLSGSFNYLT
76	37A10S714 VL CDR2	YASTRET
77	37A10S714 VL CDR3	HHHYNAPPT
80	37A10S715 heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFS DYWMDWVRQA PGKGLVWVSN IDEDGSITEY SPFVKGRFTI SRDNAKNTLY LQMNSLRAED TAVYYCTRWG RFGFDSWGQG TLVTVSS
81	37A10S715 light chain variable region	DIVMTQSPDS LAVSLGERAT INCKSSQSLL SGSFNYLTWY QQKPGQPPKL LIFYASTRQT GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCHHHYNAPP TFGPGTKVDI K
82	37A10S715 VH CDR1	GFTFSDYWMD
83	37A10S715 VH CDR2	NIDEDGSITEYSPFVKG
84	37A10S715 VH CDR3	WGRFGFDS
85	37A10S715 VL CDR1	KSSQSLLSGSFNYLT
86	37A10S715 VL CDR2	YASTRQT
87	37A10S715 VL CDR3	HHHYNAPPT
90	37A10S716 heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFS DYWMDWVRQA PGKGLVWVSN IDESGSITEY SPFVKGRFTI SRDNAKNTLY LQMNSLRAED TAVYYCTRWG RFGFDSWGQG TLVTVSS
91	37A10S716 light chain variable region	DIVMTQSPDS LAVSLGERAT INCKSSQSLL SGSFNYLTWY QQKPGQPPKL LIFYASTRHT GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCHHHYNAPP TFGPGTKVDI K
92	37A10S716 VH CDR1	GFTFSDYWMD
93	37A10S716 VH CDR2	NIDESGSITEYSPFVKG
94	37A10S716 VH CDR3	WGRFGFDS
95	37A10S716 VL CDR1	KSSQSLLSGSFNYLT
96	37A10S716 VL CDR2	YASTRHT
97	37A10S716 VL CDR3	HHHYNAPPT
100	37A10S717 heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFS DYWMDWVRQA PGKGLVWVSN IDESGSITEY SPFVKGRFTI SRDNAKNTLY LQMNSLRAED TAVYYCTRWG RFGFDSWGQG TLVTVSS
101	37A10S717 light chain variable region	DIVMTQSPDS LAVSLGERAT INCKSSQSLL SGSFNYLTWY QQKPGQPPKL LIFYASTRET GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCHHHYNAPP TFGPGTKVDI K
102	37A10S717 VH CDR1	GFTFSDYWMD
103	37A10S717 VH CDR2	NIDESGSITEYSPFVKG
104	37A10S717 VH CDR3	WGRFGFDS
105	37A10S717 VL CDR1	KSSQSLLSGSFNYLT

106	37A10S717 VL CDR2	YASTRET
107	37A10S717 VL CDR3	HHHYNAPPT
110	37A10S718 heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFS DYWMDWVRQA PGKGLVWVSN IDESGSITEY SPFVKGRFTI SRDNAKNTLY LQMNSLRAED TAVYYCTRWG RFGFDSWGQG TLVTVSS
111	37A10S718 light chain variable region	DIVMTQSPDS LAVSLGERAT INCKSSQSLL SGSFNYLTWY QQKPGQPPKL LIFYASTRQT GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCHHHYNAPP TFGPGTKVDI K
112	37A10S718 VH CDR1	GFTFSDYWMD
113	37A10S718 VH CDR2	NIDESGSITEYSPFVKG
114	37A10S718 VH CDR3	WGRFGFDS
115	37A10S718 VL CDR1	KSSQSLLSGSFNYLT
116	37A10S718 VL CDR2	YASTRQT
117	37A10S718 VL CDR3	HHHYNAPPT
120	16G10S71 heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFS DYWMDWVRQA PGKGLVWVSN IDHDGNIINF APSVKGRFTI SRDNAKNTLY LQMNSLRAED TAVYYCARWG HYAFDSWGQG TLVTVSS
121	16G10S71 light chain variable region	DIVMTQSPDS LAVSLGERAT INCKSSQSLL SSGYNYLIWY QQKPGQPPKL LIFYASTRHT GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQHHYSSPP TFGPGTKVDI K
122	16G10S71 VH CDR1	GFTFSDYWMD
123	16G10S71 VH CDR2	NIDHDGNIINFAPSVKG
124	16G10S71 VH CDR3	WGHYAFDS
125	16G10S71 VL CDR1	KSSQSLLSSGYNYLI
126	16G10S71 VL CDR2	YASTRHT
127	16G10S71 VL CDR3	QHHYSSPPT
130	16G10S72 heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFS DYWMDWVRQA PGKGLVWVSN IDHDGNIINF APSVKGRFTI SRDNAKNTLY LQMNSLRAED TAVYYCARWG HYAFDSWGQG TLVTVSS
131	16G10S72 light chain variable region	DIVMTQSPDS LAVSLGERAT INCKSSQSLL SSGYNYLIWY QQKPGQPPKL LIFYASTRET GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQHHYSSPP TFGPGTKVDI K
132	16G10S72 VH CDR1	GFTFSDYWMD
133	16G10S72 VH CDR2	NIDHDGNIINFAPSVKG
134	16G10S72 VH CDR3	WGHYAFDS
135	16G10S72 VL CDR1	KSSQSLLSSGYNYLI
136	16G10S72 VL CDR2	YASTRET
137	16G10S72 VL CDR3	QHHYSSPPT
140	16G10S73 heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFS DYWMDWVRQA PGKGLVWVSN IDHDGNIINF APSVKGRFTI SRDNAKNTLY LQMNSLRAED TAVYYCARWG HYAFDSWGQG TLVTVSS
141	16G10S73 light chain variable region	DIVMTQSPDS LAVSLGERAT INCKSSQSLL SSGYNYLIWY QQKPGQPPKL LIFYASTRQT GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQHHYSSPP TFGPGTKVDI K
142	16G10S73 VH CDR1	GFTFSDYWMD
143	16G10S73 VH CDR2	NIDHDGNIINFAPSVKG
144	16G10S73 VH CDR3	WGHYAFDS
145	16G10S73 VL CDR1	KSSQSLLSSGYNYLI
146	16G10S73 VL CDR2	YASTRQT
147	16G10S73 VL CDR3	QHHYSSPPT
150	16G10S83 heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFS DYWMDWVRQA PGKGLEWVSN IDHDGNIINF APSVKGRFTI SRDNAKNSLY LQMNSVRAED TAVYYCARWG HYAFDSWGQG TLVTVSS
151	16G10S83 light chain variable region	DIVMTQSPDS LAVSAGERVT INCKSSQSLL SSGYNYLIWY QQKPGQPPKL LIFYASTRQT GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQHHYSSPP TFGQGTKLEI K
152	16G10S83 VH CDR1	GFTFSDYWMD
153	16G10S83 VH CDR2	NIDHDGNIINFAPSVKG

154	16G10S83 VH CDR3	WGHYAFDS
155	16G10S83 VL CDR1	KSSQSLLSSGYNYLI
156	16G10S83 VL CDR2	YASTRQT
157	16G10S83 VL CDR3	QHHYSSPPT
160	35A9S79 heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFS DYWMDWVRQA PGKGLVWVSN IDEDGSIAEY SPFVKGRFTI SRDNAKNTLY LQMNSLRAED TAVYYCSRWG RFAFDSWGQG TLVTVSS
161	35A9S79 light chain variable region	DIVMTQSPDS LAVSLGERAT INCKSSQSLL SGSFNYLTWY QQKPGQPPKL LIFYASTRQT GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCHHHYNAPP TFGPGTKVDI K
162	35A9S79 VH CDR1	GFTFSDYWMD
163	35A9S79 VH CDR2	NIDEDGSIAEYSPFVKG
164	35A9S79 VH CDR3	WGRFAFDS
165	35A9S79 VL CDR1	KSSQSLLSGSFNYLT
166	35A9S79 VL CDR2	YASTRQT
167	35A9S79 VL CDR3	HHHYNAPPT
170	35A9S710 heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFS DYWMDWVRQA PGKGLVWVSN IDESGSIAEY SPFVKGRFTI SRDNAKNTLY LQMNSLRAED TAVYYCSRWG RFAFDSWGQG TLVTVSS
171	35A9S710 light chain variable region	DIVMTQSPDS LAVSLGERAT INCKSSQSLL SGSFNYLTWY QQKPGQPPKL LIFYASTRHT GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCHHHYNAPP TFGPGTKVDI K
172	35A9S710 VH CDR1	GFTFSDYWMD
173	35A9S710 VH CDR2	NIDESGSIAEYSPFVKG
174	35A9S710 VH CDR3	WGRFAFDS
175	35A9S710 VL CDR1	KSSQSLLSGSFNYLT
176	35A9S710 VL CDR2	YASTRHT
177	35A9S710 VL CDR3	HHHYNAPPT
180	35A9S89 heavy chain variable region	EVQLVESGGG LVQPGGSLRL SCAASGFTFS DYWMDWVRQA PGKGLEWVSN IDEDGSIAEY SPFVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCSRWG RFAFDSWGQG TLVTVSS
181	35A9S89 light chain variable region	DIVMTQSPDS LAVSAGERVT INCKSSQSLL SGSFNYLTWY QQKPGQPPKL LIFYASTRQT GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCHHHYNAPP TFGQGTKLEI K
182	35A9S89 VH CDR1	GFTFSDYWMD
183	35A9S89 VH CDR2	NIDEDGSIAEYSPFVKG
184	35A9S89 VH CDR3	WGRFAFDS
185	35A9S89 VL CDR1	KSSQSLLSGSFNYLT
186	35A9S89 VL CDR2	YASTRQT
187	35A9S89 VL CDR3	HHHYNAPPT
188	37A10S713 human IgG1 heavy chain	EVQLVESGGG LVQPGGSLRL SCAASGFTFS DYWMDWVRQA PGKGLVWVSN IDEDGSITEY SPFVKGRFTI SRDNAKNTLY LQMNSLRAED TAVYYCTRWG RFGFDSWGQG TLVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTVPS SSLGTQTYIC NVNHKPSNTK VDKKVEPKSC DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK
189	37A10S713 human κ light chain	DIVMTQSPDS LAVSLGERAT INCKSSQSLL SGSFNYLTWY QQKPGQPPKL LIFYASTRHT GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCHHHYNAPP TFGPGTKVDI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC

190	Rat ICOS precursor (with signal sequence); UniProt Q9R1T7	MKPYFSCVFV FCFLIKLLTG ELNDLANHRM FSFHDGGVQI SCNYPETVQQ LKMQLFKDRE VLCDLTKTKG SGNTVSIKNP MSCPYQLSNN SVSFFLDNAD SSQGSYFLCS LSIFDPPPFQ EKNLSGGYLL IYESQLCCQL KLWLPVGCAA FVAALLFGCI FIVWFAKKKY RSSVHDPNSE YMFMAAVNTN KKSRLAGMTS
191	Mature rat ICOS (without signal sequence)	ELNDLANHRM FSFHDGGVQI SCNYPETVQQ LKMQLFKDRE VLCDLTKTKG SGNTVSIKNP MSCPYQLSNN SVSFFLDNAD SSQGSYFLCS LSIFDPPPFQ EKNLSGGYLL IYESQLCCQL KLWLPVGCAA FVAALLFGCI FIVWFAKKKY RSSVHDPNSE YMFMAAVNTN KKSRLAGMTS

WHAT IS CLAIMED IS:

1. An isolated monoclonal antibody that binds to ICOS, wherein the antibody comprises:

(a) HCDR1 comprising the amino acid sequence of SEQ ID NO: 62; (b) HCDR2 comprising the amino acid sequence of SEQ ID NO: 63; (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 64; (d) LCDR1 comprising the amino acid sequence of SEQ ID NO: 65; (e) LCDR2 comprising the amino acid sequence of SEQ ID NO: 66; and (f) LCDR3 comprising the amino acid sequence of SEQ ID NO: 67.

2. The antibody of claim 1, which comprises a heavy chain variable region (VH) and a light chain variable region (V_L), wherein:

the V_H is at least 90% identical to the amino acid sequence of SEQ ID NO: 60 and the V_L is at least 90% identical to the amino acid sequence of SEQ ID NO: 61.

3. The antibody of claim 1 or claim 2, which comprises a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein:

the V_H comprises the amino acid sequence of SEQ ID NO: 60 and the V_L comprises the amino acid sequence of SEQ ID NO: 61.

4. The antibody of any one of the preceding claims, wherein the antibody is a chimeric antibody or a humanized antibody.

5. The antibody of any one of the preceding claims, wherein the antibody is an antibody fragment selected from a Fab, Fab', Fv, scFv or $(Fab')_2$ fragment.

6. The antibody of any one of claims 1 to 4, wherein the antibody is a full length antibody.

7. The antibody of claim 6, wherein the antibody comprises an IgG1 heavy chain constant region or an IgG3 heavy chain constant region.

8. The antibody of claim 6, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 188 and the light chain comprises the amino acid sequence of SEQ ID NO: 189.

9. The antibody of any one of the preceding claims, wherein administration of the antibody to a mammal results in an increase in T effector (Teff) cells in the mammal.

10. The antibody of any one of the preceding claims, wherein administration of the antibody to a mammal results in activation of T effector (Teff) cells in the mammal.

11. The antibody of claim 9 or claim 10, wherein the Teff cells are CD4+ FoxP3- T cells.

12. The antibody of claim 9 or claim 10, wherein the Teff cells are CD4+ FoxP3- T cells and CD8+ T cells.

13. The antibody of claim 9 or claim 10, wherein the Teff cells are CD8+ T cells.

14. The antibody of any one of the preceding claims, wherein administration of the antibody to a mammal results in a decrease in T regulatory (Treg) cells in the mammal.

15. The antibody of claim 14, wherein the Treg cells are CD4+ FoxP3+ T cells.

16. An isolated nucleic acid encoding the antibody of any one of claims 1 to 15.

17. A vector comprising the nucleic acid of claim 16.

18. A host cell comprising the vector of claim 17.

19. A host cell that produces the antibody of any one of claims 1 to 15.

20. A method for making an anti-ICOS antibody, comprising culturing the host cell of claim 19 under conditions suitable for expression of the antibody.

21. The method of claim 20, further comprising recovering the antibody produced by the host cell.

22. A pharmaceutical composition comprising the anti-ICOS antibody of any one of claims 1 to 15 and a pharmaceutically acceptable carrier.

23. Use of the anti-ICOS antibody of any one of claims 1 to 15 or the pharmaceutical composition of claim 22 in the manufacture of a medicament for treating cancer in a mammal.

24. The use of claim 23, wherein the cancer is selected from melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), gastric cancer, bladder cancer, diffuse large B-cell lymphoma (DLBCL), Hodgkin's lymphoma, ovarian cancer, head & neck squamous cell cancer (HNSCC), and triple negative breast cancer (TNBC).

25. The use of claim 24, wherein the cancer is selected from melanoma, gastric cancer, head & neck squamous cell cancer (HNSCC), non-small cell lung cancer (NSCLC), and triple negative breast cancer (TNBC).

26. The use of any one of claims 23 to 25, wherein the mammal is a human.

27. The use of any one of claims 23 to 26, wherein the mammal is to be administered at least one additional therapeutic agent.

28. The use of claim 27, wherein the additional therapeutic agent is to be administered concurrently or sequentially with the anti-ICOS antibody.

29. The use of claim 27 or claim 28, wherein the additional therapeutic agent is selected from an anti-PD-1 antibody and an anti-PD-L1 antibody.

30. The use of claim 27 or claim 28, wherein the additional therapeutic agent is a cancer vaccine.

31. The use of claim 30, wherein the cancer vaccine is selected from a DNA vaccine, an engineered virus vaccine, an engineered tumor cell vaccine, and a cancer vaccine developed using neoantigens.

32. Use of an antibody of any one of claims 1 to 15 or the pharmaceutical composition of claim 22 and at least one additional therapeutic agent in the manufacture of a medicament for treating cancer.

33. The use of claim 32, wherein the additional therapeutic agent is selected from an anti-PD-1 antibody and an anti-PD-L1 antibody.

34. The use of claim 32, wherein the additional therapeutic agent is a cancer vaccine.

35. The use of claim 34, wherein the cancer vaccine is selected from a DNA vaccine, an engineered virus vaccine, an engineered tumor cell vaccine, and a cancer vaccine developed using neoantigens.

36. The use of any one of claims 32 to 35, wherein the cancer is selected from melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), gastric cancer, bladder cancer, diffuse large B-cell lymphoma (DLBCL), Hodgkin's lymphoma, ovarian cancer, head & neck squamous cell cancer (HNSCC), and triple negative breast cancer (TNBC).

37. The use of claim 36, wherein the cancer is selected from melanoma, gastric cancer, head & neck squamous cell cancer (HNSCC), non-small cell lung cancer (NSCLC), and triple negative breast cancer (TNBC).

38. An antibody of any one of claims 1 to 15 or the pharmaceutical composition of claim 22 for use in treating cancer.

39. The antibody of claim 38, wherein the cancer is selected from melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), gastric cancer, bladder cancer, diffuse large B-cell lymphoma (DLBCL), Hodgkin's lymphoma, ovarian cancer, head & neck squamous cell cancer (HNSCC), and triple negative breast cancer (TNBC).

40. The antibody of claim 38, wherein the cancer is selected from melanoma, gastric cancer, head & neck squamous cell cancer (HNSCC), non-small cell lung cancer (NSCLC), and triple negative breast cancer (TNBC).

41. The antibody of any one of claims 38 to 40, wherein the mammal is a human.

42. The use of any one of claims 23 to 37, wherein Teff cells are activated following administration of the anti-ICOS antibody.

43. The use of any one of claims 23 to 37 and 42, wherein the number of Teff cells is increased following administration of the anti-ICOS antibody.

44. The use of claim 42 or claim 43, wherein the Teff cells are CD4+ FoxP3- T cells.

45. The use of any one of claims 42 to 44, wherein the Teff cells are CD4+ FoxP3- T cells and CD8+ T cells.

46. The use of any one of claims 42 to 45, wherein the Teff cells are CD8+ T cells.

47. The use of any one of claims 23 to 37 and 42 to 46, wherein the number of T regulatory (Treg) cells is decreased following administration of the anti-ICOS antibody.

48. The use of claim 47, wherein the Treg cells are CD4+ FoxP3+ T cells.

49. The use of any one of claims 23 to 26, wherein the mammal is to be administered at least one additional therapeutic agent.

50. The use of claim 49, wherein the additional therapeutic agent is to be administered concurrently or sequentially with the anti-ICOS antibody.

51. The use of claim 50, wherein the additional therapeutic agent is a PD-1 therapy.

52. The use of any one of claims 49 to 51, wherein the additional therapeutic agent is selected from an anti-PD-1 antibody and an anti-PD-L1 antibody.

53. The use of claim 49 or claim 50, wherein the additional therapeutic agent is a cancer vaccine.

54. The use of claim 53, wherein the cancer vaccine is selected from a DNA vaccine, an engineered virus vaccine, an engineered tumor cell vaccine, and a cancer vaccine developed using neoantigens.

55. The use of any one of claims 23 to 27 and 42 to 54, wherein a sample of the cancer has been determined to express ICOS.

56. The use of claim 55, wherein the sample shows 1+, 2+, or 3+ staining of ICOS by immunohistochemistry (IHC).

57. The use of any one of claims 23 to 37 and 42 to 56, wherein the sample has been determined to have a low level of PD-L1.

58. The use of any one of claims 23 to 37 and 42 to 56, wherein the sample has been determined to be PD-L1-negative.

59. The use of any one of claims 23 to 37 and 42 to 56, wherein the sample has been determined to have an elevated level of PD-L1.

60. The use of any one of claims 57 to 59, wherein PD-L1 levels are determined using IHC.