Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242).

Figure 1B shows the nucleotide sequence (SEQ ID NO: 3) and amino acid sequence (SEQ ID NO: 4) of the kappa light chain variable region of the 25F7 human monoclonal antibody. The CDR1 (SEQ ID NO: 8), CDR2 (SEQ ID NO: 9) and CDR3 (SEQ ID NO: 10) regions are delineated and the V and J germline derivations are indicated. The full-length heavy and light chain amino acid sequences antibody 25F7 are shown in SEQ ID NOs: 32 and 34, respectively.

Figure 2A shows the amino acid sequence (SEQ ID NO: 12) of the heavy chain variable region of the LAG3.5 monoclonal antibody. The CDR1 (SEQ ID NO: 15), CDR2 (SEQ ID NO: 16) and CDR3 (SEQ ID NO: 17) regions are delineated. The full-length heavy and light chain amino acid sequences antibody LAG3.5 are shown in SEQ ID NOs: 35 and 37, respectively.

Figure 2B shows the nucleotide sequence (SEQ ID NO: 13) and amino acid sequence (SEQ ID NO: 14) of the kappa light chain variable region of the LAG3.5 monoclonal antibody. The CDR1 (SEQ ID NO: 18), CDR2 (SEQ ID NO: 19) and CDR3 (SEQ ID NO: 20) regions are delineated.

Figure 3 shows the amino acid sequences of the CDR2 heavy chain variable region sequences of the LAG-3 variants LAG3.5 (SEQ ID NO: ~~16~~42), LAG3.6 (SEQ ID NO: ~~24~~43), LAG3.7 (SEQ ID NO: ~~25~~44), and LAG3.8 (SEQ ID NO: ~~26~~45), compared to the amino acid sequence of the CDR2 heavy chain variable region sequence of antibody 25F7 (LAG3.1) (SEQ ID NO: ~~6~~41) and corresponding human germline sequence (SEQ ID NO: 27). The CDR2 heavy chain variable region of LAG3.5 differs from the CDR2 heavy chain variable region of 25F7 by arginine (R) at position 54 (versus asparagine (N)) and serine (S) at position 56 (versus asparagines (N)). The remaining CDRs of LAG3.5 anf 25F7 are identical. Figure 3 also discloses SEQ ID NO: 40.

Figures 4A and 4B are graphs showing the binding activity (EC_{50} and affinity, respectively) of antibodies LAG3.1 (25F7), LAG3.2, LAG3.5, LAG3.6, LAG3.7, and LAG3.8 to activated human CD4+ T cells. Figure 4B discloses SEQ ID NOS 41, 42, 45, 44, and 43, respectively, in order of appearance.

Figures 5A, B, C, D, and E are graphs showing thermal melting curves (i.e., thermal stability) of antibodies LAG3.1 (25F7), LAG3.5, LAG3.6, LAG3.7, and LAG3.8, respectively.

Figures 6A, B, C, D, and E are graphs showing thermal reversibility curves (i.e., thermal stability) of antibodies LAG3.1 (25F7), LAG3.5, LAG3.6, LAG3.7, and LAG3.8, respectively.

Figure 7 is a graph, showing the binding activity of antibodies LAG3.1 (25F7) and LAG3.5 to activated human CD4+ T cells and antigen binding (Biacore).

Figure 8 shows the results of peptide mapping using mass-sepctrometry (chemical modifications / molecular stability) for antibodies LAG3.1 (25F7) and LAG3.5 reflecting deamidation and isomerization after incubating for 5 days under accelerated stress conditions as described herein. Figure 8 discloses SEQ ID NOS 46-52, respectively, in order of appearance.

Figure 9 is a graph comparing the hydrophilicity profiles of antibodies LAG3.1 (25F7) and LAG3.5.

Figures 10 A, B, C, and D are graphs comparing the affinity and physical stability (i.e., thermal and chemical stability) of antibodies LAG3.1 and LAG3.5 at 4C° and 40C°, i.e., both accelerated stress conditions and “real-time” stability studies, as described herein.

Figures 11 A and B are graphs comparing the percent modification of the amino acid sequences of antibodies LAG3.1 and LAG3.5 at 4C° and 40C°.

The isolated DNA encoding the V_H region can be converted to a full-length heavy chain gene by operatively linking the VH-encoding DNA to another DNA molecule encoding heavy chain constant regions (CH1, CH2 and CH3). The sequences of human heavy chain constant region genes are known in the art (*see e.g.*, Kabat *et al.* (1991), *supra*) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The heavy chain constant region can be an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region, but most preferably is an IgG1 or IgG4 constant region. For a Fab fragment heavy chain gene, the V_H-encoding DNA can be operatively linked to another DNA molecule encoding only the heavy chain CH1 constant region.

The isolated DNA encoding the V_L region can be converted to a full-length light chain gene (as well as a Fab light chain gene) by operatively linking the V_L-encoding DNA to another DNA molecule encoding the light chain constant region, CL. The sequences of human light chain constant region genes are known in the art (*see e.g.*, Kabat *et al.*, *supra*) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. In preferred embodiments, the light chain constant region can be a kappa or lambda constant region.

To create a scFv gene, the V_H- and V_L-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, *e.g.*, encoding the amino acid sequence $(Gly_4\text{-}Ser)_3$ (SEQ ID NO: 28), such that the V_H and V_L sequences can be expressed as a contiguous single-chain protein, with the V_L and V_H regions joined by the flexible linker (*see e.g.*, Bird *et al.* (1988) *Science* 242:423-426; Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883; McCafferty *et al.*, (1990) *Nature* 348:552-554).

Production of Monoclonal Antibodies of the Invention

Monoclonal antibodies (mAbs) of the present invention can be produced using the well-known somatic cell hybridization (hybridoma) technique of Kohler and Milstein (1975) *Nature* 256: 495. Other embodiments for producing monoclonal antibodies include viral or oncogenic transformation of B lymphocytes and phage display techniques. Chimeric or humanized antibodies are also well known in the art. *See* e.g., U.S. Patent Nos. 4,816,567; 5,225,539; 5,530,101; 5,585,089; 5,693,762 and 6,180,370, the contents of which are specifically incorporated herein by reference in their entirety.

Val-Cit, Ala-Val, Val-Ala-Val, Lys-Lys, Pro-Val-Gly-Val-Val (SEQ ID NO: ~~15~~39), Ala-Asn-Val, Val-Leu-Lys, Ala-Ala-Asn, Cit-Cit, Val-Lys, Lys, Cit, Ser, or Glu. The ADCs can be prepared as described in U.S. Patent Nos. 7,087,600; 6,989,452; and 7,129,261; PCT Publications WO 02/096910; WO 07/038658; WO 07/051081; WO 07/059404; WO 08/083312; and WO 08/103693; U.S. Patent Publications 20060024317; 20060004081; and 20060247295; the disclosures of which are incorporated herein by reference.

Bispecific Molecules

In another aspect, the present disclosure features bispecific molecules comprising one or more antibodies of the invention linked to at least one other functional molecule, *e.g.*, another peptide or protein (*e.g.*, another antibody or ligand for a receptor) to generate a bispecific molecule that binds to at least two different binding sites or target molecules. Thus, as used herein, "bispecific molecule" includes molecules that have three or more specificities. In a preferred embodiment, the bispecific molecule comprises a first binding specificity for LAG-3 and a second binding specificity for a triggering molecule that recruits cytotoxic effector cells that can kill a LAG-3 expressing target cell. Examples of suitable triggering molecules are CD64, CD89, CD16, and CD3. *See*, *e.g.*, Kufer *et al.*, *TRENDS in Biotechnology*, 22 (5), 238-244 (2004).

In an embodiment, a bispecific molecule has, in addition to an anti-Fc binding specificity and an anti-LAG-3 binding specificity, a third specificity. The third specificity can be for an anti-enhancement factor (EF), *e.g.*, a molecule that binds to a surface protein involved in cytotoxic activity and thereby increases the immune response against the target cell. For example, the anti-enhancement factor can bind a cytotoxic T-cell (*e.g.* via CD2, CD3, CD8, CD28, CD4, CD40, or ICAM-1) or other immune cell, resulting in an increased immune response against the target cell.

Bispecific molecules can come in many different formats and sizes. At one end of the size spectrum, a bispecific molecule retains the traditional antibody format, except that, instead of having two binding arms of identical specificity, it has two binding arms each having a different specificity. At the other extreme are bispecific molecules consisting of two single-chain antibody fragments (scFv's) linked by a peptide chain, a so-called $Bs(scFv)_2$ construct. Intermediate-sized bispecific molecules include two different F(ab) fragments linked by a peptidyl linker. Bispecific molecules of these and other formats can be prepared by genetic engineering, somatic hybridization, or

V_H n.a. LAG3.5

caggtgcagctacagcagtggggcgcaggactgttgaagccttcggagaccctgtccctcacctgcgctgtctatggtgggtc
cttcagtgattactactggaactggatccgccagcccccagggaaggggctggagtggattggggaaatcaatcatcgtggaa
gcaccaactccaacccgtccctcaagagtcgagtcaccctatcactagacacgtccaagaaccagttctccctgaagctgaggt
ctgtgaccgccgcggacacggctgtgtattactgtgcgtttggatatagtgactacgagtacaactggttcgacccctggggcc
agggaaccctggtcaccgtctcctca

12	V_H a.a. LAG3.5	

V_H a.a. LAG3.5

QVQLQQWGAGLLKPSETLSLTCAVYGGSFSDYYWNWIRQPPGKGLEWIGE
INHRGSTNSNPSLKSRVTLSLDTSKNQFSLKLRSVTAADTAVYYCAFGYS
DYEYNWFDPWGQGTLVTVSS

13	V_K n.a. LAG3.5	

V_K n.a. LAG3.5

gaaattgtgttgacacagtctccagccaccctgtctttgtctccaggggaaagagccaccctctcctgcagggccagtcagagt
attagcagctacttagcctggtaccaacagaaacctggccaggctcccaggctcctcatctatgatgcatccaacagggccact
ggcatcccagccaggttcagtggcagtgggtctgggacagacttcactctcaccatcagcagcctagagcctgaagattttgca
gtttattactgtcagcagcgtagcaactggcctctcacttttggccaggggaccaacctggagatcaaa

14	V_K a.a. LAG3.5	

V_K a.a. LAG3.5

EIVLTQSPATLSLSPGERATLSCRASQSISSYLAWYQQKPGQAPRLLIYD
ASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPLTFGQ
GTNLEIK

15	V_H CDR1 a.a. LAG3.5	**DYYWN**
16	V_H CDR2 a.a. LAG3.5	**EINHRGSTNSNPSLKS**
17	V_H CDR3 a.a. LAG3.5	GYSDYEYNWFDP
18	V_K CDR1 a.a. LAG3.5	RASQSISSYLA
19	V_K CDR2 a.a. LAG3.5	DASNRAT
20	V_K CDR3 a.a. LAG3.5	**QQRSNWPLT**
21	LAG-3 epitope	PGHPLAPG
22	LAG-3 epitope	HPAAPSSW
23	LAG-3 epitope	PAAPSSWG
24	V_H CDR2 a.a. LAG3.6	**EIIHSGSTNSNPSLKS**
25	V_H CDR2 a.a. LAG3.7	**EINHGGGTNSNPSLKS**
26	V_H CDR2 a.a. LAG3.8	**EINHIGNTNSNPSLKS**
27	V_H CDR2 a.a.HUMAN GERMLINE	GEINHSGSTNY
28		$(Gly_4\text{-}Ser)_3$