



(51) International Patent Classification:
C07K 14/725 (2006.01)

(21) International Application Number:
PCT/US2014/064383

(22) International Filing Date:
6 November 2014 (06.11.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/900,806 6 November 2013 (06.11.2013) US

(71) Applicant: **THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES** [US/US]; National Institutes of Health, Office of Technology of Transfer, 6011 Executive Boulevard, Suite 325, MSC 7660, Bethesda, MD 20852-7660 (US).

(72) Inventors: **ORENTAS, Rimas**; Lentigen Technology, Inc., 910 Clopper Rd., Ste. 200, South, Gaithersburg, MD 20878 (US). **MACKALL, Crystal**; NCI, CCR, 1W-3750 10 Center Drive, Bethesda, MD 20892 (US).

(74) Agent: **SCOTT, Gregory, K.**; Klarquist Sparkman, LLP, One World Trade Center, Suite 1600, 121 SW Salmon Street, Portland, OR 97204 (US).

(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: ALK ANTIBODIES, CONJUGATES, AND CHIMERIC ANTIGEN RECEPTORS, AND THEIR USE

(57) Abstract: Chimeric antigen receptors that specifically bind to anaplastic lymphoma kinase are disclosed. Nucleic acids, recombinant expression vectors, host cells, antibodies, antigen binding fragments, and pharmaceutical compositions, relating to the chimeric antigen receptors are also disclosed. Methods of treating or preventing cancer in a subject, and methods of making chimeric antigen receptor T cells are also disclosed.



WO 2015/069922 A2

ALK ANTIBODIES, CONJUGATES, AND CHIMERIC ANTIGEN RECEPTORS, AND THEIR USE

CROSS REFERENCE TO RELATED APPLICATIONS

5 This application claims priority to U.S. Provisional Application No. 61/900,806, filed November 6, 2013, which is incorporated by reference in its entirety.

FIELD OF THE DISCLOSURE

10 This application relates to the field of cancer, particularly to chimeric antigen receptors (CARs) that specifically bind anaplastic lymphoma kinase (ALK) and their use.

BACKGROUND

 Cancer is a public health concern. It is one of the primary causes of death in the industrialized world. Despite advances in treatments such as chemotherapy, the prognosis for many cancers, including
15 neuroblastoma, can be poor. One treatment approach includes genetic modification of T cells to express CARs that target antigens expressed on tumor cells. CARs are antigen receptors that are designed to recognize cell surface antigens in a human leukocyte antigen-independent manner. Additional treatment approaches include use of therapeutic antibodies, or conjugates thereof. However, a need exists for additional and improved treatments for cancer, particularly neuroblastoma.

20

SUMMARY

 Novel CARs that specifically bind to the extracellular domain of ALK protein are provided herein, as well as host cells (e.g., T cells) expressing the receptors, nucleic acid molecules encoding the receptors. Methods of using the disclosed CARs, host cells, and nucleic acid molecules are also provided, for example, to
25 treat a tumor in a subject.

 In some embodiments, a nucleic acid molecule encoding a CAR is provided. The CAR comprises, from N-terminus to C-terminus, an antigen binding domain, a transmembrane domain, and at least one intracellular T-cell signaling domain. The antigen binding domain comprises a heavy chain variable region and a light chain variable region comprising one of: (a) a heavy chain complementarity determining region (H-
30 CDR)1, a H-CDR2, and a H-CDR3 of the heavy chain variable region set forth as SEQ ID NO: 1, and a light chain complementarity determining region (L-CDR)1, a L-CDR2, and a L-CDR3 of the light chain variable region set forth as SEQ ID NO: 2; (b) a H-CDR1, a H-CDR2, and a H-CDR3 of the heavy chain variable region sequence set forth as SEQ ID NO: 3, and a L-CDR1, a L-CDR2, and a L-CDR3 of the light chain variable region sequence set forth as SEQ ID NO: 4; (c) a H-CDR1, a H-CDR2, and a H-CDR3 of the heavy
35 chain variable region sequence set forth as SEQ ID NO: 5, and a L-CDR1, a L-CDR2, and a L-CDR3 of the light chain variable region sequence set forth as SEQ ID NO: 6; or (d) a H-CDR1, a H-CDR2, and a H-CDR3 of the heavy chain variable region sequence set forth as SEQ ID NO: 7, and a L-CDR1, a L-CDR2, and a L-CDR3 of the light chain variable sequence region set forth as SEQ ID NO: 8. The CAR specifically binds to an extracellular domain of anaplastic lymphoma kinase.

In some embodiments, the H-CDR1, H-CDR2, and H-CDR3 comprise amino acids 26-33, 51-57, and 95-109 of SEQ ID NO: 1, respectively, and the L-CDR1, L-CDR2, and L-CDR3 comprise amino acids 27-37, 55-57, and 93-103 of SEQ ID NO: 2, respectively. In other embodiments, the H-CDR1, H-CDR2, and H-CDR3 comprise amino acids 26-33, 51-58, and 96-110 of SEQ ID NO: 3, respectively, and the L-CDR1, L-CDR2, and L-CDR3 comprise amino acids 27-36, 54-56, and 92-102 of SEQ ID NO: 4, respectively. In additional embodiments, the H-CDR1, H-CDR2, and H-CDR3 comprise amino acids 26-33, 51-58, and 96-108 of SEQ ID NO: 5, respectively, and the L-CDR1, L-CDR2, and L-CDR3 comprise amino acids 27-37, 55-57, and 93-103 of SEQ ID NO: 6, respectively. In still other embodiments, the H-CDR1, H-CDR2, and H-CDR3 comprise amino acids 26-33, 51-58, and 96-110 of SEQ ID NO: 7, respectively, and the L-CDR1, L-CDR2, and L-CDR3 comprise amino acids 27-32, 50-52, and 88-98 of SEQ ID NO: 8, respectively.

In more embodiments the heavy and light chain variable regions of the antigen binding domain includes amino acid sequences set forth as SEQ ID NO: 9 and SEQ ID NO: 10, respectively; SEQ ID NO: 11 and SEQ ID NO: 12, respectively; SEQ ID NO: 13 and SEQ ID NO: 14, respectively; or SEQ ID NO: 15 and SEQ ID NO: 16, respectively.

In some embodiments, the at least one T-cell signaling domain comprises, from N-terminus to C-terminus, (a) a CD3 zeta signaling domain; (b) a CD28 signaling domain and a CD3 zeta signaling domain; (c) a CD137 (4-1BB) signaling domain and a CD3 zeta signaling domain; (d) an OX40 signaling domain and a CD3 zeta signaling domain; (e) a CD28 signaling domain, a CD137 (4-1BB) signaling domain, and a CD3 zeta signaling domain; or (f) a CD28 signaling domain, an OX40 (CD134) signaling domain, and a CD3 zeta signaling domain.

In some embodiments, the CAR includes the amino acid sequence set forth as one of SEQ ID NOs: 43-90.

The nucleic acid molecule encoding the CAR can be included on a vector, such as a viral vector. In some embodiments, the viral vector is a lentiviral vector.

Host cells including the nucleic acid molecule encoding the CAR are also provided. In some embodiments, the host cell is a T cell, such as a primary T cell obtained from a subject.

Methods of making CAR T cells are provided. The methods include transducing a T cell with the vector or nucleic acid molecule encoding a disclosed CAR that specifically binds ALK, thereby making the CAR T cell.

Also provided are methods of treating a subject with a tumor using the disclosed CARs that specifically bind ALK. The methods include administering to the subject a therapeutically effective amount of host cells expressing a disclosed CAR that specifically binds ALK, under conditions sufficient to form an immune complex of the antigen binding domain on the CAR and the extracellular domain of ALK in the subject. In some embodiments, the host cells are T cells from the subject that have been transformed or transduced with a nucleic acid molecule or vector encoding the disclosed CAR that specifically binds ALK. In additional embodiments, the methods further include the steps of obtaining T cells from the subject, and transforming or transducing the T cells with the nucleic acid molecule or vector encoding the disclosed CAR that specifically binds ALK.

In several embodiments, the tumor comprises cell surface expression of anaplastic lymphoma kinase.

In additional embodiments, the tumor is a neuroblastoma, a rhabdomyosarcoma, or a glioblastoma.

Isolated human monoclonal neutralizing antibodies and antigen binding fragments thereof that specifically bind to ALK on the cell surface, and conjugates thereof are also provided. Nucleic acid molecules encoding the disclosed antibodies, antigen binding fragments, or conjugates, expression vectors including such nucleic acid molecules, and host cells including the nucleic acid molecules and/or expression vectors are also provided. In several embodiments, the antibodies, antigen binding fragments, conjugates, nucleic acid molecules, expression vectors and/or host cells can be used in methods of treating a subject with a tumor, for example treating a subject with a neuroblastoma.

It will be understood that the antibodies, antigen binding fragments, CARs, host cells, nucleic acids, and methods are useful beyond the specific circumstances that are described in detail herein. The foregoing and features and advantages of the disclosure will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE FIGURES

FIGS. 1A-1F show a series of graphs illustrating ALK expression on tumor cell lines. ALK expression on tumor cell lines was evaluated by flow cytometric analysis using the ALK specific monoclonal antibody (mAb) ALK 48. Cell lines analyzed were of neuroblastoma origin: (A) SY5Y, (C) LAN5, (D) KCNR, (E), IMR32; rhabdomyosarcoma origin (B) Rh18; or a control leukemia cell line (F), K562. Control is shown in grey, and ALK positive cells are shown with a solid line, gated as indicated.

FIG. 2 shows a series of schematic diagrams illustrating ALK-specific CAR constructs. CAR constructs were created by linking the variable heavy and light chain regions of four different anti-ALK scFv antibody fragments (clones 15, 43, 53, 58) with CD28 transmembrane and signaling domains (CD28), 4-1BB (also known as CD137) transmembrane and signaling domain (41BB), and/or CD3 zeta-chain intracellular signaling domains (CD3 Z). For some constructs a CH2CH3 spacer domain (CH2CH3) was also included. The names of some of the constructs used herein are listed to the left of the schematic diagrams. All constructs include heavy chain first, followed by light chain in the linear sequence except for ALK58-LH which includes the light chain first.

FIGS. 3A-3F show a series of graphs illustrating expression of ALK specific CARs on the T cell surface. Peripheral blood mononuclear cells (PBMCs) were transduced with a) ALK48, b) ALK48SH, c) non-transduced control (Mock), d) ALK58, e) ALK58SH, or f) ALK58LH, and then stained for CAR expression. The expression of cell-surface CAR was evaluated using flow cytometry. Percent transduction according to the indicated gates is listed at the top of each panel.

FIGS. 4A-4C show a series of graphs illustrating cytotoxic T Cell activity mediated by the disclosed CARs that specifically bind ALK. PBMCs were transduced with the indicated vectors: ALK48, ALK53, ALK58, and incubated in 96-well plates with ⁵¹Cr-labeled LAN5 (A), Rh18 (B), or K562 (C) target cell lines at the E:T ratios indicated. Effector number was corrected for percent transduction.

FIGS. 5A and 5B show a set of graphs illustrating cytotoxic T cell activity of SH-ALK CAR. PBMC were transduced with A) ALK58, ALK58SH, or ALK58LH, or B) ALK48 or ALK48SH and incubated in 96-well plates with ⁵¹Cr-labeled LAN5 to compare relative CAR format efficiency. Effector number was corrected for percent transduction.

FIGs. 6A-6C show a series of graphs illustrating cytokine activity of the disclosed CAR T Cells. PBMC were transduced with the indicated CAR constructs, and incubated with tumor targets (LAN5 SY5Y, Rh18, K562 as indicated in legend), and supernatants collected after 24 hours and tested for the presence of cytokine by a MesoScale mutli-analyte detection system. Results for A) IFN-gamma, B) Interleukin-2, and C) TNF-alpha are shown. Controls include mock transduced cells, tumor only, and T cells without tumor target, as indicated.

FIG. 7 is a graph illustrating effective CAR T cell therapy with CAR T-cells expressing a CAR based on the ALK48 mAb. NOD *scid* gamma (NSG) mice were inoculated with ALK positive neuroblastoma cells (SY5Y cells) to produce xenograft. Following inoculation, the mice were treated with primary human T cells transduced to express either ALK48L-28z (second generation CAR, long-format that contains a CH2CH3 spaced domain from IgG); ALK48SH-28z (second generation CAR in a short format, no spacer), or mock transduced T cells.

SEQUENCES

The nucleic and amino acid sequences listed below are shown using standard letter abbreviations for nucleotide bases, and three letter code for amino acids, as defined in 37 C.F.R. 1.822. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand. The Sequence Listing is submitted as an ASCII text file in the form of the file named "Sequence.txt" (~400 kb), which was created on October 30, 2014, and is incorporated by reference herein.

In the accompanying sequence listing:

SEQ ID NO: 1 is the amino acid sequence of the V_H of the ALK15 mAb.

DVKLQESGPGVLVAPSQSLSTICTVSGFSLTSYAVSWVRQPPGKGLEWLGIIWSGGATNYNSALKSRLSISKDNS
KSQVFLKMNGLQTDITARYYCAREHYYGSSAMDYWGQGASITVSS

SEQ ID NO: 2 is the amino acid sequence of the V_L of the ALK15 mAb.

GIVMTQSPLSLPVSLGDAQSISCRSSQSIVHSYGNTYLFWYLQKPGQSPKLLIYRVSNRFSGVPDRFSGSGSGT
NFTLKISRVEAEDMGVYYCFQGTHTVPYTFGGGTKLEIK

SEQ ID NO: 3 is the amino acid sequence of the V_H of the ALK48 mAb.

QVQLQQSGAELVKPGASVKISCKASGYAFSSYWMNVKQRPKGKLEWIGQIYPGDGDDTTYNGKFKGKATLTADK
SSSTVYMQLNSLTSEDSAVYFCVRYYYGSSGYFDYWQGTTTLTVSS

SEQ ID NO: 4 is the amino acid sequence of the V_L of the ALK48 mAb.

DVQMIQTPDSLAVSLGQRATISCRASESVDNYGISFMHWYQQKPGQSPKLLIYRASNLMSGIPARFSGSGSRD
FTLTINPVETDDVATYYCQQNNKDPPTFGGGTKLEIK

SEQ ID NO: 5 is the amino acid sequence of the V_H of the ALK53 mAb.

DVQLQESGPGVLVKTGASVKMSCTASGYTFTDHFMDWVKQSHGKSLEWIGSLNPYSGGTSYNQKFKGKATLTVDK
SSSTAYMELNSLTSDSAVYYCARHNWGAYFDYWQGTTTLTVSS

SEQ ID NO: 6 is the amino acid sequence of the V_L of the ALK53 mAb.

DIVMTQAAPSVPTPGESVSI SCRSSKSLLSHNGNTYLYWFLQRPQGQSPQRLIYYMSNLAGVPDRFSGRSGT
DFTLRISRVEAEDVGYYCMQGLEDPYTFGGGTKLEIK

SEQ ID NO: 7 is the amino acid sequence of the V_H of the ALK58 mAb.

ALQLQQSGAELVRPGASVTLSCASGYTFTDYEHWVQTPVHGLEWIGAIDPETGGTAYNQKFEGKAILTADK
SSSTAYMELRSLTSEDSPVYYCARRRYGSSSFYDWQGTTTLTVSS

SEQ ID NO: 8 is the amino acid sequence of the V_L of the ALK58 mAb.

DVQMIQTPSSLSASLGDRVTISCRASQDIGNYLNWYQQKPDGTVKLLIYYTSRLHSGVPSRFSGSGSGTEYSLT
ISNLEQEDIATYFCQQGSALPPTFGGGTKLEIN

SEQ ID NO: 9 is the amino acid sequence of a humanized V_H of the ALK15 mAb.

QVQLQQSGAEVKKPGSSSVKVSCKAS**GFSLTSYA**ISWVRQAPQGQLEWMGGI**IWSGGAT**NYAQKFQGRVTITADE
STSTAYMELSSLRSEDTAVYYC**CAREHYYGSSAMDYW**WGQGMVTV

SEQ ID NO: 10 is the amino acid sequence of a humanized V_L of the ALK15 mAb.

EIVLTQSPATLSLSPGERATLSCRAS**QSIVHSYGNTY**AWYQQKPGQAPRLLIY**RVSRATGIPARFSGSGSGTDF**
5 **TLTISSLEPEDFAVYYCFQGTHVPYTF**FGQGTKLEIKR

SEQ ID NO: 11 is the amino acid sequence of a humanized V_H of the ALK48 mAb.

QVQLQQSGAEVKKPGSSSVKVSCKAS**GYAFSSY**ISWVRQAPQGQLEWMGGQ**IYPGDGDT**NYAQKFQGRVTITADE
STSTAYMELSSLRSEDTAVYYC**VRYYYGSSGYFDYW**WGQGMVTV

SEQ ID NO: 12 is the amino acid sequence of a humanized V_L of the ALK48 mAb

10 EIVLTQSPATLSLSPGERATLSCRAS**ESVDNYGISF**AWYQQKPGQAPRLLIY**RASRATGIPARFSGSGSGTDF**
LTISSELEPEDFAVYYCQNNKDPPTFFGQGTKLEIKR

SEQ ID NO: 13 is the amino acid sequence of a humanized V_H of the ALK53 mAb.

QVQLQQSGAEVKKPGSSSVKVSCKAS**GYTFTDHF**ISWVRQAPQGQLEWMGG**LNPYSGGT**NYAQKFQGRVTITADE
STSTAYMELSSLRSEDTAVYYC**CARHNWGAYFDYW**WGQGMVTV

15 **SEQ ID NO: 14** is the amino acid sequence of a humanized V_L of the ALK53 mAb.

EIVLTQSPATLSLSPGERATLSCRAS**KSLLSHNGNTY**AWYQQKPGQAPRLLIY**YMSRATGIPARFSGSGSGTDF**
TLTISSLEPEDFAVYYCMQGLEDPYTFFGQGTKLEIKR

SEQ ID NO: 15 is the amino acid sequence of a humanized V_H of the ALK58 mAb.

QVQLQQSGAEVKKPGSSSVKVSCKAS**GYTFTDYE**ISWVRQAPQGQLEWMGG**IDPETGGT**NYAQKFQGRVTITADE
20 **STSTAYMELSSLRSEDTAVYYCARRRYGSSSF**FDYW WGQGMVTV

SEQ ID NO: 16 is the amino acid sequence of a humanized V_L of the ALK58 mAb.

EIVLTQSPATLSLSPGERATLSCRAS**QDIGNY**AWYQQKPGQAPRLLIY**YTSRATGIPARFSGSGSGTDF**
SLPEPEDFAVYYCQGSALPPTFFGQGTKLEIKR

SEQ ID NO: 17 is the amino acid sequence of a scFv including the V_H and V_L of the ALK15 mAb.

25 DVKLQESGPGVLVAPSQSL SITCTVSG**GFSLTSYAV**SWVRQPPGKGLEWLG**IWSGGAT**NYNSALKSRLSISKDNS
KSQVFLKMNGLQTDITARYY**CAREHYYGSSAMDYW**QGASITVSSGGGGSGGGSGGGSGIVMTQSPLSLPV
LGDAQASISCRSS**QSIVHSYGNTY**LFWYLQKPGQSPKLLIY**RVSNR**SGVPDRFSGSGSGTNFTLKISRVEADM
GVYY**CFQGTHVPYTF**GGGKLEIK

SEQ ID NO: 18 is the amino acid sequence of a scFv including the V_H and V_L of the ALK48 mAb.

30 QVQLQQSGAELVKPGASVKISCKAS**GYAFSSY**WMNWVKQRPKGLEWIGQ**IYPGDGDT**TYNGKFKGKATLTADK
SSSTVYMQNLSTSEDSAVYY**CVRYYYGSSGYFDYW**QGTTTLTVSSGGGGSGGGSGGGSDVQMIQTPDSLAV
SLGQRATISCRAS**ESVDNYGISF**MHWYQQKPGQSPKLLIY**RASN**LESIGIPARFSGSGSRTDFTLTINPVETDDV
ATYY**CQNNKDPPTF**GGGKLEIK

SEQ ID NO: 19 is the amino acid sequence of a scFv including the V_H and V_L of the ALK53 mAb.

35 DVQLQESGPGVLVKTGASVKMSCTAS**GYTFTDHF**MDWVKQSHGKSLEWIG**SLNPYSGGT**SYNQKFKGKATLTVDK
SSSTAYMELNLSLTSVDSAVYY**CARHNWGAYFDYW**QGTTTLTVSSGGGGSGGGSGGGSDIVMTQAAPSVPTP
GESVSI SCRSS**KSLLSHNGNTY**LYWFLQRPQGSPQRLIY**YMSN**LASGVPDRFSGRSGTDFTLRISRVEADV
VYY**CMQGLEDPYTF**GGGKLEIK

SEQ ID NO: 20 is the amino acid sequence of a scFv including the V_H and V_L of the ALK58 mAb.

40 ALQLQQSGAELVRPGASVTLSCAS**GYTFTDYE**MHWVKQTPVHGLEWIG**AIDPETGGT**AYNQKFEGKAILTADK
SSSTAYMELRSLTSEDSVYY**CARRRYGSSSF**FDYW QGTTTLTVSSGGGGSGGGSGGGSDVQMIQTPSSLSA
SLGDRVTISCRAS**QDIGNY**LNWYQQKPDGTVKLLIY**YTSR**LHSGVPSRFSGSGSGTEYSLTISNLEQEDIATYF
CQGSALPPTFGGGKLEIN

SEQ ID NO: 21 is the amino acid sequence of a scFv including humanized V_H and V_L of the ALK15

45 mAb.

QVQLQQSGAEVKKPGSSSVKVSCKAS**GFSLTSYA**ISWVRQAPQGQLEWMGGI**IWSGGAT**NYAQKFQGRVTITADE
STSTAYMELSSLRSEDTAVYYC**CAREHYYGSSAMDYW**WGQGMVTVSSGGGGSGGGSGGGSEIVLTQSPATLS
LSPGERATLSCRAS**QSIVHSYGNTY**AWYQQKPGQAPRLLIY**RVSRATGIPARFSGSGSGTDF**TLTISSLEPEDF
AVYY**CFQGTHVPYTF**FGQGTKLEIKR

SEQ ID NO: 22 is the amino acid sequence of a scFv including humanized V_H and V_L of the ALK48 mAb.

QVQLQQSGAEVKKPGSSVKVSCKAS**GYAFSSY**ISWVRQAPGQGLEWMGGQ**IYPGDGDT**NYAQKFQGRVTITADE
 5 STSTAYMELSSLRSEDTAVYYC**VRYYYGSSGYFDYW**WGQGMVTVSSGGGSGGGGSGGGGSEIVLTQSPATLS
 LSPGERATLSCRAS**ESVDNYGISF**AWYQQKPGQAPRLLIY**RAS**RATGIPARFSGSGSGTDFTLTITSSLEPEDFA
 VYYC**QQNNKDPPTTF**FGQGTKLEIKR

SEQ ID NO: 23 is the amino acid sequence of a scFv including humanized V_H and V_L of the ALK53 mAb.

QVQLQQSGAEVKKPGSSVKVSCKAS**GYTFTDHF**ISWVRQAPGQGLEWMGG**LNPYSGGT**NYAQKFQGRVTITADE
 10 STSTAYMELSSLRSEDTAVYYC**ARHNWGAYFDYW**WGQGMVTVSSGGGSGGGGSGGGGSEIVLTQSPATLSLS
 PGERATLSCRAS**KSLLSNNGNTY**AWYQQKPGQAPRLLIY**YMS**RATGIPARFSGSGSGTDFTLTITSSLEPEDFAV
 YYC**MQGLEDPYTF**FGQGTKLEIKR

SEQ ID NO: 24 is the amino acid sequence of a scFv including humanized V_H and V_L of the ALK58 mAb.

QVQLQQSGAEVKKPGSSVKVSCKAS**GYTFTDYE**ISWVRQAPGQGLEWMGG**IDPETGGT**NYAQKFQGRVTITADE
 15 STSTAYMELSSLRSEDTAVYYC**ARRRYGSSSF**FDYW WGQGMVTVSSGGGSGGGGSGGGGSEIVLTQSPATLS
 LSPGERATLSCRAS**QDIGNY**AWYQQKPGQAPRLLIY**YTS**RATGIPARFSGSGSGTDFTLTITSSLEPEDFAVYYC
QQGSALPPTTFFGQGTKLEIKR

SEQ ID NO: 25 is the amino acid sequence of a peptide linker.

20 GGGGSGGGGSGGGGS

SEQ ID NO: 26 is the amino acid sequence of an exemplary signal peptide.

LLVTSLLLCELPHPAFLIPDT

SEQ ID NO: 27 is the amino acid sequence of an exemplary CD28 transmembrane domain.

IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKPFVVLVVVGGVLACYSLLVTVAFIIFWVR

25 **SEQ ID NO: 28** is the amino acid sequence of an exemplary CD28 signaling domain.

SKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS

SEQ ID NO: 29 is the amino acid sequence of exemplary CD28 transmembrane and signaling domains.

IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKPFVVLVVVGGVLACYSLLVTVAFIIFWVRSK
 30 RSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS

SEQ ID NO: 30 is the amino acid sequence of an exemplary CD8 transmembrane domain.

TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLSLVITLYC

SEQ ID NO: 31 is the amino acid sequence of an exemplary CD8 signaling domain.

FVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLL
 35 LSLVITLYCNHRNR

SEQ ID NO: 32 is the amino acid sequence of an exemplary CD137 signaling domain.

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL

SEQ ID NO: 33 is the amino acid sequence of an exemplary CD137 signaling domain.

RFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL

40 **SEQ ID NO: 34** is the amino acid sequence of an exemplary CD3 zeta signaling domain.

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRRGRDPEMGKPRRKNPQEGLYNELQKD
 KMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 35 is the amino acid sequence of an exemplary CH2CH3 spacer domain.

EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
 45 VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTT

LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSFSVMHEALHNHYTQKSLSLSPGKKDPK

SEQ ID NO: 36 is an exemplary nucleic acid sequence encoding a CH2CH3 spacer domain.

gaaccaagtcatgcgataagaccacactgtccaccctgtccagccctgaactgctcgagggtccgtcagtggttttcccccgaagcctaaggacactct
5 gatgatctctcgaccctgaagtgactgtcgctgctgagcgtgtcacacgaggatcccagggtgaagtccaactggtatgtggacggggggaagtgcataa
tgctaagaccaagcccaggaggagaacaatacaactcaacctaccgctgggtgtccgtgctcaccgtcctcatcaagactggctgaacggaaaagagtataagt
gcaagtctccaataaggtctgccagcccctatcgaaaagaccatttcaaaaggccaaggggcagcctagagagcccaagtgtacaccttctcctcaaga
gatgagctcactaagaatcaggtcagcctgactgtctgtgaaaggcttctatccagcgcataatgcccgaatgggaagcaatggacaaccagagaacaact
acaagaccaccccgctgtgctggactccgacggctcttcttcttactcaaaagctgacgcgcgataagagccggtgggcaacaggggaatgtgtcagctgctc
10 cgtcatgcacgaggctctccataaccactacaccagaaaagcctgtcttcttccgggcaaaaaggacccaaag

SEQ ID NO: 37 is the amino acid sequence of the transmembrane and intracellular domains of an exemplary 2nd generation CAR including a CD28 transmembrane domain and a CD3 zeta signaling domain ("28z").

AAAEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKPFVWLVVVGGLACYSLLVTVAFIIFWV
15 RSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELN
LGRREEYDVLDRRRGRDPEMGGKPRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRRGKHDGLY
QGLSTATKDTYDALHMQALPPR

SEQ ID NO: 38 is an exemplary nucleic acid sequence encoding the transmembrane and intracellular domains of an exemplary 2nd generation CAR including a CD28 transmembrane domain and a CD3 zeta signaling domain ("28z").

gccccgcaattgaagtgtatgtatctctctctacctagacaatgagaagagcaatggaaccattatccatgtgaagggaacaccttgtccaagtccctattt
cccggaccttcaagcccttttgggtgctggtggtggtggggagctctgctgtctatagcttctagtaacagtggcccttattatttctgggtgaggagtaaga
ggagcaggctcctgcacagtactacatgaacatgactccccgcccccggccaccgcgaagcattaccagccctatgccccaccacgcgacttcgcag
cctatcgtccagagtgaagttcagcaggagcgcagacgcccccgctaccagcagggccagaaccagctctataacgagctcaatctaggacgaagagag
25 gactacagatgttttgacaagagacgtggccgggaccctgagatgggggaaagccgagaaggaagaacctcaggaaggcctgtacaatgaactgcagaa
agataagatggcggagccctacagttagattggatgaaagcgagcgcggaggggcaaggggcacgatggcctttaccaggggtctcagtacagccacca
aggacacctacgacgcccttcacatgcaggccctgccccctcgctaa

SEQ ID NO: 39 is the amino acid sequence of the transmembrane and intracellular domains of an exemplary 2nd generation CAR including a CD8 transmembrane domain, CD137 (4-1BB) signaling domain, and a CD3 zeta signaling domain ("BBz").

AAATTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITL
YCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSRFPEEEEEGGCELRVKFSRSADAPAYKQGQNQLYNE
LNLGRREEYDVLDRRRGRDPEMGGKPRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRRGKHDG
LYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 40 is an exemplary nucleic acid sequence encoding the transmembrane and intracellular domains of an exemplary 2nd generation CAR including a CD8 transmembrane domain, CD137 (4-1BB) signaling domain, and a CD3 zeta signaling domain ("BBz").

gccccgcaaccacgacgccagcgcgcgaccaccaacaccggcccccaccatcgctgcagccccgtccctgcgccagaggcgtgccggccagcg
gcggggggcgcagtgacacgaggggctgacttcgctgtgatatctacatctggcgcccttggccgggactgtgggtctctcctgtcactggttctc
40 acccttactgcaaacggggcagaagaaactcctgtatattcaacaaccatttatgagaccagtacaaactactcaagaggaagatggctgtagctgccgatt
tcagaagaagaagaggagatgtgaactgagagtgaagttcagcaggagcgcagacgcccccgctacaagcagggccagaaccagctctataacgag
ctcaatctaggacgaagagaggagtacgatgttttgacaagagacgtggccgggaccctgagatgggggaaagccgagaaggaagaacctcaggaag
gcctgtacaatgaactgcagaaagataagatggcggagccctacagttagattggatgaaagcgagcgcggagggggcaaggggcacgatggcctttac
cagggctcagtacagccaccaaggacacctacgacgcccttcacatgcaggccctgccccctcgctaa

SEQ ID NO: 41 is the amino acid sequence of the transmembrane and intracellular domains of an exemplary 3rd generation CAR including a CD8 transmembrane domain, a CD28 signaling domain, a CD137 (4-1BB) signaling domain, and a CD3 zeta signaling domain ("28BBz").

AAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTC
 GVLLLSLVITLYCNHRNRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRFSVVKRGR
 KKLLYIFKQPFMRPVQTTQEEDGCSRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRRE
 EYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLST
 5 ATKDTYDALHMQUALPPR

SEQ ID NO: 42 is an exemplary nucleic acid sequence encoding the transmembrane and intracellular domains of an exemplary 3rd generation CAR including a CD8 transmembrane domain, a CD28 signaling domain, a CD137 (4-1BB) signaling domain, and a CD3 zeta signaling domain ("28BBz").

gcggccgcattcgtgccggtctcctgcagcgaagccaccacgacgccagcgcgcgaccaccaacaccgcgccaccatcgctgcagccctgtc
 10 cctgcgcccagaggcgtgccggccagcggcgggggcgagtcacacgagggggcctgacttcgctgtgatactacatctggcgcccttgccggga
 cttgtgggtctctcctgtcactggtatcacccttactgaaccacaggaacaggagtaagaggagcagcctctgcacagtactacatgaacatgactcc
 ccgcccggggcccccaccgcaagcattaccagccctatgccccaccacgcgacttcgagcctatcgtcccggttctctgtttaaaggggcagaaaga
 agctcctgtatatattcaacaaccatttatgagaccagtacaaactactcaagaggaagatggctgtagctgccgatttcagaagaagaaggaggatgga
 actgagagtgaagttcagcaggagcgcagacgccccgcgtaccagcagggccagaaccagctctataacgagctcaatctaggacgaagagaggagtacg
 15 atgttttgacaagagacgtggccgggaccctgagatgggggaaagccgagaaggagaaccctcaggaaggcctgtacaatgaactgcagaaagataag
 atggcgaggcctacagtgagattgggatgaaggcgagcggcgaggggcaaggggcacgatggcctttaccagggtctcagtacagccaccaaggaca
 cctacgacgcccttcacatgcaggccctgccccctcgctaa

SEQ ID NOS: 43-90 are amino acid sequences of exemplary chimeric antigen receptors including an antigen binding domain that specifically binds to the extracellular domain of ALK protein.

20 **SEQ ID NOS: 91-114** are exemplary nucleic acid sequences encoding chimeric antigen receptors including an antigen binding domain that specifically binds to the extracellular domain of ALK protein.

SEQ ID NO: 115 is an exemplary amino acid sequence of human ALK protein (UniProt Acc. No. Q9UM73, incorporated by reference herein as present in the database on October 31, 2013). The extracellular domain is composed of amino acids 19-1038.

25 **SEQ ID NO: 116** is an exemplary nucleic acid sequence encoding human ALK protein (GENBANK Acc. No. NP_004295.2, incorporated by reference herein as present in the database on October 31, 2013).

DETAILED DESCRIPTION

The developmentally-regulated cell surface receptor tyrosine kinase ALK is known to be expressed as
 30 a tumor-associated antigen as a fusion protein resulting from a chromosomal translocation. Cancer-associated ALK was first described as a 2;5 chromosomal translocation associated with nucleophosmin (NPM) in anaplastic large cell leukemia (ALCL; Morris et al., (1994) *Science*, 263, 1281-1284). The fusion protein was composed of the intracellular domain of NPM and the intracellular kinase domain of ALK. However, ALK fusion proteins are not known to be expressed on the cell surface.

35 Chimeric antigen receptors are an example of synthetic biology used for adoptive immunotherapy for cancer, wherein a protein not encoded by the genome, is designed in the laboratory and is expressed in normal human tissues for a therapeutic effect. There are a number of CAR constructs in clinical trials, but most of the activity has been in hematologic malignancies, most notably in B cell leukemias (Lee et al., (2012) *Clin Cancer Res*, 18, 2780-2790; Sadelain et al., (2013) *Cancer Discov*, 3, 388-398). This is due in part to the
 40 acceptable safety profile of B cell antigen-specific CAR-modified T cells.

Chimeric antigen receptors are composed of an extracellular antigen binding domain, transmembrane domain, and one or more intracellular T cell signaling domains (Long et al., (2013) *Oncoimmunology*, 2, e23621). In addition to variations in these structural design elements, how these elements are linked to one

another by joining domains provides another level of variability. First-generation CARs include only the CD3 zeta chain-derived cytoplasmic signaling domain. Second generation CARs additionally include CD28 or CD137-derived signaling domains. Third generation CARs encode three signaling domains and may also include sequences derived from CD137, OX40, or GITR.

5 An overarching rule for the assembly of CAR domains into a functional chimeric receptor that is effective for cancer therapy has yet to be developed, particularly in the context of CARs for use to treat non-hematological cancers. Accordingly, recombinant scFv domains specific for the extracellular domain of ALK were designed and synthesized, and linked to CAR structural domains to create a series of CARs specific for ALK. The ALK-specific CARs have utility, for example, as a new generation of adoptive immunotherapeutic
10 agents for treatment of tumors, such as neuroblastoma.

I. Summary of Terms

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology can be found in Benjamin Lewin, *Genes VII*, published by Oxford
15 University Press, 1999; Kendrew *et al.* (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994; and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by VCH Publishers, Inc., 1995; and other similar references.

As used herein, the singular forms “a,” “an,” and “the,” refer to both the singular as well as plural, unless the context clearly indicates otherwise. For example, the term “an antigen” includes single or plural
20 antigens and can be considered equivalent to the phrase “at least one antigen.” As used herein, the term “comprises” means “includes.” Thus, “comprising an antigen” means “including an antigen” without excluding other elements. The phrase “and/or” means “and” or “or.” It is further to be understood that any and all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for descriptive purposes, unless otherwise indicated. Although
25 many methods and materials similar or equivalent to those described herein can be used, particular suitable methods and materials are described below. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. To facilitate review of the various embodiments, the following explanations of terms are provided:

30 **Administration:** To provide or give to a subject an agent, for example, a composition that includes a monoclonal antibody or antigen binding fragment that specifically binds ALK, or a CAR including the antigen binding fragment, by any effective route. Exemplary routes of administration include, but are not limited to, oral, injection (such as subcutaneous, intramuscular, intradermal, intraperitoneal, and intravenous), sublingual, rectal, transdermal (for example, topical), intranasal, vaginal, and inhalation routes.

35 **Agent:** Any substance or any combination of substances that is useful for achieving an end or result; for example, a substance or combination of substances useful for decreasing or reducing tumor growth in a subject. Agents include effector molecules and detectable markers. In some embodiments, the agent is a chemotherapeutic agent. The skilled artisan will understand that particular agents may be useful to achieve more than one result; for example, an agent may be useful as both a detectable marker and a chemotherapeutic
40 agent.

ALK inhibitor: An agent that that inhibits or decreases ALK activity, such as ALK tyrosine kinase activity. In some examples, an ALK inhibitor can be a small molecule, a protein (such as an antibody), or a nucleic acid (such as an antisense molecule). An ALK inhibitor may inhibit or decrease binding of a ligand (such as pleiotrophin) to ALK and thus decrease ALK tyrosine kinase activity. An ALK inhibitor may also directly inhibit or decrease ALK tyrosine kinase activity, for example, an ATP-competitive inhibitor (such as crizotinib). Molecules that decrease or inhibit expression of ALK, such as antisense molecules, are also ALK inhibitors. The ALK inhibitor may specifically inhibit ALK tyrosine kinase activity or may inhibit other receptor tyrosine kinase activity (such as c-Met/HGFR activity), in addition to inhibiting ALK tyrosine kinase activity. In one example, and ALK inhibitor is the protein kinase inhibitor (PKI) crizotinib. PKIs or other agents that affect ALK may render ALK positive cancers more susceptible to immune targeting with anti-ALK antibody or with CAR-expressing T cells specific for ALK.

Amino acid substitution: The replacement of one amino acid in peptide with a different amino acid.

Anaplastic lymphoma kinase (ALK): A receptor tyrosine kinase belonging to the insulin receptor superfamily. The protein includes an extracellular domain, a hydrophobic stretch corresponding to a single pass transmembrane region, and an intracellular kinase domain. Human ALK sequences are publically available, for example from the GENBANK® sequence database (*e.g.*, accession numbers NP_004295 (protein), and NM_004304 (nucleic acid), respectively, as available on October 31, 2013, which are hereby incorporated by reference in their entirety). Human ALK sequences can also be found at UniProt Acc. No Q9UM73, incorporated by reference herein as present in the database on October 31, 2013). The extracellular domain of human ALK is composed of amino acids 19-1038 of UniProt Acc. No Q9UM73. One of ordinary skill in the art can identify additional ALK nucleic acid and protein sequences, including ALK variants.

Antigen Binding Domain and Antibody: A polypeptide or polypeptides that specifically bind and recognizes an analyte (antigen) such as ALK protein or an antigenic fragment thereof. As used herein, and “antigen binding domain” can include an antibody and antigen binding fragments thereof. The term “antibody” is used herein in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), and antigen binding fragments thereof, so long as they exhibit the desired antigen-binding activity. Non-limiting examples of antibodies include, for example, intact immunoglobulins and variants and fragments thereof known in the art that retain binding affinity for the antigen.

A “monoclonal antibody” is an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, 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 epitope. 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. In some examples, a monoclonal antibody is an antibody produced by a single clone of B-lymphocytes or by a cell into which nucleic acid encoding the light and heavy variable regions of the antibody of a single antibody (or an antigen binding fragment thereof) have been transfected, or a progeny thereof. In some examples monoclonal antibodies are isolated from a subject. Monoclonal antibodies can have conservative amino acid substitutions which have substantially no effect on antigen binding or other immunoglobulin functions. Exemplary methods of production of monoclonal

antibodies are known, for example, see Harlow & Lane, *Antibodies, A Laboratory Manual*, 2nd ed. Cold Spring Harbor Publications, New York (2013).

Typically, an immunoglobulin has heavy (H) chains and light (L) chains interconnected by disulfide bonds. Immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable domain genes. There are two types of light chain, lambda (λ) and kappa (κ). There are five main heavy chain classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE.

Each heavy and light chain contains a constant region (or constant domain) and a variable region (or variable domain; see, e.g., Kindt et al. *Kuby Immunology*, 6^{sup.th} ed., W.H. Freeman and Co., page 91 (2007).) In several embodiments, the heavy and the light chain variable regions combine to specifically bind the antigen. In additional embodiments, only the heavy chain variable region is required. For example, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the absence of light chain (see, e.g., Hamers-Casterman *et al.*, *Nature*, 363:446-448, 1993; Sheriff *et al.*, *Nat. Struct. Biol.*, 3:733-736, 1996). References to “V_H” or “VH” refer to the variable region of an antibody heavy chain, including that of an antigen binding fragment, such as Fv, scFv, dsFv or Fab. References to “V_L” or “VL” refer to the variable domain of an antibody light chain, including that of an Fv, scFv, dsFv or Fab.

Light and heavy chain variable regions contain a “framework” region interrupted by three hypervariable regions, also called “complementarity-determining regions” or “CDRs” (see, e.g., Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, U.S. Department of Health and Human Services, 1991). The sequences of the framework regions of different light or heavy chains are relatively conserved within a species. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDRs in three-dimensional space.

The CDRs are primarily responsible for binding to an epitope of an antigen. The amino acid sequence boundaries of a given CDR can be readily determined using any of a number of well-known schemes, including those described by Kabat et al. (“Sequences of Proteins of Immunological Interest,” 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991; “Kabat” numbering scheme), Al-Lazikani et al., (*JMB* 273,927-948, 1997; “Chothia” numbering scheme), and Lefranc et al. (“IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains,” *Dev. Comp. Immunol.*, 27:55-77, 2003; “IMGT” numbering scheme). The CDRs of each chain are typically referred to as CDR1, CDR2, and CDR3 (from the N-terminus to C-terminus), and are also typically identified by the chain in which the particular CDR is located. Thus, a V_H CDR3 is the CDR3 from the variable domain of the heavy chain of the antibody in which it is found, whereas a V_L CDR1 is the CDR1 from the variable domain of the light chain of the antibody in which it is found. Light chain CDRs are sometimes referred to as LCDR1, LCDR2, and LCDR3. Heavy chain CDRs are sometimes referred to as LCDR1, LCDR2, and LCDR3.

An “antigen binding fragment” is a portion of a full length antibody that retains the ability to specifically recognize the cognate antigen, as well as various combinations of such portions. Non-limiting examples of antigen binding fragments include Fv, Fab, Fab', Fab'-SH, F(ab')₂; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments. Antibody fragments include antigen binding fragments either produced by the modification of whole antibodies

or those synthesized *de novo* using recombinant DNA methodologies (see, e.g., Kontermann and Dubel (Ed), Antibody Engineering, Vols. 1-2, 2nd Ed., Springer Press, 2010).

A single-chain antibody (scFv) is a genetically engineered molecule containing the V_H and V_L domains of one or more antibody(ies) linked by a suitable polypeptide linker as a genetically fused single chain molecule (see, for example, Bird *et al.*, *Science*, 242:423-426, 1988; Huston *et al.*, *Proc. Natl. Acad. Sci.*, 85:5879-5883, 1988; Ahmad *et al.*, *Clin. Dev. Immunol.*, 2012, doi:10.1155/2012/980250; Marbry, *IDrugs*, 13:543-549, 2010). The intramolecular orientation of the V_H-domain and the V_L-domain in a scFv, is typically not decisive for scFvs. Thus, scFvs with both possible arrangements (V_H-domain-linker domain-V_L-domain; V_L-domain-linker domain-V_H-domain) may be used.

In a dsFv the heavy and light chain variable chains have been mutated to introduce a disulfide bond to stabilize the association of the chains. Diabodies also are included, which are bivalent, bispecific antibodies in which V_H and V_L domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see, for example, Holliger *et al.*, *Proc. Natl. Acad. Sci.*, 90:6444-6448, 1993; Poljak *et al.*, *Structure*, 2:1121-1123, 1994).

Antibodies also include genetically engineered forms such as chimeric antibodies (such as humanized murine antibodies) and heteroconjugate antibodies (such as bispecific antibodies). See also, *Pierce Catalog and Handbook*, 1994-1995 (Pierce Chemical Co., Rockford, IL); Kubly, J., *Immunology*, 3rd Ed., W.H. Freeman & Co., New York, 1997.

Non-naturally occurring antibodies can be constructed using solid phase peptide synthesis, can be produced recombinantly, or can be obtained, for example, by screening combinatorial libraries consisting of variable heavy chains and variable light chains as described by Huse *et al.*, *Science* 246:1275-1281 (1989), which is incorporated herein by reference. These and other methods of making, for example, chimeric, humanized, CDR-grafted, single chain, and bifunctional antibodies, are well known to those skilled in the art (Winter and Harris, *Immunol. Today* 14:243-246 (1993); Ward *et al.*, *Nature* 341:544-546 (1989); Harlow and Lane, *supra*, 1988; Hilyard *et al.*, *Protein Engineering: A practical approach* (IRL Press 1992); Borrabeck, *Antibody Engineering*, 2d ed. (Oxford University Press 1995); each of which is incorporated herein by reference).

An “antibody that binds to the same epitope” as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more. Antibody competition assays are known, and an exemplary competition assay is provided herein.

A “humanized” antibody or antigen binding fragment includes a human framework region and one or more CDRs from a non-human (such as a mouse, rat, or synthetic) antibody or antigen binding fragment. The non-human antibody or antigen binding fragment providing the CDRs is termed a “donor,” and the human antibody or antigen binding fragment providing the framework is termed an “acceptor.” In one embodiment, all the CDRs are from the donor immunoglobulin in a humanized immunoglobulin. Constant regions need not be present, but if they are, they can be substantially identical to human immunoglobulin constant regions, such as at least about 85-90%, such as about 95% or more identical. Hence, all parts of a humanized antibody or

antigen binding fragment, except possibly the CDRs, are substantially identical to corresponding parts of natural human antibody sequences.

A “chimeric antibody” is an antibody which includes sequences derived from two different antibodies, which typically are of different species. In some examples, a chimeric antibody includes one or more CDRs and/or framework regions from one human antibody and CDRs and/or framework regions from another human antibody.

A “fully human antibody” or “human antibody” is an antibody which includes sequences from (or derived from) the human genome, and does not include sequence from another species. In some embodiments, a human antibody includes CDRs, framework regions, and (if present) an Fc region from (or derived from) the human genome. Human antibodies can be identified and isolated using technologies for creating antibodies based on sequences derived from the human genome, for example by phage display or using transgenic animals (see, e.g., Barbas et al. *Phage display: A Laboratory Manual*. 1st Ed. New York: Cold Spring Harbor Laboratory Press, 2004. Print.; Lonberg, Nat. Biotech., 23: 1117-1125, 2005; Lonenberg, Curr. Opin. Immunol., 20:450-459, 2008).

An antibody may have one or more binding sites. If there is more than one binding site, the binding sites may be identical to one another or may be different. For instance, a naturally-occurring immunoglobulin has two identical binding sites, a single-chain antibody or Fab fragment has one binding site, while a bispecific or bifunctional antibody has two different binding sites.

Biological sample: A sample obtained from a subject. Biological samples include all clinical samples useful for detection of disease or infection (for example, cancer) in subjects, including, but not limited to, cells, tissues, and bodily fluids, such as blood, derivatives and fractions of blood (such as serum), cerebrospinal fluid; as well as biopsied or surgically removed tissue, for example tissues that are unfixed, frozen, or fixed in formalin or paraffin. In a particular example, a biological sample is obtained from a subject having or suspected of having a tumor; for example, a subject having or suspected of having a neuroblastoma. In some examples, the subject has or is suspected of having a carcinoma.

Chemotherapeutic agent: Any chemical agent with therapeutic usefulness in the treatment of diseases characterized by abnormal cell growth. For example, chemotherapeutic agents are useful for the treatment of neuroblastoma. Particular examples of additional therapeutic agents that can be used include microtubule binding agents, DNA intercalators or cross-linkers, DNA synthesis inhibitors, DNA and RNA transcription inhibitors, antibodies, enzymes, enzyme inhibitors, gene regulators, and angiogenesis inhibitors. In one embodiment, a chemotherapeutic agent is a radioactive compound. One of skill in the art can readily identify a chemotherapeutic agent of use (see for example, Slapak and Kufe, *Principles of Cancer Therapy*, Chapter 86 in Harrison's Principles of Internal Medicine, 14th edition; Perry *et al.*, *Chemotherapy*, Ch. 17 in Abeloff, *Clinical Oncology* 2nd ed., © 2000 Churchill Livingstone, Inc; Baltzer, L., Berkery, R. (eds): *Oncology Pocket Guide to Chemotherapy*, 2nd ed. St. Louis, Mosby-Year Book, 1995; Fischer, D.S., Knobf, M.F., Durivage, H.J. (eds): *The Cancer Chemotherapy Handbook*, 4th ed. St. Louis, Mosby-Year Book, 1993; Chabner and Longo, *Cancer Chemotherapy and Biotherapy: Principles and Practice* (4th ed.). Philadelphia: Lippincott Williams & Wilkins, 2005; Skeel, *Handbook of Cancer Chemotherapy* (6th ed.). Lippincott Williams & Wilkins, 2003). Combination chemotherapy is the administration of more than one agent to treat cancer.

Chimeric Antigen Receptor (CAR): An engineered T cell receptor having an extracellular antibody-derived targeting domain (such as an scFv) joined to one or more intracellular signaling domains of a T cell receptor. A “**chimeric antigen receptor T cell**” or “**CAR T cell**” is a T cell expressing a CAR, and has antigen specificity determined by the antibody-derived targeting domain of the CAR. Methods of making CARs (e.g., for treatment of cancer) are available (see, e.g., Park *et al.*, *Trends Biotechnol.*, 29:550-557, 2011; Grupp *et al.*, *N Engl J Med.*, 368:1509-1518, 2013; Han *et al.*, *J. Hematol Oncol.*, 6:47, 2013; Haso *et al.*, (2013) *Blood*, 121, 1165-1174; PCT Pubs. WO2012/079000, WO2013/059593; and U.S. Pub. 2012/0213783, each of which is incorporated by reference herein in its entirety.)

Conditions sufficient to form an immune complex: Conditions which allow an antibody or antigen binding fragment thereof to bind to its cognate epitope to a detectably greater degree than, and/or to the substantial exclusion of, binding to substantially all other epitopes. Conditions sufficient to form an immune complex are dependent upon the format of the binding reaction and typically are those utilized in immunoassay protocols or those conditions encountered *in vivo*. See Harlow & Lane, *supra*, for a description of immunoassay formats and conditions. The conditions employed in the methods are “physiological conditions” which include reference to conditions (e.g., temperature, osmolarity, pH) that are typical inside a living mammal or a mammalian cell. While it is recognized that some organs are subject to extreme conditions, the intra-organismal and intracellular environment normally lies around pH 7 (e.g., from pH 6.0 to pH 8.0, more typically pH 6.5 to 7.5), contains water as the predominant solvent, and exists at a temperature above 0°C and below 50°C. Osmolarity is within the range that is supportive of cell viability and proliferation.

Conjugate: A complex of two molecules linked together, for example, linked together by a covalent bond. In one embodiment, an antibody is linked to an effector molecule; for example, an antibody that specifically binds to ALK covalently linked to an effector molecule. The linkage can be by chemical or recombinant means. In one embodiment, the linkage is chemical, wherein a reaction between the antibody moiety and the effector molecule has produced a covalent bond formed between the two molecules to form one molecule. A peptide linker (short peptide sequence) can optionally be included between the antibody and the effector molecule. Because conjugates can be prepared from two molecules with separate functionalities, such as an antibody and an effector molecule, they are also sometimes referred to as “chimeric molecules.”

Conservative variants: “Conservative” amino acid substitutions are those substitutions that do not substantially decrease the binding affinity of an antibody for an antigen (for example, the binding affinity of an antibody for ALK). For example, a human antibody that specifically binds ALK can include at most about 1, at most about 2, at most about 5, at most about 10, or at most about 15 conservative substitutions and specifically bind the ALK polypeptide. The term conservative variation also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid, provided that antibody retains binding affinity for ALK. Non-conservative substitutions are those that reduce an activity or binding to ALK.

Conservative amino acid substitution tables providing functionally similar amino acids are well known to one of ordinary skill in the art. The following six groups are examples of amino acids that are considered to be conservative substitutions for one another:

1) Alanine (A), Serine (S), Threonine (T);

2) Aspartic acid (D), Glutamic acid (E);

3) Asparagine (N), Glutamine (Q);

4) Arginine (R), Lysine (K);

5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and

6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

Contacting: Placement in direct physical association; includes both in solid and liquid form, which can take place either *in vivo* or *in vitro*. Contacting includes contact between one molecule and another molecule, for example the amino acid on the surface of one polypeptide, such as an antigen, that contacts another polypeptide, such as an antibody. Contacting can also include contacting a cell for example by placing an antibody in direct physical association with a cell.

Control: A reference standard. In some embodiments, the control is a negative control, such as tissue sample obtained from a patient that does not have cancer, or a tissue sample from a tissue that is non-cancerous. In other embodiments, the control is a positive control, such as a tissue sample obtained from a patient diagnosed with cancer, or a tissue sample from a cancerous tissue. In still other embodiments, the control is a historical control or standard reference value or range of values (such as a previously tested control sample, such as a group of cancer patients with known prognosis or outcome, or group of samples that represent baseline or normal values).

A difference between a test sample and a control can be an increase or conversely a decrease. The difference can be a qualitative difference or a quantitative difference, for example a statistically significant difference. In some examples, a difference is an increase or decrease, relative to a control, of at least about 5%, such as at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, at least about 300%, at least about 350%, at least about 400%, or at least about 500%.

Crizotinib: A receptor tyrosine kinase inhibitor that inhibits ALK. Crizotinib (also known as PF-02341066 or XALKORI, Pfizer) is an orally available selective ATP-competitive small molecule inhibitor of ALK and c-Met/HGFR tyrosine kinases and their oncogenic variants. See, *e.g.*, U.S. Pat. Nos. 7,230,098; 7,825,137; 7,858,643; and 8,217,057; each of which is incorporated herein by reference in its entirety. Crizotinib can be used to treat patients with ALK-positive tumors.

Decrease or Reduce: To reduce the quality, amount, or strength of something; for example a reduction in tumor burden. In one example, a therapy reduces a tumor (such as the size of a tumor, the number of tumors, the metastasis of a tumor, or combinations thereof), or one or more symptoms associated with a tumor, for example as compared to the response in the absence of the therapy. In a particular example, a therapy decreases the size of a tumor, the number of tumors, the metastasis of a tumor, or combinations thereof, subsequent to the therapy, such as a decrease of at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%. Such decreases can be measured using the methods disclosed herein.

Degenerate variant: In the context of the present disclosure, a “degenerate variant” refers to a polynucleotide encoding a protein (for example, an antibody that specifically binds ALK) that includes a sequence that is degenerate as a result of the genetic code. There are twenty natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included as

long as the amino acid sequence of the antibody that binds ALK encoded by the nucleotide sequence is unchanged.

Detectable marker: A detectable molecule (also known as a label) that is conjugated directly or indirectly to a second molecule, such as an antibody, to facilitate detection of the second molecule. For example, the detectable marker can be capable of detection by ELISA, spectrophotometry, flow cytometry, microscopy or diagnostic imaging techniques (such as CT scans, MRIs, ultrasound, fiberoptic examination, and laparoscopic examination). Specific, non-limiting examples of detectable markers include fluorophores, chemiluminescent agents, enzymatic linkages, radioactive isotopes and heavy metals or compounds (for example super paramagnetic iron oxide nanocrystals for detection by MRI). In one example, a “labeled antibody” refers to incorporation of another molecule in the antibody. For example, the label is a detectable marker, such as the 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). Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (such as ³⁵S or ¹³¹I), fluorescent labels (such as fluorescein isothiocyanate (FITC), rhodamine, lanthanide phosphors), enzymatic labels (such as horseradish peroxidase, beta-galactosidase, luciferase, alkaline phosphatase), chemiluminescent markers, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (such as a leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags), or magnetic agents, such as gadolinium chelates. In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance. Methods for using detectable markers and guidance in the choice of detectable markers appropriate for various purposes are discussed for example in Sambrook *et al.* (Molecular Cloning: A Laboratory Manual, 4th ed, Cold Spring Harbor, New York, 2012) and Ausubel *et al.* (In Current Protocols in Molecular Biology, John Wiley & Sons, New York, through supplement 104, 2013).

Detecting: To identify the existence, presence, or fact of something. General methods of detecting are known to the skilled artisan and may be supplemented with the protocols and reagents disclosed herein. For example, included herein are methods of detecting an ALK-positive tumor in a subject.

Effector molecule: A molecule intended to have or produce a desired effect; for example, a desired effect on a cell to which the effector molecule is targeted. Effector molecules include such molecules as polypeptides, radioisotopes and small molecules. Non-limiting examples of effector molecules include toxins, chemotherapeutic agents and anti-angiogenic agents. The skilled artisan will understand that some effector molecules may have or produce more than one desired effect. In one example, an effector molecule is the portion of a chimeric molecule, for example a chimeric molecule that includes a disclosed antibody or fragment thereof, that is intended to have a desired effect on a cell to which the chimeric molecule is targeted.

Epitope: An antigenic determinant. These are particular chemical groups or peptide sequences on a molecule that are antigenic, *i.e.* that elicit a specific immune response. An antibody specifically binds a particular antigenic epitope on a polypeptide. In some examples a disclosed antibody specifically binds to an epitope on ALK.

Expressed: Translation of a nucleic acid into a protein. Proteins may be expressed and remain intracellular, become a component of the cell surface membrane, or be secreted into the extracellular matrix or medium.

Expression Control Sequences: Nucleic acid sequences that regulate the expression of a heterologous nucleic acid sequence to which it is operatively linked. Expression control sequences are operatively linked to a nucleic acid sequence when the expression control sequences control and regulate the transcription and, as appropriate, translation of the nucleic acid sequence. Thus expression control sequences can include appropriate promoters, enhancers, transcription terminators, a start codon (i.e., ATG) in front of a protein-encoding gene, splicing signal for introns, maintenance of the correct reading frame of that gene to permit proper translation of mRNA, and stop codons. The term "control sequences" is intended to include, at a minimum, components whose presence can influence expression, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences. Expression control sequences can include a promoter.

A promoter is a minimal sequence sufficient to direct transcription. Also included are those promoter elements which are sufficient to render promoter-dependent gene expression controllable for cell-type specific, tissue-specific, or inducible by external signals or agents; such elements may be located in the 5' or 3' regions of the gene. Both constitutive and inducible promoters are included (see for example, Bitter *et al.*, *Methods in Enzymology* 153:516-544, 1987). For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage lambda, plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like may be used. In one embodiment, when cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (such as metallothionein promoter) or from mammalian viruses (such as the retrovirus long terminal repeat; the adenovirus late promoter; the vaccinia virus 7.5K promoter) can be used. Promoters produced by recombinant DNA or synthetic techniques may also be used to provide for transcription of the nucleic acid sequences. A polynucleotide can be inserted into an expression vector that contains a promoter sequence which facilitates the efficient transcription of the inserted genetic sequence of the host. The expression vector typically contains an origin of replication, a promoter, as well as specific nucleic acid sequences that allow phenotypic selection of the transformed cells.

Expression vector: A vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis- acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those known in the art, such as cosmids, plasmids (e.g., naked or contained in liposomes) and viruses (e.g., lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide.

Immune complex: The binding of antibody or antigen binding fragment (such as an antigen binding domain on a CAR) to a soluble antigen forms an immune complex. The formation of an immune complex can be detected through conventional methods known to the skilled artisan, for instance immunohistochemistry, immunoprecipitation, flow cytometry, immunofluorescence microscopy, ELISA, immunoblotting (for example, Western blot), magnetic resonance imaging, CT scans, X-ray and affinity chromatography. Immunological binding properties of selected antibodies may be quantified using methods well known in the art.

Inhibiting or Treating a Disease: A therapeutic intervention (for example, administration of a therapeutically effective amount of an antibody that specifically binds ALK or a conjugate thereof) that reduces a sign or symptom of a disease or pathological condition related to a disease (such as a tumor). Treatment can also induce remission or cure of a condition, such as a tumor. In particular examples, treatment includes preventing a tumor, for example by inhibiting the full development of a tumor, such as preventing development of a metastasis or the development of a primary tumor. Prevention does not require a total absence of a tumor.

Reducing a sign or symptom of a disease or pathological condition related to a disease, refers to any observable beneficial effect of the treatment. Reducing a sign or symptom associated with a tumor can be evidenced, for example, by a delayed onset of clinical symptoms of the disease in a susceptible subject (such as a subject having a tumor which has not yet metastasized), a reduction in severity of some or all clinical symptoms of the disease, a slower progression of the disease (for example by prolonging the life of a subject having tumor), a reduction in the number of relapses of the disease, an improvement in the overall health or well-being of the subject, or by other parameters well known in the art that are specific to the particular tumor. A “prophylactic” treatment is a treatment administered to a subject who does not exhibit signs of a disease or exhibits only early signs for the purpose of decreasing the risk of developing pathology.

Isolated: A biological component (such as a nucleic acid, peptide, protein or protein complex, for example an antibody) that has been substantially separated, produced apart from, or purified away from other biological components in the cell of the organism in which the component naturally occurs, that is, other chromosomal and extra-chromosomal DNA and RNA, and proteins. Thus, isolated nucleic acids, peptides and proteins include nucleic acids and proteins purified by standard purification methods. The term also embraces nucleic acids, peptides and proteins prepared by recombinant expression in a host cell, as well as, chemically synthesized nucleic acids. A isolated nucleic acid, peptide or protein, for example an antibody, can be at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% pure.

K_D: The dissociation constant for a given interaction, such as a polypeptide ligand interaction or an antibody antigen interaction. For example, for the bimolecular interaction of an antibody or antigen binding fragment (such as an ALK specific antibody or an antigen binding fragment thereof) and an antigen (such as ALK protein) it is the concentration of the individual components of the bimolecular interaction divided by the concentration of the complex.

Linker: A bi-functional molecule that can be used to link two molecules into one contiguous molecule, for example, to link an effector molecule to an antibody. In some embodiments, the provided conjugates include a linker between the effector molecule or detectable marker and an antibody. In some embodiments, the linker is cleavable under intracellular conditions, such that cleavage of the linker releases the effector molecule or detectable marker from the antibody in the intracellular environment. In yet other embodiments, the linker is not cleavable and the effector molecule or detectable marker can be released, for example, by antibody degradation. In some cases, a linker is a peptide within an antigen binding fragment (such as an Fv fragment) which serves to indirectly bond the variable heavy chain to the variable light chain.

The terms “conjugating,” “joining,” “bonding,” or “linking” refer to making two molecules into one contiguous molecule; for example, linking two polypeptides into one contiguous polypeptide, or covalently attaching an effector molecule or detectable marker radionuclide or other molecule to a polypeptide, such as an

scFv. In the specific context, the terms include reference to joining a ligand, such as an antibody moiety, to an effector molecule. The linkage can be either by chemical or recombinant means. "Chemical means" refers to a reaction between the antibody moiety and the effector molecule such that there is a covalent bond formed between the two molecules to form one molecule.

5 **Neutralizing antibody:** An antibody that is able to specifically bind to a target protein in such a way as to inhibit a biological function associated with that target protein. In general, any protein that can perform this type of specific blocking activity is considered a neutralizing protein; neutralizing antibodies are therefore a specific class of neutralizing protein.

10 **Neoplasia, cancer, or tumor:** A neoplasm is an abnormal growth of tissue or cells that results from excessive cell division. Neoplastic growth can produce a tumor. The amount of a tumor in an individual is the "tumor burden" which can be measured as the number, volume, or weight of the tumor. A tumor that does not metastasize is referred to as "benign." A tumor that invades the surrounding tissue or can metastasize (or both) is referred to as "malignant."

15 Tumors of the same tissue type are primary tumors originating in a particular organ and may be divided into tumors of different sub-types. For examples, lung carcinomas can be divided into an adenocarcinoma, small cell, squamous cell, or non-small cell tumors.

20 Examples of solid tumors, such as sarcomas (connective tissue cancer) and carcinomas (epithelial cell cancer), include fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, and other sarcomas, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colorectal carcinoma, lymphoid malignancy, pancreatic cancer, breast cancer, lung cancers, ovarian cancer, prostate cancer, hepatocellular carcinoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, medullary thyroid carcinoma, papillary thyroid carcinoma, pheochromocytomas sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, Wilms' tumor, cervical cancer, testicular tumor, seminoma, bladder carcinoma, and CNS tumors (such as a glioma, astrocytoma, glioblastoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma and retinoblastoma).

25 **Neuroblastoma:** A solid cancerous tumor that usually originates in the abdomen in adrenal gland tissue, but can also originate from nerve tissue in the neck, chest, abdomen, and pelvis. By the time it is diagnosed, the cancer has usually metastasized to the lymph nodes, liver, lungs, bones and bone marrow. Neuroblastoma is the most common heterogenous and malignant tumor of early childhood, and two thirds of individuals with neuroblastoma are diagnosed when they are younger than 5 years.

35 Neuroblastoma is derived from the neural crest and is characterized by a marked clinical heterogeneity (aggressive, unremitting growth to spontaneous remission). As classified by International Neuroblastoma Staging System (INSS) there are six stages of neuroblastoma: Stage 1 (localized resectable), Stage 2A and 2B (localized unresectable or ipsilateral lymph node involvement), Stage 3 (regional, unresectable and crossing the midline), Stage 4 (disseminated) and Stage 4S (localized with limited spread; less than one year of age) referred to as "special" neuroblastoma. (See, e.g., Hayat (Ed.), Neuroblastoma, Pediatric Cancer, Volume 1, New York: Springer, 2011.)

Nucleic acid: A polymer composed of nucleotide units (ribonucleotides, deoxyribonucleotides, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof) linked via phosphodiester bonds, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof. Thus, the term includes nucleotide polymers in which the nucleotides and the linkages
 5 between them include non-naturally occurring synthetic analogs, such as, for example and without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs), and the like. Such polynucleotides can be synthesized, for example, using an automated DNA synthesizer. The term “oligonucleotide” typically refers to short polynucleotides, generally no greater than about 50 nucleotides. It will be understood that when a nucleotide
 10 sequence is represented by a DNA sequence (i.e., A, T, G, C), this also includes an RNA sequence (i.e., A, U, G, C) in which “U” replaces “T.”

“Nucleotide” includes, but is not limited to, a monomer that includes a base linked to a sugar, such as a pyrimidine, purine or synthetic analogs thereof, or a base linked to an amino acid, as in a peptide nucleic acid (PNA). A nucleotide is one monomer in a polynucleotide. A nucleotide sequence refers to the sequence of
 15 bases in a polynucleotide.

Conventional notation is used herein to describe nucleotide sequences: the left-hand end of a single-stranded nucleotide sequence is the 5'-end; the left-hand direction of a double-stranded nucleotide sequence is referred to as the 5'-direction. The direction of 5' to 3' addition of nucleotides to nascent RNA transcripts is referred to as the transcription direction. The DNA strand having the same sequence as an mRNA is referred to
 20 as the “coding strand;” sequences on the DNA strand having the same sequence as an mRNA transcribed from that DNA and which are located 5' to the 5'-end of the RNA transcript are referred to as “upstream sequences;” sequences on the DNA strand having the same sequence as the RNA and which are 3' to the 3' end of the coding RNA transcript are referred to as “downstream sequences.”

“cDNA” refers to a DNA that is complementary or identical to an mRNA, in either single stranded or
 25 double stranded form.

“Encoding” refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (i.e., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene
 30 encodes a protein if transcription and translation of mRNA produced by that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and non-coding strand, used as the template for transcription, of a gene or cDNA can be referred to as encoding the protein or other product of that gene or cDNA. Unless otherwise specified, a “nucleotide sequence encoding an amino acid sequence” includes all
 35 nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. Nucleotide sequences that encode proteins and RNA may include introns.

A first sequence is an “antisense” with respect to a second sequence if a polynucleotide whose sequence is the first sequence specifically hybridizes with a polynucleotide whose sequence is the second sequence.

Operably linked: A first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter, such as the CMV promoter, is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein-coding regions, in the same reading frame.

Pharmaceutically acceptable carriers: The pharmaceutically acceptable carriers of use are conventional. *Remington's Pharmaceutical Sciences*, by E. W. Martin, Mack Publishing Co., Easton, PA, 19th Edition, 1995, describes compositions and formulations suitable for pharmaceutical delivery of the disclosed immunogens.

In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (*e.g.*, powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate. In particular embodiments, suitable for administration to a subject the carrier may be sterile, and/or suspended or otherwise contained in a unit dosage form containing one or more measured doses of the composition suitable to induce the desired anti- MERS-CoV immune response. It may also be accompanied by medications for its use for treatment purposes. The unit dosage form may be, for example, in a sealed vial that contains sterile contents or a syringe for injection into a subject, or lyophilized for subsequent solubilization and administration or in a solid or controlled release dosage.

Polypeptide: Any chain of amino acids, regardless of length or post-translational modification (*e.g.*, glycosylation or phosphorylation). "Polypeptide" applies to amino acid polymers including naturally occurring amino acid polymers and non-naturally occurring amino acid polymer as well as in which one or more amino acid residue is a non-natural amino acid, for example an artificial chemical mimetic of a corresponding naturally occurring amino acid. A "residue" refers to an amino acid or amino acid mimetic incorporated in a polypeptide by an amide bond or amide bond mimetic. A polypeptide has an amino terminal (N-terminal) end and a carboxy terminal (C-terminal) end. "Polypeptide" is used interchangeably with peptide or protein, and is used herein to refer to a polymer of amino acid residues. A protein can include multiple polypeptide chains; for example, mature MERS-CoV S protein includes S1 and S2 polypeptide chains.

Amino acids in a peptide, polypeptide or protein generally are chemically bound together via amide linkages (CONH). Additionally, amino acids may be bound together by other chemical bonds. For example, linkages for amino acids or amino acid analogs can include CH₂NH-, -CH₂S-, -CH₂-CH₂ -, -CH=CH-- (cis and trans), -COCH₂ --, -CH(OH)CH₂-, and -CHH₂SO- (These and others can be found in Spatola, in *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins*, B. Weinstein, eds., Marcel Dekker, New York, p. 267 (1983); Spatola, A. F., *Vega Data* (March 1983), Vol. 1, Issue 3, *Peptide Backbone Modifications* (general review); Morley, *Trends Pharm Sci* pp. 463-468, 1980; Hudson, *et al.*, *Int J Pept Prot Res* 14:177-185, 1979; Spatola *et al.* *Life Sci* 38:1243-1249, 1986; Harm *J. Chem. Soc Perkin Trans.* 1307-314, 1982; Almquist *et al.*

J. Med. Chem. 23:1392-1398, 1980; Jennings-White *et al. Tetrahedron Lett* 23:2533, 1982; Holladay *et al. Tetrahedron. Lett* 24:4401-4404, 1983; and Hruby *Life Sci* 31:189-199, 1982.

Polypeptide modifications: Polypeptides and peptides, such as the MERS-CoV S proteins disclosed herein can be modified by a variety of chemical techniques to produce derivatives having essentially the same activity as the unmodified peptides, and optionally having other desirable properties. For example, carboxylic acid groups of the protein, whether carboxyl-terminal or side chain, may be provided in the form of a salt of a pharmaceutically-acceptable cation or esterified to form a C₁-C₁₆ ester, or converted to an amide of formula NR₁R₂ wherein R₁ and R₂ are each independently H or C₁-C₁₆ alkyl, or combined to form a heterocyclic ring, such as a 5- or 6- membered ring. Amino groups of the peptide, whether amino-terminal or side chain, may be in the form of a pharmaceutically-acceptable acid addition salt, such as the HCl, HBr, acetic, benzoic, toluene sulfonic, maleic, tartaric and other organic salts, or may be modified to C₁-C₁₆ alkyl or dialkyl amino or further converted to an amide.

Hydroxyl groups of the peptide side chains can be converted to C₁-C₁₆ alkoxy or to a C₁-C₁₆ ester using well-recognized techniques. Phenyl and phenolic rings of the peptide side chains can be substituted with one or more halogen atoms, such as F, Cl, Br or I, or with C₁-C₁₆ alkyl, C₁-C₁₆ alkoxy, carboxylic acids and esters thereof, or amides of such carboxylic acids. Methylene groups of the peptide side chains can be extended to homologous C₂-C₄ alkylenes. Thiols can be protected with any one of a number of well-recognized protecting groups, such as acetamide groups. Those skilled in the art will also recognize methods for introducing cyclic structures into the peptides of this disclosure to select and provide conformational constraints to the structure that result in enhanced stability. For example, a C- or N-terminal cysteine can be added to the peptide, so that when oxidized the peptide will contain a disulfide bond, generating a cyclic peptide. Other peptide cyclizing methods include the formation of thioethers and carboxyl- and amino-terminal amides and esters.

Purified: The term purified does not require absolute purity; rather, it is intended as a relative term. Thus, for example, a purified peptide preparation is one in which the peptide or protein (such as an antibody) is more enriched than the peptide or protein is in its natural environment within a cell. In one embodiment, a preparation is purified such that the protein or peptide represents at least 50% of the total peptide or protein content of the preparation.

Recombinant: A recombinant nucleic acid is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination can be accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, for example, by genetic engineering techniques. A recombinant protein is a protein encoded by a heterologous (for example, recombinant) nucleic acid, that has been introduced into a host cell, such as a bacterial or eukaryotic cell. The nucleic acid can be introduced, for example, on an expression vector having signals capable of expressing the protein encoded by the introduced nucleic acid or the nucleic acid can be integrated into the host cell chromosome.

Sequence identity: The similarity between amino acid sequences is expressed in terms of the similarity between the sequences, otherwise referred to as sequence identity. Sequence identity is frequently measured in terms of percentage identity (or similarity or homology); the higher the percentage, the more

similar the two sequences are. Homologs or variants of a polypeptide will possess a relatively high degree of sequence identity when aligned using standard methods.

Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith and Waterman, *Adv. Appl. Math.* 2:482, 1981; Needleman and Wunsch, *J. Mol. Biol.* 48:443, 1970; Pearson and Lipman, *Proc. Natl. Acad. Sci. U.S.A.* 85:2444, 1988; Higgins and Sharp, *Gene* 73:237, 1988; Higgins and Sharp, *CABIOS* 5:151, 1989; Corpet et al., *Nucleic Acids Research* 16:10881, 1988; and Pearson and Lipman, *Proc. Natl. Acad. Sci. U.S.A.* 85:2444, 1988. Altschul et al., *Nature Genet.* 6:119, 1994, presents a detailed consideration of sequence alignment methods and homology calculations.

The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul et al., *J. Mol. Biol.* 215:403, 1990) is available from several sources, including the National Center for Biotechnology Information (NCBI, Bethesda, MD) and on the internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx. A description of how to determine sequence identity using this program is available on the NCBI website on the internet.

Homologs and variants of a V_L or a V_H of an antibody that specifically binds a polypeptide are typically characterized by possession of at least about 75%, for example at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity counted over the full length alignment with the amino acid sequence of interest. Proteins with even greater similarity to the reference sequences will show increasing percentage identities when assessed by this method, such as at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity. When less than the entire sequence is being compared for sequence identity, homologs and variants will typically possess at least 80% sequence identity over short windows of 10-20 amino acids, and may possess sequence identities of at least 85% or at least 90% or 95% depending on their similarity to the reference sequence. Methods for determining sequence identity over such short windows are available at the NCBI website on the internet. One of skill in the art will appreciate that these sequence identity ranges are provided for guidance only; it is entirely possible that strongly significant homologs could be obtained that fall outside of the ranges provided.

Terms used to describe sequence relationships between two or more nucleotide sequences or amino acid sequences include "reference sequence," "selected from," "comparison window," "identical," "percentage of sequence identity," "substantially identical," "complementary," and "substantially complementary."

For sequence comparison of nucleic acid sequences, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters are used. Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482, 1981, by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443, 1970, by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444, 1988, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual

alignment and visual inspection (see, e.g., *Current Protocols in Molecular Biology* (Ausubel et al., eds 1995 supplement)).

One example of a useful algorithm is PILEUP. PILEUP uses a simplification of the progressive alignment method of Feng & Doolittle, *J. Mol. Evol.* 35:351-360, 1987. The method used is similar to the method described by Higgins & Sharp, *CABIOS* 5:151-153, 1989. Using PILEUP, a reference sequence is compared to other test sequences to determine the percent sequence identity relationship using the following parameters: default gap weight (3.00), default gap length weight (0.10), and weighted end gaps. PILEUP can be obtained from the GCG sequence analysis software package, e.g., version 7.0 (Devereaux et al., *Nuc. Acids Res.* 12:387-395, 1984).

Another example of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and the BLAST 2.0 algorithm, which are described in Altschul et al., *J. Mol. Biol.* 215:403-410, 1990 and Altschul et al., *Nucleic Acids Res.* 25:3389-3402, 1997. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (ncbi.nlm.nih.gov). The BLASTN program (for nucleotide sequences) uses as defaults a word length (W) of 11, alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands. The BLASTP program (for amino acid sequences) uses as defaults a word length (W) of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915, 1992). An oligonucleotide is a linear polynucleotide sequence of up to about 100 nucleotide bases in length.

Signal Peptide: A short amino acid sequence (e.g., approximately 18-25 amino acids in length) that directs newly synthesized secretory or membrane proteins to and through membranes (for example, the endoplasmic reticulum membrane). Signal peptides are typically located at the N-terminus of a polypeptide and can be removed by signal peptidases after the polypeptide has crossed the membrane. Signal peptide sequences typically contain three common structural features: an N-terminal polar basic region (n-region), a hydrophobic core, and a hydrophilic c-region). An exemplary signal peptide sequence is provided as SEQ ID NO: 26.

Specifically bind: When referring to an antibody, refers to a binding reaction which determines the presence of a target protein, peptide, or polysaccharide in the presence of a heterogeneous population of proteins and other biologics. Thus, under designated conditions, an antibody binds preferentially to a particular target protein, peptide or polysaccharide (such as an epitope of ALK) and does not bind in a significant amount to other proteins or polysaccharides present in the sample or subject. Specific binding can be determined by methods known in the art. With reference to an antibody antigen complex, specific binding of the antigen and antibody has a K_d of less than about 10^{-7} Molar (M), such as less than about 10^{-8} M, 10^{-9} M, 10^{-10} M, or even less than about 10^{-11} M.

The antibodies disclosed herein specifically bind only to a defined target (or multiple targets, in the case of a bispecific antibody). Thus, an antibody that specifically binds to ALK is an antibody that binds substantially to ALK, including cells or tissue expressing ALK, substrate to which the ALK is attached, or ALK in a biological specimen. It is, of course, recognized that a certain degree of non-specific interaction may occur between an antibody or conjugate including an antibody (such as an antibody that specifically binds ALK or conjugate including such antibody) and a non-target (such as a cell that does not express ALK). Typically, specific binding results in a much stronger association between the antibody and protein or cells

bearing the antigen than between the antibody and protein or cells lacking the antigen. Specific binding typically results in greater than 2-fold, such as greater than 5-fold, greater than 10-fold, or greater than 100-fold increase in amount of bound antibody (per unit time) to a protein including the epitope or cell or tissue expressing the target epitope as compared to a protein or cell or tissue lacking this epitope. Specific binding to a protein under such conditions requires an antibody that is selected for its specificity for a particular protein. A variety of immunoassay formats are appropriate for selecting antibodies or other ligands specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. See Harlow & Lane, *Antibodies, A Laboratory Manual*, 2nd ed., Cold Spring Harbor Publications, New York (2013), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

Subject: Any mammal, such as humans, non-human primates, pigs, sheep, cows, rodents, and the like. In two non-limiting examples, a subject is a human subject or a murine subject. Thus, the term “subject” includes both human and veterinary subjects.

T Cell: A white blood cell critical to the immune response. T cells include, but are not limited to, CD4⁺ T cells and CD8⁺ T cells. A CD4⁺ T lymphocyte is an immune cell that carries a marker on its surface known as “cluster of differentiation 4” (CD4). These cells, also known as helper T cells, help orchestrate the immune response, including antibody responses as well as killer T cell responses. CD8⁺ T cells carry the “cluster of differentiation 8” (CD8) marker. In one embodiment, a CD8 T cell is a cytotoxic T lymphocyte. In another embodiment, a CD8 cell is a suppressor T cell. An **effector function of a T cell** is a specialized function of the T cell, such as cytolytic activity or helper activity including the secretion of cytokines.

T Cell Signaling Domain: An intracellular portion of a protein expressed in a T cell that transduces a T cell effector function signal (e.g., an activation signal) and directs the T cell to perform a specialized function. T cell activation can be induced by a number of factors, including binding of cognate antigen to the T cell receptor on the surface of T cells and binding of cognate ligand to co-stimulatory molecules on the surface of the T cell. A T cell co-stimulatory molecule is a cognate binding partner on a T cell that specifically binds with a co-stimulatory ligand, thereby mediating a co-stimulatory response by the T cell, such as, but not limited to, proliferation. Co-stimulatory molecules include, but are not limited to an MHC class I molecule, BTLA and a Toll ligand receptor. Activation of a T cell leads to immune response, such as T cell proliferation and differentiation (see, e.g., Smith-Garvin *et al.*, *Annu. Rev. Immunol.*, 27:591-619, 2009). Exemplary T cell signaling domains are known and described herein. Non-limiting examples include the CD3 zeta, CD8, CD28, CD27, CD154, GITR (TNFRSF18), CD134 (OX40), and CD137 (4-1BB) signaling domains.

Therapeutic agent: Used in a generic sense, it includes treating agents, prophylactic agents, and replacement agents. A therapeutic agent is used to ameliorate a specific set of conditions in a subject with a disease or a disorder.

Therapeutically effective amount: The amount of an agent (such as a ALK specific antibody, antigen binding fragment, CAR or CAR T cell, or nucleic acid molecule encoding thereof) that alone, or together with one or more additional agents, induces the desired response, such as, for example treatment of a tumor in a subject. Ideally, a therapeutically effective amount provides a therapeutic effect without causing a substantial cytotoxic effect in the subject.

In one example, a desired response is to decrease the size, volume, or number (such as metastases) of a tumor in a subject. For example, the agent or agents can decrease the size, volume, or number of tumors by a desired amount, for example by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 50%, at least 75%, at least 90%, or at least 95% as compared to a response in the absence of the agent.

5 Several preparations disclosed herein are administered in therapeutically effective amounts. A therapeutically effective amount of an antibody that specifically binds ALK or antigen binding fragment thereof, or conjugate thereof (or a composition including one or more of these molecules) that is administered to a human or veterinary subject will vary depending upon a number of factors associated with that subject, for example the overall health of the subject. A therapeutically effective amount can be determined by varying the
10 dosage and measuring the resulting therapeutic response, such as the regression of a tumor. Therapeutically effective amounts also can be determined through various *in vitro*, *in vivo* or *in situ* immunoassays. The disclosed agents can be administered in a single dose, or in several doses, as needed to obtain the desired response. However, the therapeutically effective amount of can be dependent on the source applied, the subject being treated, the severity and type of the condition being treated, and the manner of administration.

15 **Toxin:** An effector molecule that induces cytotoxicity when it contacts a cell. Specific, non-limiting examples of toxins include, but are not limited to, abrin, ricin, auristatins (such as monomethyl auristatin E (MMAE; see for example, Francisco et al., Blood, 102: 1458-1465, 2003)) and monomethyl auristatin F (MMAF; see, for example, Doronina *et al.*, BioConjugate Chem., 17: 114-124, 2006), maytansinoids (such as DM1; see, for example, Phillips et al., Cancer Res., 68:9280-9290, 2008), *Pseudomonas* exotoxin (PE, such as
20 PE35, PE37, PE38, and PE40), diphtheria toxin (DT), botulinum toxin, saporin, restrictocin or gelonin, or modified toxins thereof, or other toxic agents that directly or indirectly inhibit cell growth or kill cells. For example, PE and DT are highly toxic compounds that typically bring about death through liver toxicity. PE and DT, however, can be modified into a form for use as an immunotoxin by removing the native targeting component of the toxin (such as the domain Ia of PE and the B chain of DT) and replacing it with a different
25 targeting moiety, such as an antibody.

Transmembrane domain: An amino acid sequence that inserts into a lipid bilayer, such as the lipid bilayer of a cell or virus or virus-like particle. A transmembrane domain can be used to anchor a protein of interest (such as a CAR) to a membrane. Exemplary transmembrane domains are familiar to the person of ordinary skill in the art, and provided herein.

30 **Transformed:** A transformed cell is a cell into which a nucleic acid molecule has been introduced by molecular biology techniques. As used herein, the term transformation encompasses all techniques by which a nucleic acid molecule might be introduced into such a cell, including transfection with viral vectors, transformation with plasmid vectors, and introduction of DNA by electroporation, lipofection, and particle gun acceleration.

35 **Tumor burden:** The total volume, number, metastasis, or combinations thereof of tumor or tumors in a subject.

Under conditions sufficient for: A phrase that is used to describe any environment that permits a desired activity. In one example the desired activity is formation of an immune complex. In particular examples the desired activity is treatment of a tumor.

II. Description of Several Embodiments

Isolated monoclonal antibodies that specifically bind to ALK on the cell surface, antigen binding fragments of such antibodies, conjugates thereof, nucleic acid molecules encoding the antibodies and/or antigen binding fragments, and methods of using these molecules, are provided. Several embodiments include
 5 a chimeric antigen receptor including a disclosed antigen binding fragment that specifically binds to ALK, or a nucleic acid molecule encoding the CAR. The nucleic acid molecule can be included in an expression vector (such as a viral vector) for expression in a host cell (such as an autologous T cell). Isolated host cells (such as a T-cell) that express the nucleic acid molecules are also provided.

10 Compositions including the antibodies, antigen binding fragments, conjugates, CARs, nucleic acid molecules, and/or host cells, and a pharmaceutically acceptable carrier as also provided. The compositions can be used for research, diagnostic and therapeutic purposes, for example for treatment of a tumor (such as is a neuroblastoma, a rhabdomyosarcoma, or a glioblastoma) in a subject.

A. Chimeric Antigen Receptors (CARs)

15 Disclosed herein are CARs that are artificially constructed chimeric proteins including an extracellular antigen binding domain (e.g., single chain variable fragment (scFv)) that specifically binds to ALK), linked to a transmembrane domain, linked to one or more intracellular T-cell signaling domains. Characteristics of the disclosed CARs include their ability to redirect T-cell specificity and reactivity towards ALK expressing cells in a non-MHC-restricted manner. The non-MHC-restricted ALK recognition gives T cells expressing a
 20 disclosed CAR the ability to recognize antigen independent of antigen processing, thus bypassing a major mechanism of tumor escape.

The intracellular T cell signaling domains can include, for example, a T cell receptor signaling domain, a T cell costimulatory signaling domain, or both. The T cell receptor signaling domain refers to a portion of the CAR comprising the intracellular domain of a T cell receptor, such as the intracellular portion of
 25 the CD3 zeta protein. The costimulatory signaling domain refers to a portion of the CAR comprising the intracellular domain of a costimulatory molecule, which is a cell surface molecule other than an antigen receptor or their ligands that are required for an efficient response of lymphocytes to antigen.

In some embodiments, the CAR includes or consists of the amino acid sequence set forth as one of SEQ ID NOs: 43-90.

30

1. Extracellular Region

Several embodiments provide a CAR including an antigen binding domain that specifically binds to ALK as disclosed herein (see, e.g., section II.B below). For example, the antigen binding domain can be a scFv including the heavy chain variable region and the light chain variable region of any of the antibodies or
 35 antigen binding fragments thereof disclosed in section II.B below.

In some embodiments, the antigen binding domain can include a heavy chain variable region and a light chain variable region including the HCDR1, HCDR2, and HCDR3, and LCDR1, LCDR2, and LCDR3 of the of the heavy and light chain variable regions, respectively, of one of the ALK15, ALK48, ALK53, or ALK58 antibodies (e.g., as set forth in Table 1 or Table 2 below). In some embodiments, the antigen binding
 40 domain includes a heavy chain variable region and a light chain variable region including the amino acid

sequences set forth as SEQ ID NOs: 1 and 2, respectively; SEQ ID NOs: 3 and 4, respectively; SEQ ID NOs: 5 and 6, respectively; SEQ ID NOs: 7 and 8, respectively; SEQ ID NOs: 9 and 10, respectively; SEQ ID NOs: 11 and 12, respectively; SEQ ID NOs: 13 and 14, respectively; or SEQ ID NOs: 15 and 16, respectively.

In several embodiments, the antigen binding domain can be a scFv. In some embodiments, the scFv includes a heavy chain variable region and a light chain variable region joined by a peptide linker, such as a linker including the amino acid sequence set forth as SEQ ID NO: 25. In some such embodiments, the antigen binding domain comprises an amino acid sequence set forth as one of SEQ ID NOs: 17-24.

The CAR can include a signal peptide sequence, e.g., N-terminal to the antigen binding domain. The signal peptide sequence may comprise any suitable signal peptide sequence. In an embodiment, the signal peptide sequence is a human granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor sequence, such as an amino acid sequence including or consisting of SEQ ID NO: 26. While the signal peptide sequence may facilitate expression of the CAR on the surface of the cell, the presence of the signal peptide sequence in an expressed CAR is not necessary in order for the CAR to function. Upon expression of the CAR on the cell surface, the signal peptide sequence may be cleaved off of the CAR. Accordingly, in some embodiments, the CAR lacks a signal peptide sequence.

Between the antigen binding domain and the transmembrane domain of the CAR, there may be a spacer domain, which includes a polypeptide sequence. The spacer domain may comprise up to 300 amino acids, preferably 10 to 100 amino acids and most preferably 25 to 50 amino acids. In some embodiments, the spacer domain can include an immunoglobulin domain, such as a human immunoglobulin sequence. In an embodiment, the immunoglobulin domain comprises an immunoglobulin CH2 and CH3 immunoglobulin G (IgG1) domain sequence (CH2CH3). In this regard, the spacer domain can include an immunoglobulin domain comprising or consisting of the amino acid sequence set forth as SEQ ID NO: 35. Without being bound to a particular theory, it is believed that the CH2CH3 domain extends the antigen binding domain of the CAR away from the membrane of CAR-expressing cells and may more accurately mimic the size and domain structure of a native TCR.

2. *Transmembrane Domain*

With respect to the transmembrane domain, the CAR can be designed to comprise a transmembrane domain that is fused to the extracellular domain of the CAR. In one embodiment, the transmembrane domain that naturally is associated with one of the domains in the CAR is used.

The transmembrane domain may be derived either from a natural or from a synthetic source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein. Exemplary transmembrane domains for use in the disclosed CARs can include at least the transmembrane region(s) of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD 16, CD22, CD33, CD37, CD64, CD80, CD86, CD 134, CD137, CD 154. Alternatively the transmembrane domain may be synthetic, in which case it will comprise predominantly hydrophobic residues such as leucine and valine. In several embodiments, a triplet of phenylalanine, tryptophan and valine will be found at each end of a synthetic transmembrane domain.

Optionally, a short oligo- or polypeptide linker, preferably between 2 and 10 amino acids in length may form the linkage between the transmembrane domain and the intracellular T cell signaling domain and/or

T cell costimulatory domain of the CAR. An exemplary linker sequence includes one or more glycine-serine doublets.

In some embodiments, the transmembrane domain comprises the transmembrane domain of a T cell receptor, such as a CD8 transmembrane domain. Thus, the CAR can include a CD8 transmembrane domain including or consisting of SEQ ID NO: 30. In another embodiment, the transmembrane domain comprises the transmembrane domain of a T cell costimulatory molecule, such as CD137 or CD28. Thus, the CAR can include a CD28 transmembrane domain including or consisting of SEQ ID NO: 27.

3. *Intracellular Region*

The intracellular region of the CAR includes one or more intracellular T cell signaling domains responsible for activation of at least one of the normal effector functions of a T cell in which the CAR is expressed or placed in. Exemplary T cell signaling domains are provided herein, and are known to the person of ordinary skill in the art.

While an entire intracellular T cell signaling domain can be employed in a CAR, in many cases it is not necessary to use the entire chain. To the extent that a truncated portion of the intracellular T cell signaling domain is used, such truncated portion may be used in place of the intact chain as long as it transduces the relevant T cell effector function signal.

Examples of intracellular T cell signaling domains for use in the CAR include the cytoplasmic sequences of the T cell receptor (TCR) and co-stimulatory molecules that act in concert to initiate signal transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any synthetic sequence that has the same functional capability.

T cell receptor signaling domains regulate primary activation of the T cell receptor complex either in a stimulatory way, or in an inhibitory way. The disclosed CARs can include primary cytoplasmic signaling sequences that act in a stimulatory manner, which may contain signaling motifs that are known as immunoreceptor tyrosine-based activation motifs or ITAMs. Examples of ITAM containing primary cytoplasmic signaling sequences that can be included in a disclosed CAR include those from CD3 zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CDS, CD22, CD79a, CD79b, and CD66d proteins. It is particularly preferred that cytoplasmic signaling molecule in the CAR include an intracellular T cell signaling domain from CD3 zeta.

The intracellular region of the CAR can include the ITAM containing primary cytoplasmic signaling domain (such as CD3-zeta) by itself or combined with any other desired cytoplasmic domain(s) useful in the context of a CAR. For example, the cytoplasmic domain of the CAR can include a CD3 zeta chain portion and an intracellular costimulatory signaling domain. The costimulatory signaling domain refers to a portion of the CAR comprising the intracellular domain of a costimulatory molecule. A costimulatory molecule is a cell surface molecule other than an antigen receptor or their ligands that is required for an efficient response of lymphocytes to an antigen. Examples of such molecules include CD27, CD28, 4-1BB (CD137), OX40 (CD134), CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen 1 (LFA-1), CD2, CD7, LIGHT, NKG2C, and B7-H3. An additional example of a signaling domain that can be included in a disclosed CARs is a Tumor necrosis factor receptor superfamily member 18 (TNFRSF18; also known as glucocorticoid-induced TNFR-related protein, GITR) signaling domain.

In some embodiments, the CAR can include a CD3 zeta signaling domain, a CD8 signaling domain, a CD28 signaling domain, a CD137 signaling domain or a combination of two or more thereof. In one embodiment, the cytoplasmic domain includes the signaling domain of CD3-zeta and the signaling domain of CD28. In another embodiment, the cytoplasmic domain includes the signaling domain of CD3 zeta and the signaling domain of CD137. In yet another embodiment, the cytoplasmic domain includes the signaling domain of CD3-zeta and the signaling domain of CD28 and CD137. The order of the one or more T cell signaling domains on the CAR can be varied as needed by the person of ordinary skill in the art.

Exemplary amino acid sequences for such T cell signaling domains are provided. For example, the CD3 zeta signaling domain can include or consist of the amino acid sequence set forth as SEQ ID NO: 34, the CD8 signaling domain can include or consist of the amino acid sequence set forth as SEQ ID NO: 31, the CD28 signaling domain can include or consist of the amino acid sequence set forth as SEQ ID NO: 28, the CD137 signaling domain can include or consist of the amino acid sequences set forth as SEQ ID NO: 32 or SEQ ID NO: 33.

The cytoplasmic signaling sequences within the cytoplasmic signaling portion of the CAR of the invention may be linked to each other in a random or specified order. Optionally, a short polypeptide linker, preferably between 2 and 10 amino acids in length may form the linkage. A glycine-serine doublet provides a particularly suitable linker. Further, between the signaling domain and the transmembrane domain of the CAR, there may be a spacer domain, which includes a polypeptide sequence. The spacer domain may comprise up to 300 amino acids, preferably 10 to 100 amino acids and most preferably 25 to 50 amino acids.

4. *Additional Description of CARs*

Also provided are functional portions of the CARs described herein. The term "functional portion" when used in reference to a CAR refers to any part or fragment of the CAR, which part or fragment retains the biological activity of the CAR of which it is a part (the parent CAR). Functional portions encompass, for example, those parts of a CAR that retain the ability to recognize target cells, or detect, treat, or prevent a disease, to a similar extent, the same extent, or to a higher extent, as the parent CAR. In reference to the parent CAR, the functional portion can comprise, for instance, about 10%, 25%, 30%, 50%, 68%, 80%, 90%, 95%, or more, of the parent CAR.

The CAR or functional portion thereof, can include additional amino acids at the amino or carboxy terminus, or at both termini, which additional amino acids are not found in the amino acid sequence of the parent CAR. Desirably, the additional amino acids do not interfere with the biological function of the CAR or functional portion, e.g., recognize target cells, detect cancer, treat or prevent cancer, etc. More desirably, the additional amino acids enhance the biological activity, as compared to the biological activity of the parent CAR.

Also provided are functional variants of the CARs described herein, which have substantial or significant sequence identity or similarity to a parent CAR, which functional variant retains the biological activity of the CAR of which it is a variant. Functional variants encompass, for example, those variants of the CAR described herein (the parent CAR) that retain the ability to recognize target cells to a similar extent, the same extent, or to a higher extent, as the parent CAR. In reference to the parent CAR, the functional variant can, for instance, be at least about 30%, about 50%, about 75%, about 80%, about 85%, about 90%, about 91

%, about 92%, about 93%, about 94%, about 95%, about 96%), about 97%, about 98%, about 99% or more identical in amino acid sequence to the parent CAR.

A functional variant can, for example, comprise the amino acid sequence of the parent CAR with at least one conservative amino acid substitution. Alternatively or additionally, the functional variants can
 5 comprise the amino acid sequence of the parent CAR with at least one non-conservative amino acid substitution. In this case, it is preferable for the non-conservative amino acid substitution to not interfere with or inhibit the biological activity of the functional variant. The non-conservative amino acid substitution may enhance the biological activity of the functional variant, such that the biological activity of the functional variant is increased as compared to the parent CAR.

10 The CARs (including functional portions and functional variants) can be of any length, i.e., can comprise any number of amino acids, provided that the CARs (or functional portions or functional variants thereof) retain their biological activity, e.g., the ability to specifically bind to antigen, detect diseased cells in a mammal, or treat or prevent disease in a mammal, etc. For example, the CAR can be about 50 to about 5000 amino acids long, such as 50, 70, 75, 100, 125, 150, 175, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or more
 15 amino acids in length.

The CARs (including functional portions and functional variants of the invention) can comprise synthetic amino acids in place of one or more naturally-occurring amino acids. Such synthetic amino acids are known in the art, and include, for example, aminocyclohexane carboxylic acid, norleucine, α -amino n-decanoic acid, homoserine, S-acetylaminomethyl-cysteine, trans-3- and trans-4-hydroxyproline, 4- aminophenylalanine,
 20 4- nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β -phenylserine β -hydroxyphenylalanine, phenylglycine, α -naphthylalanine, cyclohexylalanine, cyclohexylglycine, indoline-2-carboxylic acid, 1,2,3,4- tetrahydroisoquinoline-3-carboxylic acid, aminomalonic acid, aminomalonic acid monoamide, N'-benzyl-N'-methyl-lysine, N',N'-dibenzyl-lysine, 6-hydroxylysine, ornithine, α -aminocyclopentane carboxylic acid, α -aminocyclohexane carboxylic acid, α -aminocycloheptane carboxylic
 25 acid, -(2-amino-2-norbornane)-carboxylic acid, γ -diaminobutyric acid, α,β -diaminopropionic acid, homophenylalanine, and α -tert-butylglycine.

The CARs (including functional portions and functional variants) can be glycosylated, amidated, carboxylated, phosphorylated, esterified, N-acylated, cyclized via, e.g., a disulfide bridge, or converted into an acid addition salt and/or optionally dimerized or polymerized, or conjugated.

30 Methods of generating chimeric antigen receptors, T cells including such receptors, and their use (e.g., for treatment of cancer) are known in the art and further described herein (see, e.g., Brentjens et al., 2010, *Molecular Therapy*, 18:4, 666-668; Morgan et al., 2010, *Molecular Therapy*, published online February 23, 2010, pages 1 -9; Till et al., 2008, *Blood*, 112:2261 -2271; Park *et al.*, *Trends Biotechnol.*, 29:550-557, 2011; Grupp et al., *N Engl J Med.*, 368:1509-1518, 2013; Han et al., *J. Hematol Oncol.*, 6:47, 2013; Tumaini et al.,
 35 *Cytotherapy*, 15, 1406-1417, 2013; Haso et al., (2013) *Blood*, 121, 1165-1174; PCT Pubs. WO2012/079000, WO2013/126726; and U.S. Pub. 2012/0213783, each of which is incorporated by reference herein in its entirety). For example, a nucleic acid molecule encoding a disclosed chimeric antigen binding receptor can be included in an expression vector (such as a lentiviral vector) used to transduce a host cell, such as a T cell, to make the disclosed CAR. In some embodiments, methods of using the chimeric antigen receptor include
 40 isolating T cells from a subject, transducing the T cells with an expression vector (such as a lentiviral vector)

encoding the chimeric antigen receptor, and administering the CAR-expressing T cells to the subject for treatment, for example for treatment of a tumor in the subject.

B. Antibodies and Antigen Binding Fragments

5 Isolated monoclonal antibodies that specifically bind to ALK on the cell surface, and antigen binding fragments thereof are provided. The antibodies can be fully human and/or neutralizing.

In several embodiments, the monoclonal antibodies include a heavy chain comprising a heavy chain complementarity determining region (HCDR)1, a HCDR2 and an HCDR3, and a light chain comprising a light chain complementarity determining region (LCDR) 1, LCDR2 and LCDR3. The disclosed antibodies
10 specifically bind to an epitope of ALK and are neutralizing. In some embodiments, the ALK specific antibodies include a variable heavy (V_H) and a variable light (V_L) chain and specifically bind ALK. In several embodiments, the antibody or antigen binding fragment thereof includes heavy and light chain variable regions including the HCDR1, HCDR2, and HCDR3, and LCDR1, LCDR2, and LCDR3, respectively, of one of the ALK15, ALK48, ALK53, or ALK58 antibodies.

15 The discussion of monoclonal antibodies below refers to isolated monoclonal antibodies that include heavy and light chain variable domains including at least one complementarity determining region (CDR), such as a CDR1, CDR2 and CDR3. The person of ordinary skill in the art will understand that various CDR numbering schemes (such as the Kabat, Chothia or IMGT numbering schemes) can be used to determine CDR positions. The amino acid sequence and the CDR positions of the heavy and light chain of the ALK15,
20 ALK48, ALK53, or ALK58 monoclonal antibodies according to the IMGT and Kabat numbering schemes are shown in Table 1 (IMGT) and Table 2 (Kabat). The person of skill in the art will readily understand use of various CDR numbering schemes when referencing particular amino acids of the antibodies disclosed herein.

Table 1. IMGT CDR sequences of ALK specific antibodies

ALK15					
	SEQ ID NO: 1	A.A. Sequence		SEQ ID NO: 2	A.A. Sequence
HCDR1	26-33	GFSLTSYA	LCDR1	27-37	QSIVHSYGNTY
HCDR2	51-57	IWSGGAT	LCDR2	55-57	RVS
HCDR3	95-109	CAREHYYGSSAMD YW	LCDR3	93-103	CFQGTHVPYTF
ALK48					
	SEQ ID NO: 3	A.A. Sequence		SEQ ID NO: 4	A.A. Sequence
HCDR1	26-33	GYAFSSYW	LCDR1	27-36	ESVDNYGISF
HCDR2	51-58	IYPGDGDT	LCDR2	54-56	RAS
HCDR3	96-110	CVRYYYGSSGYFDY W	LCDR3	92-102	CQQNNKDPPTF
ALK53					
	SEQ ID NO: 5	A.A. Sequence		SEQ ID NO: 6	A.A. Sequence
HCDR1	26-33	GYTFTDHF	LCDR1	27-37	KSLLSHNGNTY

HCDR2	51-58	LNPYSGGT	LCDR2	55-57	YMS
HCDR3	96-108	CARHNWGAYFDYW	LCDR3	93-103	CMQGLEDPYTF
ALK58					
	SEQ ID NO: 7	A.A. Sequence		SEQ ID NO: 8	A.A. Sequence
HCDR1	26-33	GYTFTDYE	LCDR1	27-32	QDIGNY
HCDR2	51-58	IDPETGGT	LCDR2	50-52	YTS
HCDR3	96-110	CARRRYYGSSSFDY W	LCDR3	88-98	CQQGSALPPTF

In some embodiments, the antibody includes IMGT CDRs, such as those listed in Table 1. For example, in some embodiments, the antibody includes a heavy chain variable region including a HCDR1, HCDR2, and/or HCDR3 including amino acids amino acids 26-33, 51-57, and 95-109 of SEQ ID NO: 1, respectively. In further embodiments, the antibody includes a heavy chain variable region including a HCDR1, HCDR2, and/or HCDR3 including amino acids amino acids 26-33, 51-58, and 96-110 of SEQ ID NO: 3, respectively. In additional embodiments, the antibody includes a heavy chain variable region including a HCDR1, HCDR2, and/or HCDR3 including amino acids 26-33, 51-58, and 96-108 of SEQ ID NO: 5, respectively. In more embodiments, the antibody includes a heavy chain variable region including a HCDR1, HCDR2, and/or HCDR3 including amino acids 26-33, 51-58, and 96-110 of SEQ ID NO: 7, respectively.

In some embodiments, the antibody includes a light chain variable region including a LCDR1, LCDR2, and/or LCDR3 including amino acids 27-37, 55-57, and 93-103 of SEQ ID NO: 2, respectively. In further embodiments, the antibody includes a light chain variable region including a LCDR1, LCDR2, and/or LCDR3 including amino acids 27-36, 54-56, and 92-102 of SEQ ID NO: 4, respectively. In additional embodiments, the antibody includes a light chain variable region including a LCDR1, LCDR2, and/or LCDR3 including amino acids 27-37, 55-57, and 93-103 of SEQ ID NO: 6, respectively. In more embodiments, the antibody includes a light chain variable region including a LCDR1, LCDR2, and/or LCDR3 including amino acids 27-32, 50-52, and 88-98 of SEQ ID NO: 8, respectively.

In some embodiments, the antibody includes a heavy chain variable region including a HCDR1, HCDR2, and HCDR3 including amino acids 26-33, 51-57, and 95-109 of SEQ ID NO: 1, respectively, and a light chain variable region including a LCDR1, LCDR2, and LCDR3 including amino acids 27-37, 55-57, and 93-103 of SEQ ID NO: 2, respectively. In further embodiments, the antibody includes a heavy chain variable region including a HCDR1, HCDR2, and HCDR3 including amino acids 26-33, 51-58, and 96-110 of SEQ ID NO: 3, respectively, and a light chain variable region including a LCDR1, LCDR2, and LCDR3 including amino acids 27-36, 54-56, and 92-102 of SEQ ID NO: 4, respectively. In additional embodiments, the antibody includes a heavy chain variable region including a HCDR1, HCDR2, and HCDR3 including amino acids 26-33, 51-58, and 96-108 of SEQ ID NO: 5, respectively, and a light chain variable region including a LCDR1, LCDR2, and LCDR3 including amino acids 27-37, 55-57, and 93-103 of SEQ ID NO: 6, respectively. In more embodiments, the antibody includes a heavy chain variable region including a HCDR1, HCDR2, and HCDR3 including amino acids 26-33, 51-58, and 96-110 of SEQ ID NO: 7, respectively, and a light chain variable region including a LCDR1, LCDR2, and LCDR3 including amino acids 27-32, 50-52, and 88-98 of SEQ ID NO: 8, respectively.

Table 2. Kabat CDR sequences of ALK specific antibodies

ALK15					
	SEQ ID NO: 1	A.A. Sequence		SEQ ID NO: 2	A.A. Sequence
HCDR1	31-35	SYAVS	LCDR1	24-39	RSSQSIVHSYGN TYLF
HCDR2	50-65	IIWSGGATNYNSALKS	LCDR2	55-61	RVSNRFS
HCDR3	98-108	EHYYGSSAMDY	LCDR3	94-102	FQGTHVPYT
ALK48					
	SEQ ID NO: 3	A.A. Sequence		SEQ ID NO: 4	A.A. Sequence
HCDR1	31-35	SYWMN	LCDR1	24-38	RASESVDNYSIS FMH
HCDR2	50-66	QIYPGDGDTTYNGKF KG	LCDR2	54-60	RASNLES
HCDR3	99-109	YYYGSSGYFDY	LCDR3	93-101	QQNNKDPPT
ALK53					
	SEQ ID NO: 5	A.A. Sequence		SEQ ID NO: 6	A.A. Sequence
HCDR1	31-35	DHFMD	LCDR1	24-39	RSSKSLLHSNG NTYLY
HCDR2	50-66	SLNPYSGGTSYNQKFK G	LCDR2	55-61	YMSNLAS
HCDR3	99-107	HNWGAYFDY	LCDR3	94-102	MQGLEDPYT
ALK58					
	SEQ ID NO: 7	A.A. Sequence		SEQ ID NO: 8	A.A. Sequence
HCDR1	31-35	DYEMH	LCDR1	24-34	RASQDIGNYLN
HCDR2	50-66	AIDPETGGTAYNQKFE G	LCDR2	50-56	YTSRLHS
HCDR3	99-109	RRYYGSSSFDY	LCDR3	89-97	QQGSALPPT

In some embodiments, the antibody includes Kabat CDRs, such as those listed in Table 2. In some
5 embodiments, the antibody includes a heavy chain variable region including a HCDR1, HCDR2, and/or
HCDR3 including amino acids 31-35, 50-65, and 98-108 of SEQ ID NO: 1, respectively. In further
embodiments, the antibody includes a heavy chain variable region including a HCDR1, HCDR2, and/or
HCDR3 including amino acids 31-35, 50-66, and 99-109 of SEQ ID NO: 3, respectively. In additional
embodiments, the antibody includes a heavy chain variable region including a HCDR1, HCDR2, and/or
10 HCDR3 including amino acids 31-35, 50-66, and 99-107 of SEQ ID NO: 5, respectively. In more
embodiments, the antibody includes a heavy chain variable region including a HCDR1, HCDR2, and/or
HCDR3 including amino acids 31-35, 50-66, and 99-109 of SEQ ID NO: 7, respectively.

In some embodiments, the antibody includes a light chain variable region including a LCDR1,
LCDR2, and/or LCDR3 including amino acids 24-39, 55-61, and 94-102 of SEQ ID NO: 2, respectively. In

further embodiments, the antibody includes a light chain variable region including a LCDR1, LCDR2, and/or LCDR3 including amino acids 24-38, 55-60, and 93-101 of SEQ ID NO: 4, respectively. In additional embodiments, the antibody includes a light chain variable region including a LCDR1, LCDR2, and/or LCDR3 including amino acids 24-39, 55-61, and 94-102 of SEQ ID NO: 6, respectively. In more embodiments, the antibody includes a light chain variable region including a LCDR1, LCDR2, and/or LCDR3 including amino acids 24-34, 50-56, and 89-97 of SEQ ID NO: 8, respectively.

In some embodiments, the antibody includes a heavy chain variable region including a HCDR1, HCDR2, and HCDR3 including amino acids 31-35, 50-65, and 98-108 of SEQ ID NO: 1, respectively, and a light chain variable region including a LCDR1, LCDR2, and LCDR3 including amino acids 24-39, 55-61, and 94-102 of SEQ ID NO: 2, respectively. In further embodiments, the antibody includes a heavy chain variable region including a HCDR1, HCDR2, and HCDR3 including amino acids 31-35, 50-66, and 99-109 of SEQ ID NO: 3, respectively, and a light chain variable region including a LCDR1, LCDR2, and LCDR3 including amino acids 24-38, 55-60, and 93-101 of SEQ ID NO: 4, respectively. In additional embodiments, the antibody includes a heavy chain variable region including a HCDR1, HCDR2, and HCDR3 including amino acids 31-35, 50-66, and 99-107 of SEQ ID NO: 5, respectively, and a light chain variable region including a LCDR1, LCDR2, and LCDR3 including amino acids 24-39, 55-61, and 94-102 of SEQ ID NO: 6, respectively. In more embodiments, the antibody includes a heavy chain variable region including a HCDR1, HCDR2, and HCDR3 including amino acids 31-35, 50-66, and 99-109 of SEQ ID NO: 7, respectively, and a light chain variable region including a LCDR1, LCDR2, and LCDR3 including amino acids 24-34, 50-56, and 89-97 of SEQ ID NO: 8, respectively.

In some embodiments, the antibody includes a heavy chain variable region including an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth as one of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, or 15. In more embodiments, the antibody includes a light chain variable region including an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth as one of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, or 16. In additional embodiments, the antibody includes a heavy chain variable region including the amino acid sequence set forth as one of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, or 15. In more embodiments, the antibody includes a light chain variable region including the amino acid sequence set forth as one of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, or 16.

In additional embodiments, the antibody includes a heavy chain variable region including an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth as SEQ ID NO: 1, and a light chain variable region including an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth as SEQ ID NO: 2. In additional embodiments, the antibody includes a heavy chain variable region including an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth as SEQ ID NO: 3, and a light chain variable region including an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth as SEQ ID NO: 4. In additional embodiments, the antibody includes a heavy chain variable region including an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth as SEQ ID NO: 5, and a light chain variable region including an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth as SEQ ID NO: 6. In additional embodiments, the antibody includes a heavy chain variable region including an amino acid sequence at least

95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth as SEQ ID NO: 7, and a light chain variable region including an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth as SEQ ID NO: 8.

In additional embodiments, the antibody includes a heavy chain variable region including an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth as SEQ ID NO: 9, and a light chain variable region including an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth as SEQ ID NO: 10. In additional embodiments, the antibody includes a heavy chain variable region including an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth as SEQ ID NO: 11, and a light chain variable region including an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth as SEQ ID NO: 12. In additional embodiments, the antibody includes a heavy chain variable region including an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth as SEQ ID NO: 13, and a light chain variable region including an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth as SEQ ID NO: 14. In additional embodiments, the antibody includes a heavy chain variable region including an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth as SEQ ID NO: 15, and a light chain variable region including an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth as SEQ ID NO: 16.

In some embodiments, the antibody includes a heavy chain variable region and a light chain variable region including the amino acid sequences set forth as SEQ ID NOs: 1 and 2, respectfully. In some embodiments, the antibody includes a heavy chain variable region and a light chain variable region including the amino acid sequences set forth as SEQ ID NOs: 3 and 4, respectfully. In some embodiments, the antibody includes a heavy chain variable region and a light chain variable region including the amino acid sequences set forth as SEQ ID NOs: 5 and 6, respectfully. In some embodiments, the antibody includes a heavy chain variable region and a light chain variable region including the amino acid sequences set forth as SEQ ID NOs: 7 and 8, respectfully. In some embodiments, the antibody includes a heavy chain variable region and a light chain variable region including the amino acid sequences set forth as SEQ ID NOs: 9 and 10, respectfully. In some embodiments, the antibody includes a heavy chain variable region and a light chain variable region including the amino acid sequences set forth as SEQ ID NOs: 11 and 12, respectfully. In some embodiments, the antibody includes a heavy chain variable region and a light chain variable region including the amino acid sequences set forth as SEQ ID NOs: 13 and 14, respectfully. In some embodiments, the antibody includes a heavy chain variable region and a light chain variable region including the amino acid sequences set forth as SEQ ID NOs: 15 and 16, respectfully.

In several embodiments, the antibody can specifically bind ALK with an affinity of at least about 1.0×10^{-8} M, at least about 5.0×10^{-8} M, at least about 1.0×10^{-9} M, at least about 5.0×10^{-9} M, at least about 1.0×10^{-10} M, at least about 5.0×10^{-10} M, or at least about 1.0×10^{-11} M.

The monoclonal antibodies can be human monoclonal antibodies. Chimeric antibodies are also provided. The antibodies can include any suitable framework region, such as (but not limited to) a human framework region. Human framework regions, and mutations that can be made in a human antibody framework regions, are known in the art (see, for example, in U.S. Patent No. 5,585,089, which is incorporated

herein by reference). Alternatively, a heterologous framework region, such as, but not limited to a mouse framework region, can be included in the heavy or light chain of the antibodies. (See, for example, Jones *et al.*, *Nature* 321:522, 1986; Riechmann *et al.*, *Nature* 332:323, 1988; Verhoeven *et al.*, *Science* 239:1534, 1988; Carter *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 89:4285, 1992; Sandhu, *Crit. Rev. Biotech.* 12:437, 1992; and Singer *et al.*, *J. Immunol.* 150:2844, 1993.)

In some embodiments, an antibody that specifically binds ALK as disclosed herein includes up to 10 amino acid substitutions (such as up to 1, 2, 3, 4, 5, 6, 7, 8, or up to 9 amino acid substitutions) in the framework regions of the heavy chain of the antibody, or the light chain of the antibody, or the heavy and light chains of the antibody.

The antibodies or antigen binding fragments disclosed herein can be derivatized or linked to another molecule (such as another peptide or protein). In general, the antibodies or portion thereof is derivatized such that the binding to ALK is not affected adversely by the derivatization or labeling. For example, the antibody can be functionally linked (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (for example, a bi-specific antibody or a diabody), a detectable marker, an effector molecule, or a protein or peptide that can mediate association of the antibody or antibody portion with another molecule (such as a streptavidin core region or a polyhistidine tag).

One type of derivatized antibody is produced by crosslinking two or more antibodies (of the same type or of different types, such as to create bispecific antibodies). Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups separated by an appropriate spacer (such as m-maleimidobenzoyl-N-hydroxysuccinimide ester) or homobifunctional (such as disuccinimidyl suberate). Such linkers are available from Pierce Chemical Company, Rockford, Ill.

The monoclonal antibodies disclosed herein can be of any isotype. The monoclonal antibody can be, for example, an IgM or an IgG antibody, such as IgG₁, IgG₂, IgG₃ or an IgG₄. However, in other embodiments, the disclosed monoclonal antibodies are not an IgG. The class of an antibody that specifically binds ALK can be switched with another (for example, IgG can be switched to IgM), according to well-known procedures. For example, a nucleic acid molecule encoding the V_L or V_H of a disclosed antibody can be operatively linked to a nucleic acid sequence encoding a C_L or C_H from a different class of immunoglobulin molecule. This can be achieved using a vector or nucleic acid molecule that comprises a C_L or C_H chain, as known in the art. For example, an antibody that specifically binds ALK, that was originally IgG may be class switched to an IgM. Class switching can be used to convert one IgG subclass to another, such as from IgG₁ to IgG₂, IgG₃, or IgG₄.

In some examples, the disclosed antibodies are oligomers of antibodies, such as dimers, trimers, tetramers, pentamers, hexamers, septamers, octomers and so on. In some examples, the antibodies are pentamers.

In several embodiments, the constant region of the antibody includes one or more amino acid substitutions to optimize *in vivo* half-life of the antibody. The serum half-life of IgG Abs is regulated by the neonatal Fc receptor (FcRn). Thus, in several embodiments, the antibody includes an amino acid substitution that increases binding to the FcRn. Several such substitutions are known to the person of ordinary skill in the art, such as substitutions at IgG constant regions T250Q and M428L (see, e.g., Hinton *et al.*, *J Immunol.*, 176:346-356, 2006); M428L and N434S (see, e.g., Zalevsky, *et al.*, *Nature Biotechnology*, 28:157-159, 2010); N434A (see, e.g., Petkova *et al.*, *Int. Immunol.*, 18:1759-1769, 2006); T307A, E380A, and N434A (see, e.g.,

Petkova *et al.*, *Int. Immunol.*, 18:1759-1769, 2006); and M252Y, S254T, and T256E (see, e.g., Dall'Acqua *et al.*, *J. Biol. Chem.*, 281:23514-23524, 2006).

In some embodiments, the constant region of the antibody includes one or more amino acid substitutions to optimize Antibody-dependent cell-mediated cytotoxicity (ADCC). ADCC is mediated primarily through a set of closely related Fcγ receptors. In some embodiments, the antibody includes one or more amino acid substitutions that increase binding to FcγRIIIa. Several such substitutions are known to the person of ordinary skill in the art, such as substitutions at IgG constant regions S239D and I332E (see, e.g., Lazar *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 103:4005-4010, 2006); and S239D, A330L, and I332E (see, e.g., Lazar *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 103:4005-4010, 2006).

Combinations of the above substitutions are also included, to generate an IgG constant region with increased binding to FcRn and FcγRIIIa. The combinations increase antibody half-life and ADCC.

Antigen binding fragments of the antibodies that specifically bind to ALK are also encompassed by the present disclosure, such as single-domain antibodies (for example, VH domain antibodies), Fab, F(ab')₂, and Fv. These antigen binding fragments retain the ability to specifically bind ALK. These fragments include:

(1) Fab, the fragment which contains a monovalent antigen-binding fragment of an antibody molecule, can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain;

(2) Fab', the fragment of an antibody molecule can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain; two Fab' fragments are obtained per antibody molecule;

(3) (Fab')₂, the fragment of the antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction; F(ab')₂ is a dimer of two Fab' fragments held together by two disulfide bonds;

(4) Fv, a genetically engineered fragment containing the variable region of the light chain and the variable region of the heavy chain expressed as two chains;

(5) Single chain antibody (such as scFv), a genetically engineered molecule containing the variable region of the light chain, the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule;

(6) A dimer of a single chain antibody (scFV₂), defined as a dimer of a scFV (also known as a "mini-antibody"); and

(7) VH single-domain antibody, an antigen binding fragment consisting of the heavy chain variable domain.

Methods of making these fragments are known in the art (see for example, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York, 1988).

In some embodiments, the antigen binding fragments are Fv antibodies, which are typically about 25 kDa and contain a complete antigen-binding site with three CDRs per each heavy chain and each light chain. To produce these antibodies, the V_H and the V_L can be expressed from two individual nucleic acid constructs in a host cell. If the V_H and the V_L are expressed non-contiguously, the chains of the Fv antibody are typically held together by noncovalent interactions. However, these chains tend to dissociate upon dilution, so methods have been developed to crosslink the chains through glutaraldehyde, intermolecular disulfides, or a peptide

linker. Thus, in one example, the Fv can be a disulfide stabilized Fv (dsFv), wherein the heavy chain variable region and the light chain variable region are chemically linked by disulfide bonds.

In an additional example, the Fv fragments include V_H and V_L chains connected by a peptide linker. These single-chain antigen binding proteins (scFv) are prepared by constructing a structural gene including DNA sequences encoding the V_H and V_L domains connected by an oligonucleotide. The structural gene is inserted into an expression vector, which is subsequently introduced into a host cell such as *E. coli*. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing scFvs are known in the art (see Whitlow *et al.*, *Methods: a Companion to Methods in Enzymology*, Vol. 2, page 97, 1991; Bird *et al.*, *Science* 242:423, 1988; U.S. Patent No. 4,946,778; Pack *et al.*, *Bio/Technology* 11:1271, 1993; and Sandhu, *supra*). Dimers of a single chain antibody (scFV₂), are also contemplated.

Antigen binding fragments can be prepared by proteolytic hydrolysis of the antibody or by expression in *E. coli* of DNA encoding the fragment. Antigen binding fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods. For example, antigen binding fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted F(ab')₂. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly (see U.S. Patent No. 4,036,945 and U.S. Patent No. 4,331,647, and references contained therein; Nisonhoff *et al.*, *Arch. Biochem. Biophys.* 89:230, 1960; Porter, *Biochem. J.* 73:119, 1959; Edelman *et al.*, *Methods in Enzymology*, Vol. 1, page 422, Academic Press, 1967; and Coligan *et al.* at sections 2.8.1-2.8.10 and 2.10.1-2.10.4).

Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

In some cases, antigen binding fragments can be prepared by proteolytic hydrolysis of the antibody or by expression in a host cell (such as *E. coli*) of DNA encoding the fragment. Antigen binding fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods. For example, antigen binding fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted F(ab')₂. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly (see U.S. Patent No. 4,036,945 and U.S. Patent No. 4,331,647).

Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

One of skill will realize that conservative variants of the antibodies can be produced. Such conservative variants employed in antigen binding fragments, such as dsFv fragments or in scFv fragments, will retain critical amino acid residues necessary for correct folding and stabilizing between the V_H and the V_L regions, and will retain the charge characteristics of the residues in order to preserve the low pI and low

toxicity of the molecules. Amino acid substitutions (such as at most one, at most two, at most three, at most four, or at most five amino acid substitutions) can be made in the V_H or the V_L regions to increase yield. In particular examples, the V_H sequence is one of SEQ ID NO: 1, 3, 5, or 7. In other examples, the V_L sequence is one of SEQ ID NO: 2, 4, 7, or 8. Conservative amino acid substitution tables providing functionally similar amino acids are well known to one of ordinary skill in the art. The following six groups are examples of amino acids that are considered to be conservative substitutions for one another:

- 1) Alanine (A), Serine (S), Threonine (T);
- 2) Aspartic acid (D), Glutamic acid (E);
- 3) Asparagine (N), Glutamine (Q);
- 4) Arginine (R), Lysine (K);
- 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and
- 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

Also included are antibodies that bind to the same epitope on ALK to which the ALK specific antibodies provided herein bind. Antibodies that bind to such an epitope can be identified based on their ability to cross-compete (for example, to competitively inhibit the binding of, in a statistically significant manner) with the ALK specific antibodies provided herein in ALK binding assays (such as those described in the Examples). An antibody "competes" for binding when the competing antibody inhibits ALK binding of an antibody of the invention by more than 50%, in the presence of competing antibody concentrations higher than $10^6 \times K_D$ of the competing antibody. In a certain embodiment, the antibody that binds to the same epitope on ALK as the antibodies of the present invention is a human monoclonal antibody. Such human monoclonal antibodies can be prepared and isolated as described herein.

Additionally, to increase binding affinity of the antibody, the V_L and V_H segments can be randomly mutated, such as within H-CDR3 region or the L-CDR3 region, in a process analogous to the *in vivo* somatic mutation process responsible for affinity maturation of antibodies during a natural immune response. Thus *in vitro* affinity maturation can be accomplished by amplifying V_H and V_L regions using PCR primers complementary to the H-CDR3 or L-CDR3, respectively. In this process, the primers have been "spiked" with a random mixture of the four nucleotide bases at certain positions such that the resultant PCR products encode V_H and V_L segments into which random mutations have been introduced into the V_H and/or V_L CDR3 regions. These randomly mutated V_H and V_L segments can be tested to determine the binding affinity for ALK. In particular examples, the V_H amino acid sequence is one of SEQ ID NOs: 1, 3, 5, or 7. In other examples, the V_L amino acid sequence is SEQ ID NOs: 2, 4, 6, or 8.

C. Conjugates

Monoclonal antibodies specific for ALK, or antigen binding fragments thereof, can be conjugated to an agent, such as an effector molecule or detectable marker, using any number of means known to those of skill in the art. Both covalent and noncovalent attachment means may be used. Conjugates include, but are not limited to, molecules in which there is a covalent linkage of an effector molecule or a detectable marker to an antibody or antigen binding fragment that specifically binds ALK. One of skill in the art will appreciate that various effector molecules and detectable markers can be used, including (but not limited to) chemotherapeutic

agents, anti-angiogenic agents, toxins, radioactive agents such as ^{125}I , ^{32}P , ^{14}C , ^3H and ^{35}S and other labels, target moieties and ligands, etc.

The choice of a particular effector molecule or detectable marker depends on the particular target molecule or cell, and the desired biological effect. Thus, for example, the effector molecule can be a cytotoxin that is used to bring about the death of a particular target cell (such as a tumor cell).

The procedure for attaching an effector molecule or detectable marker to an antibody or antigen binding fragment varies according to the chemical structure of the effector. Polypeptides typically contain a variety of functional groups; such as carboxylic acid (COOH), free amine ($-\text{NH}_2$) or sulfhydryl ($-\text{SH}$) groups, which are available for reaction with a suitable functional group on an antibody to result in the binding of the effector molecule or detectable marker. Alternatively, the antibody or antigen binding fragment is derivatized to expose or attach additional reactive functional groups. The derivatization may involve attachment of any of a number of known linker molecules such as those available from Pierce Chemical Company, Rockford, IL. The linker can be any molecule used to join the antibody or antigen binding fragment to the effector molecule or detectable marker. The linker is capable of forming covalent bonds to both the antibody or antigen binding fragment and to the effector molecule or detectable marker. Suitable linkers are well known to those of skill in the art and include, but are not limited to, straight or branched-chain carbon linkers, heterocyclic carbon linkers, or peptide linkers. Where the antibody or antigen binding fragment and the effector molecule or detectable marker are polypeptides, the linkers may be joined to the constituent amino acids through their side groups (such as through a disulfide linkage to cysteine) or to the alpha carbon amino and carboxyl groups of the terminal amino acids.

In several embodiments, the linker can include a spacer element, which, when present, increases the size of the linker such that the distance between the effector molecule or the detectable marker and the antibody or antigen binding fragment is increased. Exemplary spacers are known to the person of ordinary skill, and include those listed in U.S. Pat. Nos. 7,964,566, 498,298, 6,884,869, 6,323,315, 6,239,104, 6,034,065, 5,780,588, 5,665,860, 5,663,149, 5,635,483, 5,599,902, 5,554,725, 5,530,097, 5,521,284, 5,504,191, 5,410,024, 5,138,036, 5,076,973, 4,986,988, 4,978,744, 4,879,278, 4,816,444, and 4,486,414, as well as U.S. Pat. Pub. Nos. 20110212088 and 20110070248, each of which is incorporated by reference in its entirety.

In some embodiments, the linker is cleavable under intracellular conditions, such that cleavage of the linker releases the effector molecule or detectable marker from the antibody or antigen binding fragment in the intracellular environment. In yet other embodiments, the linker is not cleavable and the effector molecule or detectable marker is released, for example, by antibody degradation. In some embodiments, the linker is cleavable by a cleaving agent that is present in the intracellular environment (for example, within a lysosome or endosome or caveola). The linker can be, for example, a peptide linker that is cleaved by an intracellular peptidase or protease enzyme, including, but not limited to, a lysosomal or endosomal protease. In some embodiments, the peptide linker is at least two amino acids long or at least three amino acids long. However, the linker can be 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids long, such as 1-2, 1-3, 2-5, 3-10, 3-15, 1-5, 1-10, 1-15, amino acids long. Proteases can include cathepsins B and D and plasmin, all of which are known to hydrolyze dipeptide drug derivatives resulting in the release of active drug inside target cells (see, for example, Dubowchik and Walker, 1999, *Pharm. Therapeutics* 83:67-123). For example, a peptide linker that is

cleavable by the thiol-dependent protease cathepsin-B, can be used (for example, a Phenylalanine -Leucine or a Glycine- Phenylalanine -Leucine-Glycine linker). Other examples of such linkers are described, for example, in U.S. Pat. No. 6,214,345, incorporated herein by reference. In a specific embodiment, the peptide linker cleavable by an intracellular protease is a Valine-Citruline linker or a Phenylalanine-Lysine linker (see, for example, U.S. Pat. No. 6,214,345, which describes the synthesis of doxorubicin with the Valine-Citruline linker).

In other embodiments, the cleavable linker is pH-sensitive, i.e., sensitive to hydrolysis at certain pH values. Typically, the pH-sensitive linker is hydrolyzable under acidic conditions. For example, an acid-labile linker that is hydrolyzable in the lysosome (for example, a hydrazone, semicarbazone, thiosemicarbazone, cis-aconitic amide, orthoester, acetal, ketal, or the like) can be used. (See, for example, U.S. Pat. Nos. 5,122,368; 5,824,805; 5,622,929; Dubowchik and Walker, 1999, *Pharm. Therapeutics* 83:67-123; Neville et al., 1989, *Biol. Chem.* 264:14653-14661.) Such linkers are relatively stable under neutral pH conditions, such as those in the blood, but are unstable at below pH 5.5 or 5.0, the approximate pH of the lysosome. In certain embodiments, the hydrolyzable linker is a thioether linker (such as, for example, a thioether attached to the therapeutic agent via an acylhydrazone bond (see, for example, U.S. Pat. No. 5,622,929).

In other embodiments, the linker is cleavable under reducing conditions (for example, a disulfide linker). A variety of disulfide linkers are known in the art, including, for example, those that can be formed using SATA (N-succinimidyl-S-acetylthioacetate), SPDP (N-succinimidyl-3-(2-pyridyldithio)propionate), SPDB (N-succinimidyl-3-(2-pyridyldithio)butyrate) and SMPT (N-succinimidyl-oxycarbonyl-alpha-methyl-alpha-(2-pyridyl-dithio)toluene)-, SPDB and SMPT. (See, for example, Thorpe et al., 1987, *Cancer Res.* 47:5924-5931; Wawrzynczak et al., In *Immunoconjugates: Antibody Conjugates in Radioimager and Therapy of Cancer* (C. W. Vogel ed., Oxford U. Press, 1987); Phillips et al., *Cancer Res.* 68:92809290, 2008). See also U.S. Pat. No. 4,880,935.)

In yet other specific embodiments, the linker is a malonate linker (Johnson et al., 1995, *Anticancer Res.* 15:1387-93), a maleimidobenzoyl linker (Lau et al., 1995, *Bioorg-Med-Chem.* 3(10):1299-1304), or a 3'-N-amide analog (Lau et al., 1995, *Bioorg-Med-Chem.* 3(10):1305-12).

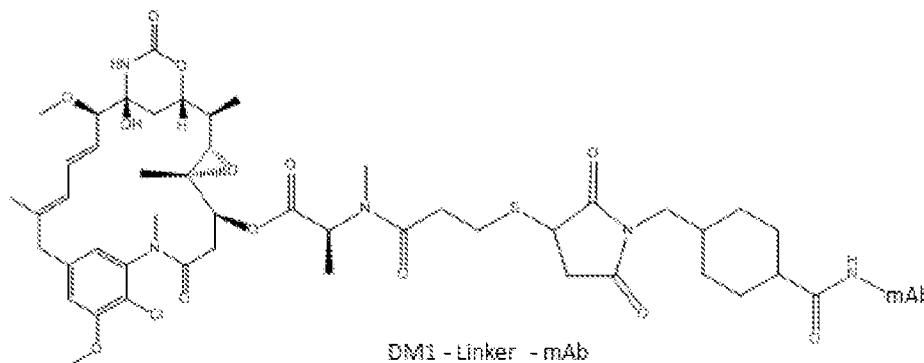
In yet other embodiments, the linker is not cleavable and the effector molecule or detectable marker is released by antibody degradation. (See U.S. Publication No. 2005/0238649 incorporated by reference herein in its entirety).

In several embodiments, the linker is resistant to cleavage in an extracellular environment. For example, no more than about 20%, no more than about 15%, no more than about 10%, no more than about 5%, no more than about 3%, or no more than about 1% of the linkers, in a sample of conjugate, are cleaved when the conjugate is present in an extracellular environment (for example, in plasma). Whether or not a linker is resistant to cleavage in an extracellular environment can be determined, for example, by incubating the conjugate containing the linker of interest with plasma for a predetermined time period (for example, 2, 4, 8, 16, or 24 hours) and then quantitating the amount of free effector molecule or detectable marker present in the plasma. A variety of exemplary linkers that can be used in conjugates are described in WO 2004-010957, U.S. Publication No. 2006/0074008, U.S. Publication No. 20050238649, and U.S. Publication No. 2006/0024317, each of which is incorporated by reference herein in its entirety.

In several embodiments, conjugates of an antibody or antigen binding fragment and one or more small molecule toxins, such as a calicheamicin, maytansinoids, dolastatins, auristatins, a trichothecene, and CC1065, and the derivatives of these toxins that have toxin activity, are provided.

Maytansine compounds suitable for use as maytansinoid toxin moieties are well known in the art, and can be isolated from natural sources according to known methods, produced using genetic engineering techniques (see Yu et al (2002) PNAS 99:7968-7973), or maytansinol and maytansinol analogues prepared synthetically according to known methods. Maytansinoids are mitototic inhibitors which act by inhibiting tubulin polymerization. Maytansine was first isolated from the east African shrub *Maytenus serrata* (U.S. Pat. No. 3,896,111). Subsequently, it was discovered that certain microbes also produce maytansinoids, such as maytansinol and C-3 maytansinol esters (U.S. Pat. No. 4,151,042). Synthetic maytansinol and derivatives and analogues thereof are disclosed, for example, in U.S. Pat. Nos. 4,137,230; 4,248,870; 4,256,746; 4,260,608; 4,265,814; 4,294,757; 4,307,016; 4,308,268; 4,308,269; 4,309,428; 4,313,946; 4,315,929; 4,317,821; 4,322,348; 4,331,598; 4,361,650; 4,364,866; 4,424,219; 4,450,254; 4,362,663; and 4,371,533, each of which is incorporated herein by reference. Conjugates containing maytansinoids, methods of making same, and their therapeutic use are disclosed, for example, in U.S. Pat. Nos. 5,208,020; 5,416,064; 6,441,163 and European Patent EP 0 425 235 B1, the disclosures of which are hereby expressly incorporated by reference.

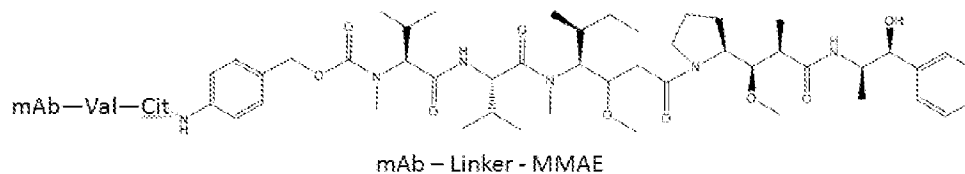
In one example, the conjugate includes a monoclonal antibody that specifically binds ALK (or antigen binding fragment thereof), a non-reducible thioester linker and the maytansinoid toxin DM1; for example the conjugate can include the structure set forth as (wherein "mAb" refers to the monoclonal antibody or antigen binding fragment thereof):



In some embodiments, the effector molecule is an auristatin, such as auristatin E (also known in the art as a derivative of dolastatin-10) or a derivative thereof. The auristatin can be, for example, an ester formed between auristatin E and a keto acid. For example, auristatin E can be reacted with paraacetyl benzoic acid or benzoylvaleric acid to produce AEB and AEVB, respectively. Other exemplary auristatins include AFP, MMAF, and MMAE. The synthesis and structure of exemplary auristatins are described in U.S. Patent Application Publication No. 2003/0083263; International Patent Publication No. WO 04/010957, International Patent Publication No. WO 02/088172, and U.S. Pat. Nos. 7, 498,298, 6,884,869, 6,323,315; 6,239,104; 6,034,065; 5,780,588; 5,665,860; 5,663,149; 5,635,483; 5,599,902; 5,554,725; 5,530,097; 5,521,284; 5,504,191; 5,410,024; 5,138,036; 5,076,973; 4,986,988; 4,978,744; 4,879,278; 4,816,444; and 4,486,414, each of which is incorporated by reference herein in its entirety. Auristatins have been shown to interfere with microtubule dynamics and nuclear and cellular division and have anticancer activity. Auristatins bind tubulin

and can exert a cytotoxic or cytostatic effect on cells. There are a number of different assays, known in the art, which can be used for determining whether an auristatin or resultant conjugate exerts a cytostatic or cytotoxic effect on a desired cell line.

In one example, the conjugate includes a monoclonal antibody that specifically binds ALK (or antigen binding fragment thereof), a cleavable linker including a Valine-Citruline peptide cleavage site, a spacer, and the toxin MMAE; for example the conjugate can include the structure set forth as (wherein "mAb" refers to the monoclonal antibody or antigen binding fragment thereof):



Additional toxins can be employed with antibodies that specifically bind ALK, and antigen binding fragment of these antibodies. Exemplary toxins include *Pseudomonas* exotoxin (PE), ricin, abrin, diphtheria toxin and subunits thereof, ribotoxin, ribonuclease, saporin, and calicheamicin, as well as botulinum toxins A through F. These toxins are well known in the art and many are readily available from commercial sources (for example, Sigma Chemical Company, St. Louis, MO). Contemplated toxins also include variants of the toxins (see, for example, see, U.S. Patent Nos. 5,079,163 and 4,689,401). In some embodiments, these conjugates are of use for the treatment of a tumor, such as a neuroblastoma.

Saporin is a toxin derived from *Saponaria officinalis* that disrupts protein synthesis by inactivating the 60S portion of the ribosomal complex (Stirpe et al., *Bio/Technology*, 10:405-412, 1992). However, the toxin has no mechanism for specific entry into cells, and therefore requires conjugation to an antibody or antigen binding fragment that recognizes a cell-surface protein that is internalized in order to be efficiently taken up by cells.

Diphtheria toxin is isolated from *Corynebacterium diphtheriae*. Typically, diphtheria toxin for use in immunotoxins is mutated to reduce or to eliminate non-specific toxicity. A mutant known as CRM107, which has full enzymatic activity but markedly reduced non-specific toxicity, has been known since the 1970's (Laird and Groman, *J. Virol.* 19:220, 1976), and has been used in human clinical trials. See, U.S. Patent No. 5,792,458 and U.S. Patent No. 5,208,021.

Ricin is the lectin RCA60 from *Ricinus communis* (Castor bean). For examples of ricin, see, U.S. Patent No. 5,079,163 and U.S. Patent No. 4,689,401. *Ricinus communis* agglutinin (RCA) occurs in two forms designated RCA₆₀ and RCA₁₂₀ according to their molecular weights of approximately 65 and 120 kD, respectively (Nicholson & Blaustein, *J. Biochim. Biophys. Acta* 266:543, 1972). The A chain is responsible for inactivating protein synthesis and killing cells. The B chain binds ricin to cell-surface galactose residues and facilitates transport of the A chain into the cytosol (Olsnes et al., *Nature* 249:627-631, 1974 and U.S. Patent No. 3,060,165).

Ribonucleases have also been conjugated to targeting molecules for use as immunotoxins (see Suzuki et al., *Nat. Biotech.* 17:265-70, 1999). Exemplary ribotoxins such as α -sarcin and restrictocin are discussed in, for example Rathore et al., *Gene* 190:31-5, 1997; and Goyal and Batra, *Biochem.* 345 Pt 2:247-54, 2000.

Calicheamicins were first isolated from *Micromonospora echinospora* and are members of the enediyne antitumor antibiotic family that cause double strand breaks in DNA that lead to apoptosis (see, for example Lee et al., *J. Antibiot.* 42:1070-87, 1989). The drug is the toxic moiety of an immunotoxin in clinical trials (see, for example, Gillespie et al., *Ann. Oncol.* 11:735-41, 2000).

5 Abrin includes toxic lectins from *Abrus precatorius*. The toxic principles, abrin a, b, c, and d, have a molecular weight of from about 63 and 67 kD and are composed of two disulfide-linked polypeptide chains A and B. The A chain inhibits protein synthesis; the B chain (abrin-b) binds to D-galactose residues (see, Funatsu et al., *Agr. Biol. Chem.* 52:1095, 1988; and Olsnes, *Methods Enzymol.* 50:330-335, 1978).

10 In one embodiment, the toxin is *Pseudomonas* exotoxin (PE) (U.S. Patent No. 5,602,095). As used herein, PE includes full-length native (naturally occurring) PE or a PE that has been modified. Such modifications can include, but are not limited to, elimination of domain Ia, various amino acid deletions in domains Ib, II and III, single amino acid substitutions and the addition of one or more sequences at the carboxyl terminus (for example, see Siegall et al., *J. Biol. Chem.* 264:14256-14261, 1989). PE employed with the provided antibodies can include the native sequence, cytotoxic fragments of the native sequence, and

15 conservatively modified variants of native PE and its cytotoxic fragments. Cytotoxic fragments of PE include those which are cytotoxic with or without subsequent proteolytic or other processing in the target cell. Cytotoxic fragments of PE include PE40, PE38, and PE35. For additional description of PE and variants thereof, see for example, U.S. Patent Nos. 4,892,827; 5,512,658; 5,602,095; 5,608,039; 5,821,238; and 5,854,044; PCT Publication No. WO 99/51643; Pai et al., *Proc. Natl. Acad. Sci. USA*, 88:3358-3362, 1991; Kondo et al., *J. Biol. Chem.*, 263:9470-9475, 1988; Pastan et al., *Biochim. Biophys. Acta*, 1333:C1-C6, 1997.

20 Also contemplated herein are protease-resistant PE variants and PE variants with reduced immunogenicity, such as, but not limited to PE-LR, PE-6X, PE-8X, PE-LR/6X and PE-LR/8X (see, for example, Weldon et al., *Blood* 113(16):3792-3800, 2009; Onda et al., *Proc. Natl. Acad. Sci. USA*, 105(32):11311-11316, 2008; and PCT Publication Nos. WO 2007/016150, WO 2009/032954 and WO 25 2011/032022, which are herein incorporated by reference).

 In some examples, the PE is a variant that is resistant to lysosomal degradation, such as PE-LR (Weldon et al., *Blood* 113(16):3792-3800, 2009; PCT Publication No. WO 2009/032954). In other examples, the PE is a variant designated PE-LR/6X (PCT Publication No. WO 2011/032022). In other examples, the PE is a variant designated PE-LR/8M (PCT Publication No. WO 2011/032022).

30 A monoclonal antibody that specifically binds ALK (or antigen binding fragment thereof) can also be conjugated with a detectable marker; for example, a detectable marker capable of detection by ELISA, spectrophotometry, flow cytometry, microscopy or diagnostic imaging techniques (such as computed tomography (CT), computed axial tomography (CAT) scans, magnetic resonance imaging (MRI), nuclear magnetic resonance imaging (NMRI), magnetic resonance tomography (MTR), ultrasound, fiberoptic examination, and laparoscopic examination). Specific, non-limiting examples of detectable markers include

35 fluorophores, chemiluminescent agents, enzymatic linkages, radioactive isotopes and heavy metals or compounds (for example super paramagnetic iron oxide nanocrystals for detection by MRI). For example, useful detectable markers include fluorescent compounds, including fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-naphthalenesulfonyl chloride, phycoerythrin, lanthanide phosphors and the like.

40 Bioluminescent markers are also of use, such as luciferase, Green fluorescent protein (GFP), Yellow

fluorescent protein (YFP). An antibody or antigen binding fragment can also be conjugated with enzymes that are useful for detection, such as horseradish peroxidase, β -galactosidase, luciferase, alkaline phosphatase, glucose oxidase and the like. When an antibody or antigen binding fragment is conjugated with a detectable enzyme, it can be detected by adding additional reagents that the enzyme uses to produce a reaction product that can be discerned. For example, when the agent horseradish peroxidase is present the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is visually detectable. An antibody or antigen binding fragment may also be conjugated with biotin, and detected through indirect measurement of avidin or streptavidin binding. It should be noted that the avidin itself can be conjugated with an enzyme or a fluorescent label.

An antibody or antigen binding fragment may be conjugated with a paramagnetic agent, such as gadolinium. Paramagnetic agents such as superparamagnetic iron oxide are also of use as labels. Antibodies can also be conjugated with lanthanides (such as europium and dysprosium), and manganese. An antibody or antigen binding fragment may also be labeled with a predetermined polypeptide epitopes recognized by a secondary reporter (such as leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags).

An antibody or antigen binding fragment can also be conjugated with a radiolabeled amino acid. The radiolabel may be used for both diagnostic and therapeutic purposes. For instance, the radiolabel may be used to detect ALK and ALK expressing cells by x-ray, emission spectra, or other diagnostic techniques. Further, the radiolabel may be used therapeutically as a toxin for treatment of tumors in a subject, for example for treatment of a neuroblastoma. Examples of labels for polypeptides include, but are not limited to, the following radioisotopes or radionucleotides: ^3H , ^{14}C , ^{15}N , ^{35}S , ^{90}Y , ^{99}Tc , ^{111}In , ^{125}I , ^{131}I .

Means of detecting such detectable markers are well known to those of skill in the art. Thus, for example, radiolabels may be detected using photographic film or scintillation counters, fluorescent markers may be detected using a photodetector to detect emitted illumination. Enzymatic labels are typically detected by providing the enzyme with a substrate and detecting the reaction product produced by the action of the enzyme on the substrate, and colorimetric labels are detected by simply visualizing the colored label.

The average number of effector molecule or detectable marker moieties per antibody or antigen binding fragment in a conjugate can range, for example, from 1 to 20 moieties per antibody or antigen binding fragment. In certain embodiments, the average number of effector molecule or detectable marker moieties per antibody or antigen binding fragment in a conjugate range from 1 to about 8; from about 2 to about 6; from about 3 to about 5; from about 3 to about 4; from about 3.1 to about 3.9; from about 3.2 to about 3.8; from about 3.2 to about 3.7; from about 3.2 to about 3.6; from about 3.3 to about 3.8; or from about 3.3 to about 3.7. See, for example, U.S. Pat. No. 7,498,298, incorporated by reference herein in its entirety.

The loading (for example, effector molecule/antibody ratio) of an conjugate may be controlled in different ways, for example, by: (i) limiting the molar excess of effector molecule-linker intermediate or linker reagent relative to antibody, (ii) limiting the conjugation reaction time or temperature, (iii) partial or limiting reductive conditions for cysteine thiol modification, (iv) engineering by recombinant techniques the amino acid sequence of the antibody such that the number and position of cysteine residues is modified for control of the number or position of linker-effector molecule attachments (such as thioMab or thioFab prepared as disclosed in WO2006/03448, incorporated by reference herein in its entirety).

D. Nucleotides, Expression, Vectors, and Host Cells

Nucleic acids encoding the amino acid sequences of antibodies, antibody binding fragments, conjugates, and CARs that specifically bind ALK are provided. Nucleic acids encoding these molecules can readily be produced by one of skill in the art, using the amino acid sequences provided herein (such as the CDR sequences, heavy chain and light chain sequences), sequences available in the art (such as framework sequences), and the genetic code. One of skill in the art can readily use the genetic code to construct a variety of functionally equivalent nucleic acids, such as nucleic acids which differ in sequence but which encode the same antibody sequence, or encode a conjugate or fusion protein including the V_L and/or V_H nucleic acid sequence.

Nucleic acid sequences encoding the of antibodies, antibody binding fragments, conjugates, and CARs that specifically bind ALK can be prepared by any suitable method including, for example, cloning of appropriate sequences or by direct chemical synthesis by methods such as the phosphotriester method of Narang *et al.*, *Meth. Enzymol.* 68:90-99, 1979; the phosphodiester method of Brown *et al.*, *Meth. Enzymol.* 68:109-151, 1979; the diethylphosphoramidite method of Beaucage *et al.*, *Tetra. Lett.* 22:1859-1862, 1981; the solid phase phosphoramidite triester method described by Beaucage & Caruthers, *Tetra. Letts.* 22(20):1859-1862, 1981, for example, using an automated synthesizer as described in, for example, Needham-VanDevanter *et al.*, *Nucl. Acids Res.* 12:6159-6168, 1984; and, the solid support method of U.S. Patent No. 4,458,066. Chemical synthesis produces a single stranded oligonucleotide. This can be converted into double stranded DNA by hybridization with a complementary sequence or by polymerization with a DNA polymerase using the single strand as a template. One of skill would recognize that while chemical synthesis of DNA is generally limited to sequences of about 100 bases, longer sequences may be obtained by the ligation of shorter sequences.

Exemplary nucleic acids can be prepared by cloning techniques. Examples of appropriate cloning and sequencing techniques, and instructions sufficient to direct persons of skill through many cloning exercises are known (see, e.g., Sambrook *et al.* (Molecular Cloning: A Laboratory Manual, 4th ed, Cold Spring Harbor, New York, 2012) and Ausubel *et al.* (In Current Protocols in Molecular Biology, John Wiley & Sons, New York, through supplement 104, 2013). Product information from manufacturers of biological reagents and experimental equipment also provide useful information. Such manufacturers include the SIGMA Chemical Company (Saint Louis, MO), R&D Systems (Minneapolis, MN), Pharmacia Amersham (Piscataway, NJ), CLONTECH Laboratories, Inc. (Palo Alto, CA), Chem Genes Corp., Aldrich Chemical Company (Milwaukee, WI), Glen Research, Inc., GIBCO BRL Life Technologies, Inc. (Gaithersburg, MD), Fluka Chemical-Biochemika Analytika (Fluka Chemie AG, Buchs, Switzerland), Invitrogen (Carlsbad, CA), and Applied Biosystems (Foster City, CA), as well as many other commercial sources known to one of skill.

Nucleic acids can also be prepared by amplification methods. Amplification methods include polymerase chain reaction (PCR), the ligase chain reaction (LCR), the transcription-based amplification system (TAS), the self-sustained sequence replication system (3SR). A wide variety of cloning methods, host cells, and *in vitro* amplification methodologies are well known to persons of skill.

In some embodiments, the nucleic acid molecule encodes a CAR as provided herein for expression in a T cell to generate a chimeric antigen receptor T cell. The nucleic acid molecule encoding the chimeric

antigen binding receptor can be included in a vector (such as a lentiviral vector) for expression in a host cell, such as a T cell. Exemplary cells include a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), and a regulatory T cell. Methods of generating nucleic acid molecules encoding chimeric antigen receptors and T cells including such receptors are known in the art (see, e.g., Brentjens et al., 2010, *Molecular Therapy*, 18:4, 666-668; Morgan et al., 2010, *Molecular Therapy*, published online February 23, 2010, pages 1 -9; Till et al., 2008, *Blood*, 112:2261 -2271; Park et al., *Trends Biotechnol.*, 29:550-557, 2011; Grupp et al., *N Engl J Med.*, 368:1509-1518, 2013; Han et al., *J. Hematol Oncol.*, 6:47, 2013; PCT Pub. WO2012/079000, WO2013/126726; and U.S. Pub. 2012/0213783, each of which is incorporated by reference herein in its entirety.)

The nucleic acid molecules can be expressed in a recombinantly engineered cell such as bacteria, plant, yeast, insect and mammalian cells. The antibodies, antigen binding fragments, and conjugates can be expressed as individual V_H and/or V_L chain (linked to an effector molecule or detectable marker as needed), or can be expressed as a fusion protein. Methods of expressing and purifying antibodies and antigen binding fragments are known and further described herein (see, e.g., Al-Rubeai (ed), *Antibody Expression and Production*, Springer Press, 2011). An immunoadhesin can also be expressed. Thus, in some examples, nucleic acids encoding a V_H and V_L, and immunoadhesin are provided. The nucleic acid sequences can optionally encode a leader sequence.

To create a scFv the V_H- and V_L-encoding DNA fragments can be operatively linked to another fragment encoding a flexible linker, e.g., encoding the amino acid sequence (Gly₄-Ser)₃, 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 domains joined by the flexible linker (see, e.g., Bird et al., *Science* 242:423-426, 1988; Huston et al., *Proc. Natl. Acad. Sci. USA* 85:5879-5883, 1988; McCafferty et al., *Nature* 348:552-554, 1990; Kontermann and Dubel (Ed), *Antibody Engineering*, Vols. 1-2, 2nd Ed., Springer Press, 2010; Harlow and Lane, *Antibodies: A Laboratory Manual*, 2nd, Cold Spring Harbor Laboratory, New York, 2013.). Optionally, a cleavage site can be included in a linker, such as a furin cleavage site.

The nucleic acid encoding a V_H and/or the V_L optionally can encode an Fc domain (immunoadhesin). The Fc domain can be an IgA, IgM or IgG Fc domain. The Fc domain can be an optimized Fc domain, as described in U.S. Published Patent Application No. 20100/093979, incorporated herein by reference. In one example, the immunoadhesin is an IgG₁ Fc.

The single chain antibody may be monovalent, if only a single V_H and V_L are used, bivalent, if two V_H and V_L are used, or polyvalent, if more than two V_H and V_L are used. Bispecific or polyvalent antibodies may be generated that bind specifically to ALK and another antigen, such as, but not limited to CD3. The encoded V_H and V_L optionally can include a furin cleavage site between the V_H and V_L domains.

One or more DNA sequences encoding the antibodies, antibody binding fragments, conjugates, and CARs can be expressed *in vitro* by DNA transfer into a suitable host cell. The cell may be prokaryotic or eukaryotic. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. Methods of stable transfer, meaning that the foreign DNA is continuously maintained in the host, are known in the art. Hybridomas expressing the antibodies of interest are also encompassed by this disclosure.

Polynucleotide sequences encoding the amino acid sequences of CARs, antibodies, antibody binding fragments, and conjugates that specifically bind ALK can be operatively linked to expression control sequences. For example, the expression of nucleic acids encoding the proteins described herein can be achieved by operably linking the DNA or cDNA to a promoter (which is either constitutive or inducible), followed by incorporation into an expression cassette. The promoter can be any promoter of interest, including a cytomegalovirus promoter and a human T cell lymphotropic virus promoter (HTLV)-1. Optionally, an enhancer, such as a cytomegalovirus enhancer, is included in the construct. The cassettes can be suitable for replication and integration in either prokaryotes or eukaryotes. Typical expression cassettes contain specific sequences useful for regulation of the expression of the DNA encoding the protein. For example, the expression cassettes can include appropriate promoters, enhancers, transcription and translation terminators, initiation sequences, a start codon (*i.e.*, ATG) in front of a protein-encoding gene, splicing signal for introns, sequences for the maintenance of the correct reading frame of that gene to permit proper translation of mRNA, and stop codons. The vector can encode a selectable marker, such as a marker encoding drug resistance (for example, ampicillin or tetracycline resistance).

To obtain high level expression of a cloned gene, it is desirable to construct expression cassettes which contain, at the minimum, a strong promoter to direct transcription, a ribosome binding site for translational initiation (internal ribosomal binding sequences), and a transcription/translation terminator. For *E. coli*, this includes a promoter such as the T7, trp, lac, or lambda promoters, a ribosome binding site, and preferably a transcription termination signal. For eukaryotic cells, the control sequences can include a promoter and/or an enhancer derived from, for example, an immunoglobulin gene, HTLV, SV40 or cytomegalovirus, and a polyadenylation sequence, and can further include splice donor and/or acceptor sequences (for example, CMV and/or HTLV splice acceptor and donor sequences). The cassettes can be transferred into the chosen host cell by well-known methods such as transformation or electroporation for *E. coli* and calcium phosphate treatment, electroporation or lipofection for mammalian cells. Cells transformed by the cassettes can be selected by resistance to antibiotics conferred by genes contained in the cassettes, such as the amp, gpt, neo and hyg genes.

For purposes of producing a recombinant CAR, the host cell may be a mammalian cell. The host cell may be a human cell. In some embodiments, the host cell may be a peripheral blood lymphocyte (PBL) or a peripheral blood mononuclear cell (PBMC), or a T cell. The T cell can be any T cell, such as a cultured T cell, *e.g.*, a primary T cell, or a T cell from a cultured T cell line, *e.g.*, Jurkat, SupT1, etc., or a T cell obtained from a mammal. If obtained from a mammal, the T cell can be obtained from numerous sources, including but not limited to blood, bone marrow, lymph node, the thymus, or other tissues or fluids. T cells can also be enriched for or purified. The T cell may be a human T cell. The T cell may be a T cell isolated from a human. The T cell can be any type of T cell and can be of any developmental stage, including but not limited to, CD4⁺/CD8⁺ double positive T cells, CD4⁺ helper T cells, *e.g.*, Th₁ and Th₂ cells, CD8⁺ T cells (*e.g.*, cytotoxic T cells), tumor infiltrating cells, memory T cells, naive T cells, and the like. The T cell may be a CD8⁺ T cell or a CD4⁺ T cell.

Also provided is a population of cells comprising at least one host cell described herein. The population of cells can be a heterogeneous population comprising the host cell comprising any of the recombinant expression vectors described, in addition to at least one other cell, *e.g.*, a host cell (*e.g.*, a T cell), which does not comprise any of the recombinant expression vectors, or a cell other than a T cell, *e.g.*, a B cell,

a macrophage, a neutrophil, an erythrocyte, a hepatocyte, an endothelial cell, an epithelial cell, a muscle cell, a brain cell, etc. Alternatively, the population of cells can be a substantially homogeneous population, in which the population comprises mainly host cells (e.g., consisting essentially of) comprising the recombinant expression vector. The population also can be a clonal population of cells, in which all cells of the population are clones of a single host cell comprising a recombinant expression vector, such that all cells of the population comprise the recombinant expression vector. In one embodiment of the invention, the population of cells is a clonal population comprising host cells comprising a recombinant expression vector as described herein.

Modifications can be made to a nucleic acid encoding a polypeptide described herein without diminishing its biological activity. Some modifications can be made to facilitate the cloning, expression, or incorporation of the targeting molecule into a fusion protein. Such modifications are well known to those of skill in the art and include, for example, termination codons, a methionine added at the amino terminus to provide an initiation site, additional amino acids placed on either terminus to create conveniently located restriction sites, or additional amino acids (such as poly His) to aid in purification steps. In addition to recombinant methods, the immunoconjugates, effector moieties, and antibodies of the present disclosure can also be constructed in whole or in part using standard peptide synthesis well known in the art.

Once expressed, the antibodies, antigen binding fragments, and conjugates can be purified according to standard procedures in the art, including ammonium sulfate precipitation, affinity columns, column chromatography, and the like (see, generally, Simpson ed., *Basic methods in Protein Purification and Analysis: A laboratory Manual*, Cold Harbor Press, 2008). The antibodies, antigen binding fragment, and conjugates need not be 100% pure. Once purified, partially or to homogeneity as desired, if to be used therapeutically, the polypeptides should be substantially free of endotoxin.

Methods for expression of the antibodies, antigen binding fragments, and conjugates, and/or refolding to an appropriate active form, from mammalian cells, and bacteria such as *E. coli* have been described and are well-known and are applicable to the antibodies disclosed herein. See, e.g., Harlow and Lane, *Antibodies: A Laboratory Manual*, 2nd, Cold Spring Harbor Laboratory, New York, 2013, Simpson ed., *Basic methods in Protein Purification and Analysis: A laboratory Manual*, Cold Harbor Press, 2008, and Ward *et al.*, *Nature* 341:544, 1989. Often, functional heterologous proteins from *E. coli* or other bacteria are isolated from inclusion bodies and require solubilization using strong denaturants, and subsequent refolding. During the solubilization step, as is well known in the art, a reducing agent must be present to separate disulfide bonds. An exemplary buffer with a reducing agent is: 0.1 M Tris pH 8, 6 M guanidine, 2 mM EDTA, 0.3 M DTE (dithioerythritol). Reoxidation of the disulfide bonds can occur in the presence of low molecular weight thiol reagents in reduced and oxidized form, as described in Saxena *et al.*, *Biochemistry* 9: 5015-5021, 1970, and especially as described by Buchner *et al.*, *supra*.

Isolation and purification of recombinantly expressed polypeptide can be carried out by conventional means including preparative chromatography and immunological separations. Once expressed, the conjugate, antibody, or antigen binding fragment thereof, can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, and the like (see, generally, R. Scopes, *Protein Purification*, Springer-Verlag, N.Y., 1982). Substantially pure compositions of at least about 90 to 95% homogeneity are disclosed herein, and 98 to 99% or more homogeneity can be used for

pharmaceutical purposes. Once purified, partially or to homogeneity as desired, if to be used therapeutically, the polypeptides should be substantially free of endotoxin.

Methods for expression of single chain antibodies and refolding to an appropriate active form, including single chain antibodies, from bacteria such as *E. coli* have been described and are well-known and are applicable to the antibodies disclosed herein. See, Buchner *et al.*, *Anal. Biochem.* 205:263-270, 1992; Pluckthun, *Biotechnology* 9:545, 1991; Huse *et al.*, *Science* 246:1275, 1989 and Ward *et al.*, *Nature* 341:544, 1989, all incorporated by reference herein. Often, functional heterologous proteins from *E. coli* or other bacteria are isolated from inclusion bodies and require solubilization using strong denaturants, and subsequent refolding. During the solubilization step, as is well known in the art, a reducing agent must be present to separate disulfide bonds. An exemplary buffer with a reducing agent is: 0.1 M Tris pH 8, 6 M guanidine, 2 mM EDTA, 0.3 M DTE (dithioerythritol). Reoxidation of the disulfide bonds can occur in the presence of low molecular weight thiol reagents in reduced and oxidized form, as described in Saxena *et al.*, *Biochemistry*, 9: 5015-5021, 1970, incorporated by reference herein, and especially as described by Buchner *et al.*, *supra*. Renaturation is typically accomplished by dilution (for example, 100-fold) of the denatured and reduced protein into refolding buffer. An exemplary buffer is 0.1 M Tris, pH 8.0, 0.5 M L-arginine, 8 mM oxidized glutathione (GSSG), and 2 mM EDTA.

As a modification to the two chain antibody purification protocol, the heavy and light chain regions are separately solubilized and reduced and then combined in the refolding solution. An exemplary yield is obtained when these two proteins are mixed in a molar ratio such that a 5 fold molar excess of one protein over the other is not exceeded. Excess oxidized glutathione or other oxidizing low molecular weight compounds can be added to the refolding solution after the redox-shuffling is completed.

In addition to recombinant methods, the antibodies, antigen binding fragments, and/or conjugates can also be constructed in whole or in part using standard peptide synthesis. Solid phase synthesis of the polypeptides can be accomplished by attaching the C-terminal amino acid of the sequence to an insoluble support followed by sequential addition of the remaining amino acids in the sequence. Techniques for solid phase synthesis are described by Barany & Merrifield, *The Peptides: Analysis, Synthesis, Biology*. Vol. 2: *Special Methods in Peptide Synthesis, Part A*. pp. 3-284; Merrifield *et al.*, *J. Am. Chem. Soc.* 85:2149-2156, 1963, and Stewart *et al.*, *Solid Phase Peptide Synthesis*, 2nd ed., Pierce Chem. Co., Rockford, Ill., 1984. Proteins of greater length may be synthesized by condensation of the amino and carboxyl termini of shorter fragments. Methods of forming peptide bonds by activation of a carboxyl terminal end (such as by the use of the coupling reagent N, N'-dicyclohexylcarbodiimide) are well known in the art.

E. Methods of Treatment

A therapeutically effective amount of a disclosed antibody, antigen binding fragment, conjugate, CAR or T cell expressing a CAR can be administered to a subject to treat a tumor in the subject. A subject can be selected for treatment that has, is suspected of having or is at risk of developing a tumor, such as a neuroblastoma, a rhabdomyosarcoma, or a glioblastoma. Subjects that can benefit from the disclosed methods include human and veterinary subjects.

In some examples, a disclosed antibody, antigen binding fragment, conjugate, CAR or T cell expressing a CAR disclosed herein can be administered to a subject to slow or inhibit the growth or metastasis

of a tumor. In these applications, a therapeutically effective amount of a disclosed antibody, antigen binding fragment, conjugate, CAR or T cell expressing a CAR or composition is administered to a subject in an amount and under conditions sufficient to form an immune complex with ALK, thereby slowing or inhibiting the growth or the metastasis of a tumor, or to inhibit a sign or a symptom of a tumor. Examples of suitable subjects include those diagnosed with or suspecting of having cancer (for example, a subject having a tumor), for example a subject having a neuroblastoma.

The therapeutically effective amount will depend upon the severity of the disease and the general state of the patient's health. A therapeutically effective amount is that which provides either subjective relief of a symptom(s) or an objectively identifiable improvement as noted by the clinician or other qualified observer. In one embodiment, a therapeutically effective amount is the amount necessary to inhibit tumor growth (such as growth of a neuroblastoma), or the amount that is effective at reducing a sign or a symptom of the tumor. The therapeutically effective amount of the agents administered can vary depending upon the desired effects and the subject to be treated. In some examples, therapeutic amounts are amounts which eliminate or reduce the patient's tumor burden, or which prevent or reduce the proliferation of metastatic cells.

In some non-limiting embodiments, a therapeutically effective amount of T cells expressing one or more ALK-specific chimeric antigen receptors as described herein can be administered to a subject in need thereof, for example a subject with an ALK-positive tumor. The therapeutically effective amount of the CAR T cells administered to the subject will depend upon the severity of the disease and the general state of the patient's health. In some embodiments, the subject is administered from 1×10^5 to 1×10^7 (such as from 1×10^5 to 1×10^6 , from 1×10^6 to 1×10^7 , from 5×10^5 to 5×10^6 , from 5×10^5 to 1×10^6 , from 7×10^5 to 3×10^6 , from 8×10^5 to 2×10^6 , or about 5×10^5 , 6×10^5 , 7×10^5 , 8×10^5 , 9×10^5 , 1×10^6 , 2×10^6 , 3×10^6 , 4×10^6 , or 5×10^6) CART cells/kg in a single dose, in multiple doses (e.g., 2, 3, or 4, doses) or spread over multiple doses (e.g., 2, 3, or 4 doses). The T cells can be autologous T cells that have been obtained from the subject and transduced or transformed with a vector (such as a lentiviral vector) or nucleic acid molecule encoding the ALK-specific CAR. Methods of making such T cells are known and disclosed herein.

Methods of generating chimeric antigen receptors, T cells including such receptors, and their use (e.g., for treatment of cancer) are known in the art and further described herein (see, e.g., Brentjens et al., 2010, *Molecular Therapy*, 18:4, 666-668; Morgan et al., 2010, *Molecular Therapy*, published online February 23, 2010, pages 1 -9; Till et al., 2008, *Blood*, 112:2261 -2271; Park et al., *Trends Biotechnol.*, 29:550-557, 2011; Grupp et al., *N Engl J Med.*, 368:1509-1518, 2013; Han et al., *J. Hematol Oncol.*, 6:47, 2013; Tumaini et al., *Cytotherapy*, 15, 1406-1417, 2013; Haso et al., (2013) *Blood*, 121, 1165-1174; PCT Pubs. WO2012/079000, WO2013/126726; and U.S. Pub. 2012/0213783, each of which is incorporated by reference herein in its entirety).

In some embodiments, the disclosed methods include isolating T cells from a subject, transducing the T cells with an expression vector (such as a lentiviral vector) encoding the chimeric antigen receptor, and administering the CAR-expressing T cells to the subject for treatment, for example for treatment of an ALK-positive tumor in the subject.

Subjects can be screened prior to initiating the disclosed therapies, for example to determine whether the subject has a tumor. The presence of a tumor indicates that the tumor can be treated using the methods provided herein. In some embodiments, a subject with an ALK-positive tumor is selected for treatment, for

example, by detecting ALK expression and/or activity in a biological sample obtained from the subject. In some embodiments, cell surface expression of ALK is detected to identify an ALK positive tumor. For example ALK nucleic acids (such as an ALK gene, cDNA, or mRNA), ALK proteins, or ALK kinase activity, can be detected, and in some examples quantified. The detected ALK in the biological sample is compared to a control (such as a normal, non-melanoma sample, for example a normal skin sample). An increase in the amount of expressed ALK (such as ALK nucleic acids (for example an ALK gene, cDNA, or mRNA), ALK proteins, or ALK kinase activity in the biological sample relative to the control indicates the presence of an ALK positive tumor, and can be used to select a subject for treatment with one or more of the agents disclosed herein. For example, an increase in the test sample of at least 10%, at least 20%, at least 30%, at least 50%, at least 75%, at least 80%, at least 90%, at least 100%, at least 200% or even greater than 500%, relative to the control, indicates the subject (such as a human subject) is likely to respond favorably to treatment with one or more of the agents disclosed herein. Suitable methods for detecting and/or monitoring an ALK-positive tumor in a subject (such as an ALK-positive neuroblastoma) can be selected by a treating physician. In one embodiment, a sample is obtained from a subject, and the presence of a cell that expresses ALK is assessed *in vitro*. In another embodiment, the antibodies disclosed herein can be used to detect cells that express ALK *in vivo*. In some examples, *in vivo* detection of a cell that expresses ALK detects a tumor in the subject.

Any method of administration can be used for the disclosed therapeutic agents, including local and systemic administration. For example topical, oral, intravascular such as intravenous, intramuscular, intraperitoneal, intranasal, intradermal, intrathecal and subcutaneous administration can be used. The particular mode of administration and the dosage regimen will be selected by the attending clinician, taking into account the particulars of the case (for example the subject, the disease, the disease state involved, and whether the treatment is prophylactic). In cases in which more than one agent or composition is being administered, one or more routes of administration may be used; for example, a chemotherapeutic agent may be administered orally and an antibody or antigen binding fragment or conjugate or composition may be administered intravenously. Methods of administration include injection for which the conjugates, antibodies, antigen binding fragments, or compositions are provided in a nontoxic pharmaceutically acceptable carrier such as water, saline, Ringer's solution, dextrose solution, 5% human serum albumin, fixed oils, ethyl oleate, or liposomes. In some embodiments, local administration of the disclosed compounds can be used, for instance by applying the antibody or antigen binding fragment to a region of tissue from which a tumor has been removed, or a region suspected of being prone to tumor development. In some embodiments, sustained intra-tumoral (or near-tumoral) release of the pharmaceutical preparation that includes a therapeutically effective amount of the antibody or antigen binding fragment may be beneficial. In other examples, the conjugate is applied as an eye drop topically to the cornea, or intravitreally into the eye.

The disclosed therapeutic agents can be formulated in unit dosage form suitable for individual administration of precise dosages. In addition, the disclosed therapeutic agents may be administered in a single dose or in a multiple dose schedule. A multiple dose schedule is one in which a primary course of treatment may be with more than one separate dose, for instance 1-10 doses, followed by other doses given at subsequent time intervals as needed to maintain or reinforce the action of the compositions. Treatment can involve daily or multi-daily doses of compound(s) over a period of a few days to months, or even years. Thus, the dosage

regime will also, at least in part, be determined based on the particular needs of the subject to be treated and will be dependent upon the judgment of the administering practitioner.

Typical dosages of the antibodies or conjugates can range from about 0.01 to about 30 mg/kg, such as from about 0.1 to about 10 mg/kg.

5 In particular examples, the subject is administered a therapeutic composition that includes one or more of the conjugates, antibodies, compositions, CAR T cells or additional agents, on a multiple daily dosing schedule, such as at least two consecutive days, 10 consecutive days, and so forth, for example for a period of weeks, months, or years. In one example, the subject is administered the conjugates, antibodies, compositions or additional agents for a period of at least 30 days, such as at least 2 months, at least 4 months, at least 6
10 months, at least 12 months, at least 24 months, or at least 36 months.

In some embodiments, the disclosed methods include providing surgery, radiation therapy, and/or chemotherapeutics to the subject in combination with a disclosed antibody, antigen binding fragment, conjugate, CAR or T cell expressing a CAR (for example, sequentially, substantially simultaneously, or simultaneously). Methods and therapeutic dosages of such agents and treatments are known to those skilled in
15 the art, and can be determined by a skilled clinician. Preparation and dosing schedules for the additional agent may be used according to manufacturer's instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in Chemotherapy Service, (1992) Ed., M. C. Perry, Williams & Wilkins, Baltimore, Md.

In some embodiments, the combination therapy can include administration of a therapeutically
20 effective amount of an additional ALK inhibitor to a subject (such as a subject having ALK-positive tumor). The ALK inhibitor can be is a small molecule inhibitor, such as crizotinib (Pfizer, New York, NY), AP26113 (Ariad Pharmaceuticals, Cambridge, MA), CH5424802 (Chugai Pharmaceutical, Tokyo, Japan), LDK378 (Novartis, Basel, Switzerland), ASP3026 (Astellas Pharma, Northbrook, IL), X-396 (Xcovery, West Palm Beach, FL), or retaspimycin (Infinity Pharmaceuticals, Cambridge, MA). Additional ALK inhibitors include
25 3-39 (Novartis), GSK1838705A (GlaxoSmithKline, Boston, MA), and CEP-28122 (Cephalon, Frazer, PA). In another example, an ALK inhibitor is an anti-ALK antibody, such as a humanized anti-ALK antibody.

Methods and therapeutic dosages of such agents and treatments are known to those of ordinary skill in the art, and for example, can be determined by a skilled clinician. In a non-limiting example, a therapeutically effective amount of crizotinib is administered to a subject having a tumor that is identified as ALK-positive. In
30 some examples, a therapeutically effective amount of crizotinib can be about 50-2000 mg/day (such as about 50, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000 mg/day), administered orally in one or two doses per day. In some examples, the methods include orally administering 200 mg of crizotinib to the subject once or twice per day if the sample from the subject is scored as ALK-positive. In other examples, the methods include orally administering 250 mg of
35 crizotinib to the subject once or twice per day if the sample from the subject is scored as ALK-positive. Dosages and dosing schedules of crizotinib for a subject can be determined by a skilled clinician, taking into account additional factors such as tumor site, tumor stage, tumor grade, patient treatment history, patient performance and nutritional status, concomitant health problems, social and logistic factors, previous primary tumors, and patient preference. Crizotinib may be administered on a continuous dosing schedule or

administered for one or more cycles (for example, one or more cycles of 21-28 days). Treatment may repeat every 21-28 days if administered in cycles.

Non-limiting examples of additional therapeutic agents that can be used with the combination therapy include microtubule binding agents, DNA intercalators or cross-linkers, DNA synthesis inhibitors, DNA and RNA transcription inhibitors, antibodies, enzymes, enzyme inhibitors, gene regulators, and angiogenesis inhibitors. These agents (which are administered at a therapeutically effective amount) and treatments can be used alone or in combination. For example, any suitable anti-cancer or anti-angiogenic agent can be administered in combination with the antibodies, conjugates disclosed herein. Methods and therapeutic dosages of such agents are known to those skilled in the art, and can be determined by a skilled clinician.

Additional chemotherapeutic agents include, but are not limited to alkylating agents, such as nitrogen mustards (for example, chlorambucil, chlormethine, cyclophosphamide, ifosfamide, and melphalan), nitrosoureas (for example, carmustine, fotemustine, lomustine, and streptozocin), platinum compounds (for example, carboplatin, cisplatin, oxaliplatin, and BBR3464), busulfan, dacarbazine, mechlorethamine, procarbazine, temozolomide, thiotepa, and uramustine; antimetabolites, such as folic acid (for example, methotrexate, pemetrexed, and raltitrexed), purine (for example, cladribine, clofarabine, fludarabine, mercaptopurine, and tioguanine), pyrimidine (for example, capecitabine), cytarabine, fluorouracil, and gemcitabine; plant alkaloids, such as podophyllum (for example, etoposide, and teniposide), taxane (for example, docetaxel and paclitaxel), vinca (for example, vinblastine, vincristine, vindesine, and vinorelbine); cytotoxic/antitumor antibiotics, such as anthracycline family members (for example, daunorubicin, doxorubicin, epirubicin, idarubicin, mitoxantrone, and valrubicin), bleomycin, rifampicin, hydroxyurea, and mitomycin; topoisomerase inhibitors, such as topotecan and irinotecan; monoclonal antibodies, such as alemtuzumab, bevacizumab, cetuximab, gemtuzumab, rituximab, panitumumab, pertuzumab, and trastuzumab; photosensitizers, such as aminolevulinic acid, methyl aminolevulinate, porfimer sodium, and verteporfin; and other agents, such as alitretinoin, altretamine, amsacrine, anagrelide, arsenic trioxide, asparaginase, axitinib, bexarotene, bevacizumab, bortezomib, celecoxib, denileukin diftitox, erlotinib, estramustine, gefitinib, hydroxycarbamide, imatinib, lapatinib, pazopanib, pentostatin, masoprocol, mitotane, pegaspargase, tamoxifen, sorafenib, sunitinib, vemurafenib, vandetanib, and tretinoin. Selection and therapeutic dosages of such agents are known to those skilled in the art, and can be determined by a skilled clinician.

The combination therapy may provide synergy and prove synergistic, that is, the effect achieved when the active ingredients used together is greater than the sum of the effects that results from using the compounds separately. A synergistic effect may be attained when the active ingredients are: (1) co-formulated and administered or delivered simultaneously in a combined, unit dosage formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by some other regimen. When delivered in alternation, a synergistic effect may be attained when the compounds are administered or delivered sequentially, for example by different injections in separate syringes. In general, during alternation, an effective dosage of each active ingredient is administered sequentially, i.e. serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together.

In one embodiment, an effective amount of an antibody or antigen binding fragment that specifically binds to ALK or a conjugate thereof is administered to a subject having an ALK positive tumor following anti-cancer treatment. After a sufficient amount of time has elapsed to allow for the administered antibody or

antigen binding fragment or conjugate to form an immune complex with ALK on an endothelial cell, the immune complex is detected. The presence (or absence) of the immune complex indicates the effectiveness of the treatment. For example, an increase in the immune complex compared to a control taken prior to the treatment indicates that the treatment is not effective, whereas a decrease in the immune complex compared to a control taken prior to the treatment indicates that the treatment is effective.

F. Compositions

Compositions are provided that include one or more of the disclosed antibodies, antigen binding fragments, conjugates, CARs, or T cells expressing a CAR that specifically bind to ALK, in a carrier (such as a pharmaceutically acceptable carrier). The compositions can be prepared in unit dosage forms for administration to a subject. The amount and timing of administration are at the discretion of the treating clinician to achieve the desired outcome. The compositions can be formulated for systemic (such as intravenous) or local (such as intra-tumor) administration. In one example, a disclosed antibody, antigen binding fragment, conjugate, CAR or T cell expressing a CAR, is formulated for parenteral administration, such as intravenous administration. Compositions including a conjugate, antibody or antigen binding fragment as disclosed herein are of use, for example, for the treatment and detection of a tumor, for a neuroblastoma. In some examples, the compositions are useful for the treatment or detection of a carcinoma. The compositions including a conjugate, antibody or antigen binding fragment as disclosed herein are also of use, for example, for the detection of pathological angiogenesis.

The compositions for administration can include a solution of the conjugate, antibody or antigen binding fragment dissolved in a pharmaceutically acceptable carrier, such as an aqueous carrier. A variety of aqueous carriers can be used, for example, buffered saline and the like. These solutions are sterile and generally free of undesirable matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of antibody or antigen binding fragment or conjugate in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the subject's needs. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in the art.

A typical composition for intravenous administration includes about 0.01 to about 30 mg/kg of antibody or antigen binding fragment or conjugate per subject per day (or the corresponding dose of a conjugate including the antibody or antigen binding fragment). Actual methods for preparing administrable compositions will be known or apparent to those skilled in the art and are described in more detail in such publications as *Remington's Pharmaceutical Science, 19th ed.*, Mack Publishing Company, Easton, PA (1995).

Antibodies, antigen binding fragments, or conjugates may be provided in lyophilized form and rehydrated with sterile water before administration, although they are also provided in sterile solutions of known concentration. The antibody or antigen binding fragment or conjugate solution is then added to an infusion bag containing 0.9% sodium chloride, USP, and in some cases administered at a dosage of from 0.5 to 15 mg/kg of body weight. Considerable experience is available in the art in the administration of antibody or

antigen binding fragment and conjugate drugs; for example, antibody drugs have been marketed in the U.S. since the approval of RITUXAN® in 1997. Antibodies, antigen binding fragments and conjugates can be administered by slow infusion, rather than in an intravenous push or bolus. In one example, a higher loading dose is administered, with subsequent, maintenance doses being administered at a lower level. For example, an initial loading dose of 4 mg/kg antibody or antigen binding fragment (or the corresponding dose of a conjugate including the antibody or antigen binding fragment) may be infused over a period of some 90 minutes, followed by weekly maintenance doses for 4-8 weeks of 2 mg/kg infused over a 30 minute period if the previous dose was well tolerated.

Controlled release parenteral formulations can be made as implants, oily injections, or as particulate systems. For a broad overview of protein delivery systems see, Banga, A.J., *Therapeutic Peptides and Proteins: Formulation, Processing, and Delivery Systems*, Technomic Publishing Company, Inc., Lancaster, PA, (1995). Particulate systems include microspheres, microparticles, microcapsules, nanocapsules, nanospheres, and nanoparticles. Microcapsules contain the therapeutic protein, such as a cytotoxin or a drug, as a central core. In microspheres the therapeutic is dispersed throughout the particle. Particles, microspheres, and microcapsules smaller than about 1 µm are generally referred to as nanoparticles, nanospheres, and nanocapsules, respectively. Capillaries have a diameter of approximately 5 µm so that only nanoparticles are administered intravenously. Microparticles are typically around 100 µm in diameter and are administered subcutaneously or intramuscularly. See, for example, Kreuter, J., *Colloidal Drug Delivery Systems*, J. Kreuter, ed., Marcel Dekker, Inc., New York, NY, pp. 219-342 (1994); and Tice & Tabibi, *Treatise on Controlled Drug Delivery*, A. Kydonieus, ed., Marcel Dekker, Inc. New York, NY, pp. 315-339, (1992).

Polymers can be used for ion-controlled release of the antibody or antigen binding fragment or conjugate compositions disclosed herein. Various degradable and nondegradable polymeric matrices for use in controlled drug delivery are known in the art (Langer, *Accounts Chem. Res.* 26:537-542, 1993). For example, the block copolymer, polaxamer 407, exists as a viscous yet mobile liquid at low temperatures but forms a semisolid gel at body temperature. It has been shown to be an effective vehicle for formulation and sustained delivery of recombinant interleukin-2 and urease (Johnston *et al.*, *Pharm. Res.* 9:425-434, 1992; and Pec *et al.*, *J. Parent. Sci. Tech.* 44(2):58-65, 1990). Alternatively, hydroxyapatite has been used as a microcarrier for controlled release of proteins (Ijntema *et al.*, *Int. J. Pharm.* 112:215-224, 1994). In yet another aspect, liposomes are used for controlled release as well as drug targeting of the lipid-capsulated drug (Betageri *et al.*, *Liposome Drug Delivery Systems*, Technomic Publishing Co., Inc., Lancaster, PA (1993)). Numerous additional systems for controlled delivery of therapeutic proteins are known (see U.S. Patent No. 5,055,303; U.S. Patent No. 5,188,837; U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; U.S. Patent No. 4,957,735; U.S. Patent No. 5,019,369; U.S. Patent No. 5,055,303; U.S. Patent No. 5,514,670; U.S. Patent No. 5,413,797; U.S. Patent No. 5,268,164; U.S. Patent No. 5,004,697; U.S. Patent No. 4,902,505; U.S. Patent No. 5,506,206; U.S. Patent No. 5,271,961; U.S. Patent No. 5,254,342 and U.S. Patent No. 5,534,496).

G. Kits

Kits are also provided. For example, kits for treating a tumor in a subject, or making a CAR T cell that expresses one or more of the CARs disclosed herein. The kits will typically include a disclosed antibody,

antigen binding fragment, conjugate, nucleic acid molecule, CAR or T cell expressing a CAR as disclosed herein. More than one of the disclosed antibodies, antigen binding fragments, conjugates, nucleic acid molecules, CARs or T cells expressing a CAR can be included in the kit.

The kit can include a container and a label or package insert on or associated with the container.

5 Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container typically holds a composition including one or more of the disclosed antibodies, antigen binding fragments, conjugates, nucleic acid molecules, CARs or T cells expressing a CAR. In several embodiments the container may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection
10 needle). A label or package insert indicates that the composition is used for treating the particular condition.

The label or package insert typically will further include instructions for use of a disclosed antibodies, antigen binding fragments, conjugates, nucleic acid molecules, CARs or T cells expressing a CAR, for example, in a method of treating or preventing a tumor or of making a CAR T cell. The package insert typically includes instructions customarily included in commercial packages of therapeutic products that
15 contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products. The instructional materials may be written, in an electronic form (such as a computer diskette or compact disk) or may be visual (such as video files). The kits may also include additional components to facilitate the particular application for which the kit is designed. Thus, for example, the kit may additionally contain means of detecting a label (such as enzyme substrates for enzymatic
20 labels, filter sets to detect fluorescent labels, appropriate secondary labels such as a secondary antibody, or the like). The kits may additionally include buffers and other reagents routinely used for the practice of a particular method. Such kits and appropriate contents are well known to those of skill in the art.

EXAMPLES

25 The following examples are provided to illustrate particular features of certain embodiments, but the scope of the claims should not be limited to those features exemplified.

Example 1

Targeting Cell-Surface ALK with CARs Derived from Anti-ALK Antibodies

30 The identification of unique or over-expressed cell-surface proteins on tumor cells that are absent on normal tissues, has been challenging for pediatric malignancies. The cell surface tyrosine kinase ALK (CD246, anaplastic lymphoma kinase) is unique in that it is expressed in native, mutated, or over-expressed forms on the plasma membrane surface of neuroblastoma. Antibodies that bind to ALK were identified, their variable regions sequenced, and used to construct chimeric antigen receptors (CARs). The ability to successfully
35 transduce primary T lymphocytes with retroviral gene vectors expressing a series of ALK-specific CARs is disclosed below. T lymphocytes transduced with ALK-specific CARs were demonstrated to mediate cytolytic activity against ALK-expressing tumors as well as to produce cytokines. In exploring different iterations of CAR protein domain structure it was found that the antibody-derived ALK binding sequences were quite robust. The synthetic scFv domains created from the heavy and light variable domain sequences of
40 immunoglobulin could be interchanged with respect to their orientation in the context of CAR tertiary protein

structure. Moreover, ALK-specific scFv functioned whether expressed in a short format (as a single domain proximal to the T cell membrane) or in long format (extended away from the plasma membrane using an IgG1-derived spacer domain composed of CH2 and CH3). ALK-specific CARs may serve as a new means to treat pediatric solid tumors.

5

Introduction:

The developmentally-regulated cell surface receptor tyrosine kinase ALK (anaplastic lymphoma kinase) is expressed as a tumor-associated antigen in either a full-length form or as a fusion protein resulting from a chromosomal translocation. Full-length ALK plays a role in mesodermal differentiation in *drosophila*, eye development in *c. elegans*, neural-crest derived iridophore development in zebrafish, and in the developing nervous system in mammals (Palmer et al., (2009) *Biochem J*, 420, 345-361). Cancer-associated ALK was first described as a 2;5 chromosomal translocation associated with nucleophosmin (NPM) in anaplastic large cell leukemia (ALCL; Morris et al., (1994) *Science*, 263, 1281-1284). The fusion protein was composed of the intracellular domain of NPM and the intracellular kinase domain of ALK. ALK is expressed on neuroblastoma as an intact protein, an amplified protein, or as a mutated protein that continues to signal through its kinase domain. ALK can also be found as a fusion partner to EML4 in up 9% of non-small cell lung carcinomas (NSCLC; Sasaki et al., (2010) *Eur J Cancer*, 46, 1773-1780). The range of ALK-associated translocations has been recently reviewed (Chiarle et al., (2008) *Nat Rev Cancer*, 8, 11-23). Activating mutations of full-length ALK are found in 8-12% of primary neuroblastoma cases (Mosse et al., (2008) *Nature*, 455, 930-935; George et al., (2008) *Nature*, 455, 975-9785). Importantly, ALK has also been shown to account for the long sought after "second-hit" in familial neuroblastoma using whole genome scans of patient pedigrees (Mosse et al., (2008) *Nature*, 455, 930-935).

Chimeric antigen receptors are an example of synthetic biology, wherein a protein not encoded by the genome, is designed in the laboratory and is expressed in normal human tissues for a therapeutic effect by adoptive immunotherapy of cancer. When Eshchar et al., demonstrated that mature human T lymphocytes from the peripheral blood could be activated by synthetic chimeric antigen receptor molecules, the era of designer cytotoxic T lymphocytes began (Eshchar et al., (1990) *Br J Cancer Suppl*, 10, 27-29). There are a number of CAR constructs in clinical trials, but most of the activity has been in hematologic malignancies, most notably in B cell leukemias (Lee et al., (2012) *Clin Cancer Res*, 18, 2780-2790; Sadelain et al., (2013) *Cancer Discov*, 3, 388-398). This is due in part to the acceptable safety profile of B cell antigen-specific CAR-modified T cells. Elimination of B cell leukemia by infused CAR-modified T cells that target B cell antigens is also been accompanied by a subsequent B cell aplasia. More caution is warranted in targeting solid tumors, in that cross-reactive tissue antigen reactivity may have a much more severe outcome.

Chimeric antigen receptors are composed of an extracellular binding domain, spacer domain, transmembrane domains, and intracellular T cell signaling domains (Long et al., (2013) *Oncoimmunology*, 2, e23621). In addition to these possible variations in structural design elements, how these elements are linked to one another by joining domains introduced another level of variability. The impact of including or not including an IgG derived spacer domain, the constant heavy chain regions 2 and 3 (CH2HC3), was explored. Initial inclusion of this domain allows for rapid assessment of T cell transduction of CAR retroviral expression vectors, as not all CARs bind to protein L. CARs are classified according to the number of signaling domains

40

they encode. First-generation CARs include only the CD3 zeta chain-derived cytoplasmic signaling domain. Second generation CARs include CD28 or CD137-derived signaling domains. Third generation CARs encode three signaling domains and may also include sequences derived from CD137, OX40, or GITR. An overarching rule for the assemble of CAR domains into a functional chimeric receptor has yet to be developed, and thus, starting with hybridomas encoding ALK-specific antibodies, the variable regions were sequenced, synthetic scFv domains designed, and linked to CAR structural domains to create a series of CARs specific for ALK. It was found that these synthetic scFv domain were functional with or without CH2CH3 spacers, and that the order of the heavy and light chains were interchangeable in the construct tested. The cytolytic activity of anti-ALK CAR-modified T cells was encouraging and in vivo analysis of CAR activity is currently underway. ALK-specific CARs have the potential to enter the clinical arena as a new generation of adoptive immunotherapeutic approaches for pediatric solid tumors.

Materials and Methods

Cell Lines and Antibodies. Cell lines used in these studies were: Sy5Y, LAN5, K562, Rh18, IMR32, KCNR. Cells were cultured in RPMI-1640 supplemented with 2 mM lglu, 10 mM HEPES, Pen/Strep, (Gibco, Life Technologies, Grand Island, NY) and 10% FBS (Omega Scientific, Tarzana, CA). Anti-ALK antibodies were produced as described previously: ALK15A (IgG2b, weak agonist, membrane proximal binding), ALK48B (IgG2a, agonist, membrane proximal binding), ALK53A, ALK58A (binds to a membrane distal epitope) (Mazot et al., *Oncogene*, 30, 2017-2025, 2011; Moog-Lutz et al., *J Biol. Chem.*, 280, 26039-26048, 2005). Staining for ALK expression on tumor lines was performed as follows: 2.5µL primary antibody (1 mg/ml conc.) per 1 million cells was added to cells on ice in FACS buffer, FB, (PBS, 0.5%BSA, 0.02% NaN3) for 20 minutes, cells were washed twice, then stained with 5µL FITC-F(ab')₂ Fragment Goat Anti-Mouse IgG + IgM (H+L) secondary antibody (Jackson ImmunoResearch, 1 mg/ml) for 20 minutes, washed, then analyzed by flow cytometry.

CAR expression on transduced T cells was also measured by flow cytometry. CARs containing an IgG1 CH2CH3 domain were detected with PE-F(ab')₂ 2 goat antihuman Fc fragment-specific antibody (Jackson ImmunoResearch, Inc., West Grove, PA), 0.5 ug per million cells in FB, stained for 20 minutes on ice. All CAR structural formats could be stained with protein L, as previously described (Zheng et al., (2012) *J Transl Med*, 10, 29). 500 ng Biotin-Protein L (Thermo Fisher) per million cells in FACS buffer were incubated on ice for 20 min., washed twice, then stained with 250 ng SA-PE (BD Biosciences), 10 minutes in ice. All incubation were in a 0.1 ml volume.

CAR construct synthesis and vector production. To sequence the variable regions of the heavy and light chain, PCR primers were used to amplify hybridoma cell line derived cDNA. PCR primer sequences specific for murine IgG were from Kettleborough, et al. (Kettleborough et al., (1993) *Eur J Immunol*, 23, 206-211). Frozen hybridoma cell pellets were resuspended in RLY buffer, passed over QIAshredder columns, and total RNA isolated on RNeasy spin columns (Qiagen GmbH, Hilden, Germany) as per manufacturer's protocol. PCR products were coned into a TopoTA vector (Invitrogen), and 10 independent bacterial colonies DNA sequenced. Sequences were aligned and once consensus was reached, cognate chains were linked with a (GGGGS)x3 sequence. ALK scFv encoding plasmids were sequence optimized and synthesized by DNA2.0 (Menlo Park, CA). Engineered restriction sites were used to introduce scFv sequences into retroviral

expression vectors that included various structural elements as described previously (Haso et al., (2013) *Blood*, 121, 1165-1174, which is incorporated by reference in its entirety).

T Cell Activation and Transduction. De-identified PBMC were obtained from the NIH Clinical Center, Department of Transfusion Medicine, under approved protocol. PBMC were activated by culture for 3 days in the presence of 40 U/ml IL-2 and anti- CD3/CD28 beads (Dynabeads, Human T-Activator CD3/CD28, Life Technologies, Grand Island, NY) in AIM-V media (Life Technologies) supplemented with 2 mM lglu, 10 mM HEPES, Pen/Strep, (Gibco, Life Technologies, Grand Island, NY) and 5% FBS (Omega Scientific, Tarzana, CA). Cells exposed to RV containing supernatants on day 3 and 4 in media containing 300 U/ml rIL-2, beads magnetically removed on day 5, and cells were expanded for 5 more days in media containing 300 U/ml IL-2, then analyzed for transduction.

T Cell Functional Assays. T cell cytolytic function was assayed in standard ⁵¹Cr release assays as described elsewhere (Haso et al., (2013) *Blood*, 121, 1165-1174). Cytokine release was assayed by co-incubating 25,000 T cells with 25,000 tumor cell targets in cRPMI. At 24 hours culture media was collected and cytokines measured (Human TH1/TH2 multiplex, Meso Scale Discovery, Rockville, MD).

Xenograft Assays. NSG mice were injected s.c. on day 0 with 5x10⁶ human neuroblastoma cells (SY5Y *in vivo* passaged and re-cultured as a new line, SY5Y.P1). On day 6, 5.1 x10⁶ ALK48-28z CAR(+)ve T cells were injected r.o. (34% positive/15 million total). CAR-treated mice were given a cocktail of (0.9 µg IL-7 + 6.6 µg M25 anti-IL7 mAb) i.p. every 2-3 days, for the time period indicated above. Survival statistics were calculated using Log-rank (Mantel-Cox) analysis (Prism Software, Inc.). On day 13, 3.2 x10⁶ ALK48SH-transduced T cells were given. The experiment was ended on day 64 due to graft vs host disease-like symptoms, standardly encountered in the NSG model system.

Results

Expression of ALK on Tumor Cell Lines. As reported previously, ALK MAbs 7, 48, 53, and 58 were able to detected ALK expression on tumor cell lines by flow cytometry. The ALK-specific MAb was used to define the expression of ALK on a series of neuroblastoma cell lines, and a rhabdomyosarcoma cell line. FIG. 1 illustrates that the neuroblastoma cell lines SY5Y, LAN5, KCNR, IMR32, all express cell surface ALK, as does the rhabdomyosarcoma cell line Rh18. The differences in expression were quite varied with the SY5Y staining the brightest for ALK expression. This was true when the other MAbs specific for ALK were tested as well.

Creation of ALK CAR. CAR expression on activated primary human T lymphocytes can be efficiently induced by transduction with retroviral gene vectors. A series of CAR expression vectors was constructed in order to test if the heavy and light immunoglobulin chains of anti-ALK monoclonal antibodies could be used to construct synthetic scFv, and then to link these synthetic scFV to CAR structural and T cell signaling domains, FIG. 2. In some constructs a CH2CH3 structural domain was included. This domain extends the scFV away from the plasma membrane extracellular surface, and also allows for the efficient detection of transduced T cells with anti-IgG Fc-specific antibody. "SH" (or "short") indicates that the extracellular domain of the CAR does not include a CH2CH3 spacer domain.

Expression of ALK CAR on transduced T cells. Transduced PBMC were analyzed for the CAR expression by flow cytometry. PBMC were activated with OKT3/CD28 beads in the presence of IL-2 low

lever IL-2, exposed to retroviral vector (RV)-containing supernatant for two consecutive days and then expand for 5-6 more days in high level (300 u/ml) IL-2. Cells were then assessed for CAR expression by direct staining with anti-Fc antibody if the construct contained a CH2CH3 domain, or by indirect staining with protein L, FIG. 3. All ALK-specific CARs generated were able to bind protein L.

5 The ALK48 and ALK58-derived CARs showed the best overall cell surface expression levels, either in the long or short formats. The order of the heavy and light chains was switched in a short CAR format for ALK58. No difference in expression level at the surface was seen when the order of the heavy and light variable domains were switched in the synthetic CAR construct, FIG. 3F. Also, no significant differences were seen in expression level of the short versus the long CAR for either clone 58 or 48, indicating that the scFv
10 domain itself may drive overall expression levels. This was certainly true for the ALK53 CAR, which showed only 9% transduction, although it was generated under identical conditions, and included the exact same structural domains as ALK48 and ALK58.

Lytic Activity of ALK CARs. The in vitro biological activity of anti-ALK CARs was assessed using 51Cr-release assays for cytolysis. Tumor cell targets were radiolabeled and then incubated with CAR-
15 transduced T cells. Both the neuroblastoma cell line LAN5 and the rhabdomyosarcoma cell line Rh18 were lysed by all three CAR constructs tested in the CH2CH3-containing format, FIG. 4. The control cell leukemia cell line K562, which is not ALK positive and is included as a control for NK cell activity, was not lysed. The effector to target ratio was normalized such that the E:T target ratio reflected the number of CAR positive T cells in the assay. The strong lytic activity of ALK53 at lower E:T ratios is intriguing, but higher E:T ratios
20 could not be tested due to very low levels of transduction. As with analysis of expression level, the shorter format ALK CAR constructs were equally able to lyse tumor lines, FIG. 5. Even when the heavy and light chains were reversed in order, ALK59LH, efficient lysis was seen, FIG. 5B.

Cytokine Data. T cells transduced with ALK48, ALK53, and ALK58 were also tested for the production of cytokine upon 24-hour co-culture with tumor cell lines, FIG. 6. Strong IFN-gamma production
25 was noted with lower levels of IL-2 and IFNalpha. The ability to produce interferon as well as the ability to lyse tumor cell lines is a strong indicator that the synthetically constructed scFv are active as CARs upon interaction with tumor cell lines expressing cell-surface ALK.

Xenograft Data. NOD scid gamma (NSG) mice were inoculated with ALK+ human neuroblastoma cells (SY5Y cells). On day 6, the mice were treated with human T cells transduced to express the murine ALK
30 specific ALK48-28z CAR (SEQ ID NO: 49, long form CAR with CH2CH3 spacer domain) or mock treated T cells. The ALK48-28z vector-transduced T cells showed significant disease control (p<0.005) when compared to the control. In a separate set of mice, on day 13 post inoculation (and without any treatment on day 6), the mice were treated with ALK48SH-28z (SEQ ID NO: 50, short form CAR without spacer domain) transduced T cells. In these mice, there was no effect on survival, likely due to the delayed time of infusion and low
35 numbers.

The mice also received biweekly injections of IL-7 complexed to anti-IL7 antibody to promote T cell persistence. The experiment was terminated at day 66 due to graft-versus host disease arising in the ALK48L-28z treated mice, none of which were sacrificed due to tumor growth. The other two groups all died of tumor. The onset on graft-versus host disease is commonly seen in xenograft models with human T cells, and may be
40 exacerbated by the addition of IL-7 to the system.

Discussion

The extracellular aspect of the ALK tyrosine kinase provides a ready target for the adoptive immunotherapy of cancer with chimeric antigen receptor (CAR)-modified T cells. In glioblastoma stem cell lines, ALK was shown to be a critical factor for both self-renewal and tumorigenic capacity (Koyama-Nasu et al., (2013) *Oncogene*, May 20). ALK expression induced gene expression signatures similar to those seen in both ESC and myc-driven signaling pathways. Moreover overexpression of pleiotrophin (an ALK ligand) was shown to be driven by SOX2 expression in cancer stem cells, and thus may serve as an autocrine stimulatory factor. In rhabdomyosarcoma, frequent ALK expression as well as ALK amplification has been detected at the genomic level (Nishimura et al., (2013) *Cancer Sci*, 104, 856-864). Interestingly, that ability of patients to produce antibodies to ALK may correlate with better outcomes in ALCL (Ait-Tahar et al., (2010) *Blood*, 115, 3314-3319). If this is a measure of a patient's general immune capacity, or a specific ALK-targeted effect is not clear, but it may indicate that in postnatal individuals ALK can be safely targeted by antibody. In rat studies, ALK expression appears to peak just at birth in the dorsal root ganglia (DRG), and to recede thereafter (Chiarle et al., (2008) *Nat Rev Cancer*, 8, 11-23, Degoutin et al., (2009) *Eur J Neurosci*, 29, 275-286). ALK is expressed on a subset of neurons in the DRG that co-express TrkA and ret. In a detailed analysis of embryonic development in mice, ALK expression is seen in numerous tissues, but near term becomes progressively restricted to the CNS (Vernersson et al., (2006) *Gene Expr Patterns*, 6, 448-461).

In 2005, Moog-Lutz, et al., described the production of monoclonal antibodies (mAb) against the extracellular domain of ALK (Moog-Lutz et al., *J Biol. Chem.*, 280, 26039-26048, 2005). These studies led to the description of ALK as both a 220 kD and 140 kDa cleaved form at the cell surface, and two of the antibodies created bound with nM affinity and were able to induce differentiation and activation of both PC12 and HEK293 cells transfected with ALK. These mAbs are the basis of the CARs reported here. The two clones worked with most were derived from antibody 58 which binds to only the 220 KDa form of ALK, and antibody 48, which binds to both. From this data it can be inferred clone 48 binds closer to the cell surface membrane, while clone 58 binds to the more distal cleaved region (Moog-Lutz et al., (2012) *PLoS One*, 7, e33581). Both mAb 48 and 58 induce phosphorylation of the receptor upon binding, and are thus considered to be activating (Mazot et al., (2011) *Oncogene*, 30, 2017-2025).

The description of point mutations in ALK has led to detailed analysis of the intracellular versus intracellular residence of the protein. In general, mutations in ALK appear to increase the number of intracellular ALK molecules. However, even wild-type ALK can be detected both within and on the surface of neuroblastoma cell lines (Mazot, et al., (2012) *PLoS One*, 7, e3358121).

The binding moiety of CARs can be derived from a B-cell derived scFv expression libraries, or can be assembled synthetically from monoclonal antibody producing hybridoma cDNA. A number of CARs specific for ALK were generated using hybridoma derived cDNA as the starting material, FIG. 2. The expression levels of these CARs on transduced T cells varied widely. In some constructs a distinct and bright population was seen, as in ALK58, in others a weaker and less distinct expression was seen, as in ALK48, while in others, T cells with high levels of transduction were not generated at all, as in ALK53 (FIG. 2). This indicates that the synthetic scFv sequences themselves have a bearing on the expression level of the CAR. In the case of the hybridoma-derived CARs, it was demonstrated that the addition of the CH2CH3 domain did not affect lytic

activity, FIG. 5. In fact all the CARs tested had strong levels of cytolysis against tumor cell lines, as well as cytokine production once data was normalized for transduction efficiency, FIGs. 4 and 6. In conclusion, a series of highly active CARs was generated that may serve as a platform for the pre-clinical testing of ALK specific adoptive immunotherapy of malignancies expressing cell-surface ALK.

5

Example 2

Exemplary CAR Sequences

This example illustrated exemplary amino acid and nucleic acid sequences of ALK-specific CARs.

All the sequences have an N-terminal signal peptide (SP; SEQ ID NO: 26). All the sequences have a scFv sequence, a transmembrane (TM) sequence and a CD3 zeta signaling sequence. Sequences that refer to “SH” *do not* have a CH2CH3 spacer domain.

For the CAR extracellular domain, the following nomenclature is used:

“short” CAR extracellular domain, without a CH2CH3 spacer

15	ALK15SH	SP - murine ALK15 scFv
	ALK48SH	SP - murine ALK48 scFv
	ALK53SH	SP - murine ALK53 scFv
	ALK58SH	SP - murine ALK58 scFv
	hALK15SH	SP - humanized ALK15 scFv
	hALK48SH	SP - humanized ALK48 scFv
20	hALK53SH	SP - humanized ALK53 scFv
	hALK58SH	SP - humanized ALK58 scFv

“long” CAR extracellular domain, with a CH2CH3 spacer

25	ALK15	SP - murine ALK15 scFv – CH2CH3 spacer
	ALK48	SP - murine ALK48 scFv – CH2CH3 spacer
	ALK53	SP - murine ALK53 scFv – CH2CH3 spacer
	ALK58	SP - murine ALK58 scFv – CH2CH3 spacer
	hALK15	SP - humanized ALK15 scFv – CH2CH3 spacer
	hALK48	SP - humanized ALK48 scFv – CH2CH3 spacer
30	hALK53	SP - humanized ALK53 scFv – CH2CH3 spacer
	hALK58	SP - humanized ALK58 scFv – CH2CH3 spacer

For the CAR transmembrane domain and intracellular domains, the following nomenclature is used:

35	28z	CD28 transmembrane - CD28 signaling - CD3 zeta signaling
	BBz	CD8 transmembrane - 4-1BB/CD137 signaling - CD3 zeta signaling
	28BBz	CD8 transmembrane - CD28 signaling – 4-1BB/CD137 signaling - CD3 zeta signaling

ALK15-28z (SEQ ID NO: 43):

LLVTSLLLCELPHPAFLIPD TDV KLQESGPGLVAPSQSL SITCTVSGFSLTSYAVSWVRQPPGKGLEWL
 40 GIWSSGGATNYNSALKSRLSISKDNSKSQVFLKMNGLQTDDTARYYCAREHYYGSSAMDYWGQGASI
 TVSSGGGGSGGGSGGGSGIVMTQSPLSLPVSLGDQASISCRSSQSIVHSYGNTYLFWYLQKPGQSP
 KLLIYRVSNRFGVDPDRFSGSGSGTNTFLKISRVEAEDMGVYYCFQGTHVPYTFGGGTGLEIKEPKSCD
 KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR
 45 EEQYNSTYRVVSVLT V L H Q D W L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R D E L T K N Q V S L
 T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y
 T Q K S L S L S P G K K D P K A A A I E V M Y P P P Y L D N E K S N G T I I H V K G K H L C P S P L F P G P S K P F W V L V V V G G V L A
 C Y S L L V T V A F I I F W V R S K R S R L L H S D Y M N M T P R R P G P T R K H Y Q P Y A P P R D F A A Y R S R V K F S R S A D A P A

YQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK
GERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR

ALK15SH-28z (SEQ ID NO: 44):

5 LLVTSLLLCELPHPAFLIPD TDV KLQESGPGLVAPSQSL SITCTVSGFSLTSYAVSWVRQPPGKGLEWL
GIIWSSGATNYNSALKSRLSISKD NSKSQVFLKMNGLQTDDTARYYCAREHYYGSSAMDYWGQGASI
TVSSGGGGSGGGSGGGSGGIVMTQSPLSLPVSLGDQASISCRSSQSIVHSYGNTYLFWYLQKPGQSP
KLLIYRVSNRFSGV PDRFSGSGSGTNFTLKISRVEAEDMGVYYCFQGTHVPYTFGGGT KLEIKAAAEV
MYPPPYLDNEKSNGTIIHVKGKHLCPSP LFPGPSKPFVWL VVVGGVLACYSLLVTVAFIIFWVRSKRSR
10 LLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREE
YDVL DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTA
TKDTYDALHMQALPPR

ALK15-BBz (SEQ ID NO: 45):

15 LLVTSLLLCELPHPAFLIPD TDV KLQESGPGLVAPSQSL SITCTVSGFSLTSYAVSWVRQPPGKGLEWL
GIIWSSGATNYNSALKSRLSISKD NSKSQVFLKMNGLQTDDTARYYCAREHYYGSSAMDYWGQGASI
TVSSGGGGSGGGSGGGSGGIVMTQSPLSLPVSLGDQASISCRSSQSIVHSYGNTYLFWYLQKPGQSP
KLLIYRVSNRFSGV PDRFSGSGSGTNFTLKISRVEAEDMGVYYCFQGTHVPYTFGGGT KLEIKEPKSCD
KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR
20 *EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL*
TCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGSEFLYSLKLTVDKSRWQQGNVFSCSVMEALHNHY
TQKSLSLSPGKKDPKAAATTPAPRPPTAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPL
AGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSADA
PAYKQQQNQLYNELNLGRREEYDVL DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG
25 MKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR

ALK15SH-BBz (SEQ ID NO: 46):

LLVTSLLLCELPHPAFLIPD TDV KLQESGPGLVAPSQSL SITCTVSGFSLTSYAVSWVRQPPGKGLEWL
GIIWSSGATNYNSALKSRLSISKD NSKSQVFLKMNGLQTDDTARYYCAREHYYGSSAMDYWGQGASI
30 TVSSGGGGSGGGSGGGSGGIVMTQSPLSLPVSLGDQASISCRSSQSIVHSYGNTYLFWYLQKPGQSP
KLLIYRVSNRFSGV PDRFSGSGSGTNFTLKISRVEAEDMGVYYCFQGTHVPYTFGGGT KLEIKAAATT
TPAPRPPTAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRG
RKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSADAPAYKQQQNQLYNELNLGR
REEYDVL DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGL
35 STATKDTYDALHMQALPPR

ALK15-28BBz (SEQ ID NO: 47):

LLVTSLLLCELPHPAFLIPD TDV KLQESGPGLVAPSQSL SITCTVSGFSLTSYAVSWVRQPPGKGLEWL
GIIWSSGATNYNSALKSRLSISKD NSKSQVFLKMNGLQTDDTARYYCAREHYYGSSAMDYWGQGASI
40 TVSSGGGGSGGGSGGGSGGIVMTQSPLSLPVSLGDQASISCRSSQSIVHSYGNTYLFWYLQKPGQSP
KLLIYRVSNRFSGV PDRFSGSGSGTNFTLKISRVEAEDMGVYYCFQGTHVPYTFGGGT KLEIKEPKSCD
KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR
EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGSEFLYSLKLTVDKSRWQQGNVFSCSVMEALHNHY
45 *TQKSLSLSPGKKDPKAAAFVPVFLPAKPTTTPAPRPPTAPTIASQPLSLRPEACRPAAGGAVHTRGLDF*
ACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFA
AYRSRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSADAPAYQQGQ
NQLYNELNLGRREEYDVL DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR
GKGGHDGLYQGLSTATKDTYDALHMQALPPR
50

ALK15SH-28BBz (SEQ ID NO: 48):

LLVTSLLLCELPHPAFLIPD TDV KLQESGPGLVAPSQSL SITCTVSGFSLTSYAVSWVRQPPGKGLEWL
GIIWSSGATNYNSALKSRLSISKD NSKSQVFLKMNGLQTDDTARYYCAREHYYGSSAMDYWGQGASI
TVSSGGGGSGGGSGGGSGGIVMTQSPLSLPVSLGDQASISCRSSQSIVHSYGNTYLFWYLQKPGQSP
55 KLLIYRVSNRFSGV PDRFSGSGSGTNFTLKISRVEAEDMGVYYCFQGTHVPYTFGGGT KLEIKAAAFV

PVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLS
 LVITLYCNHRNRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRFSVVKRGRKKLLYIF
 KQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL
 KRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTY
 5 DALHMQUALPPR

ALK48-28z (SEQ ID NO: 49):

LLVTSLLLCELPHPAFLIPDTQVQLQQSGAELVKPGASVKISCKASGYAFSSYWMNWVKQRPKGKLE
 WIGQIYPGDGDTTYNGKFKGKATLTADKSSSTVYMQNLSTSEDSAVYFCVRYYYGSSGYFDYWGQ
 10 GTTLTVSSGGGGSGGGGSGGGGSDVQMIQTPDSLAVSLGQRATISCRASESDNYGISFMHWYQQKP
 GQSPKLLIYRASNLESGIPARFSGSGSRTDFLTINPVETDDVATYYCQQNNKDPPTFGGGTKLEIKRAE
 PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
 KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTK
 NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEA
 15 LHNHYTQKSLSLSPGKKDPKAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFLPGPSKPFVWL VVV
 GGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRS
 ADAPAYQQGQNQLYNELNLGRREEYDVLKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYS
 EIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQUALPPR

20 ALK48SH-28z (SEQ ID NO: 50):

LLVTSLLLCELPHPAFLIPDTQVQLQQSGAELVKPGASVKISCKASGYAFSSYWMNWVKQRPKGKLE
 WIGQIYPGDGDTTYNGKFKGKATLTADKSSSTVYMQNLSTSEDSAVYFCVRYYYGSSGYFDYWGQ
 GTTLTVSSGGGGSGGGGSGGGGSDVQMIQTPDSLAVSLGQRATISCRASESDNYGISFMHWYQQKP
 GQSPKLLIYRASNLESGIPARFSGSGSRTDFLTINPVETDDVATYYCQQNNKDPPTFGGGTKLEIKRA
 25 AAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFLPGPSKPFVWL VVVGGVLACYSLLVTVAFIIFWVR
 SKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNL
 GRREEYDVLKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQ
 GLSTATKDTYDALHMQUALPPR

30 ALK48-BBz (SEQ ID NO: 51):

LLVTSLLLCELPHPAFLIPDTQVQLQQSGAELVKPGASVKISCKASGYAFSSYWMNWVKQRPKGKLE
 WIGQIYPGDGDTTYNGKFKGKATLTADKSSSTVYMQNLSTSEDSAVYFCVRYYYGSSGYFDYWGQ
 GTTLTVSSGGGGSGGGGSGGGGSDVQMIQTPDSLAVSLGQRATISCRASESDNYGISFMHWYQQKP
 GQSPKLLIYRASNLESGIPARFSGSGSRTDFLTINPVETDDVATYYCQQNNKDPPTFGGGTKLEIKRAE
 35 PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
 KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTK
 NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEA
 LHNHYTQKSLSLSPGKKDPKAAATTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI
 WAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFS
 40 RSADAPAYKQGQNQLYNELNLGRREEYDVLKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEA
 YSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQUALPPR

ALK48SH-BBz (SEQ ID NO: 52):

LLVTSLLLCELPHPAFLIPDTQVQLQQSGAELVKPGASVKISCKASGYAFSSYWMNWVKQRPKGKLE
 45 WIGQIYPGDGDTTYNGKFKGKATLTADKSSSTVYMQNLSTSEDSAVYFCVRYYYGSSGYFDYWGQ
 GTTLTVSSGGGGSGGGGSGGGGSDVQMIQTPDSLAVSLGQRATISCRASESDNYGISFMHWYQQKP
 GQSPKLLIYRASNLESGIPARFSGSGSRTDFLTINPVETDDVATYYCQQNNKDPPTFGGGTKLEIKRA
 AATTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLY
 CKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYKQGQNQLYNEL
 50 NLGRREEYDVLKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGL
 YQGLSTATKDTYDALHMQUALPPR

ALK48-28BBz (SEQ ID NO: 53):

LLVTSLLLCELPHPAFLIPDTQVQLQQSGAELVKPGASVKISCKASGYAFSSYWMNWVKQRPKGKLE
 55 WIGQIYPGDGDTTYNGKFKGKATLTADKSSSTVYMQNLSTSEDSAVYFCVRYYYGSSGYFDYWGQ

GTTLTVSSGGGSGGGGSGGGGSDVQMIQTPDSLAVSLGQRATISCRASESDNYGISFMHWYQQKP
 GQSPKLLIYRASNLSEGI PARFSGSGSRTDFLTINPVETDDVATYYCQQNNKDPPTFGGGTKLEIKRAE
 PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNA
 5 KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTK
 NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVSMHEA
 LHNHYTQKSLSLSPGKKDPKAAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT
 RGLDFACDIYWAPLAGTCGVLLLSLVITLYCNHRNRSKRSRLHSDYMNMTPRRPGPTRKHYPYAP
 PRDFAAYRSRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPA
 10 YQQGQNQLYNELNLGRREEYDVLDRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK
 GERRRGKGGHDGLYQGLSTATKDTYDALHMQUALPPR

ALK48SH-28BBz (SEQ ID NO: 54):

LLVTSLLLCELPHPAFLIPDTPVQLQESGAELVKPGASVKISCKASGYAFSSYWMNWVKQRPKGKLE
 WIGQIYPGDGDTTYNGKFKGKATLTADKSSSTVYMQLNSLTSEDSAVYFCVRYYYGSSGYFDYWGG
 15 GTTLTVSSGGGSGGGGSGGGGSDVQMIQTPDSLAVSLGQRATISCRASESDNYGISFMHWYQQKP
 GQSPKLLIYRASNLSEGI PARFSGSGSRTDFLTINPVETDDVATYYCQQNNKDPPTFGGGTKLEIKRA
 AAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCG
 VLLLSLVITLYCNHRNRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRFSVVKRGRK
 KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREE
 20 YDVLDRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTA
 TKDTYDALHMQUALPPR

ALK53-28z (SEQ ID NO: 55):

LLVTSLLLCELPHPAFLIPDTPVQLQESGPVLVKTGASVKMSCTASGYTFTDHFMDWVKQSHGKSLE
 25 WIGSLNPYSGGTSYNQKFKGKATLTVDKSSSTAYMELNSLTSDSAVYYCARHNWGAYFDYWGGQ
 TTTLTVSSGGGSGGGGSGGGGSDIVMTQAAPSPVPTPGESVSISCRSSKSLHNSNGNTYLWFLQRP
 QSPQRLIYMSNLASGVDPDRFSGRSGTDFTLRISRVEAEDVGYYCMQGLEDPYTFGGGTKEIKEP
 KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
 30 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKN
 QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVSMHEAL
 HNHYTQKSLSLSPGKKDPKAAAEVMPYPYLDNEKSNGTIIHVKGKHLCPSPFLPGPSKPFVWL VVVG
 GVLACYSLLVTVAFIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSA
 DAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE
 35 IGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQUALPPR

ALK53SH-28z (SEQ ID NO: 56):

LLVTSLLLCELPHPAFLIPDTPVQLQESGPVLVKTGASVKMSCTASGYTFTDHFMDWVKQSHGKSLE
 WIGSLNPYSGGTSYNQKFKGKATLTVDKSSSTAYMELNSLTSDSAVYYCARHNWGAYFDYWGGQ
 40 TTTLTVSSGGGSGGGGSGGGGSDIVMTQAAPSPVPTPGESVSISCRSSKSLHNSNGNTYLWFLQRP
 QSPQRLIYMSNLASGVDPDRFSGRSGTDFTLRISRVEAEDVGYYCMQGLEDPYTFGGGTKEIKAA
 AIEVMPYPYLDNEKSNGTIIHVKGKHLCPSPFLPGPSKPFVWL VVVGGVLACYSLLVTVAFIIFWVRS
 KRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLG
 RREEYDVLDRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQG
 45 LSTATKDTYDALHMQUALPPR

ALK53-BBz (SEQ ID NO: 57):

LLVTSLLLCELPHPAFLIPDTPVQLQESGPVLVKTGASVKMSCTASGYTFTDHFMDWVKQSHGKSLE
 WIGSLNPYSGGTSYNQKFKGKATLTVDKSSSTAYMELNSLTSDSAVYYCARHNWGAYFDYWGGQ
 50 TTTLTVSSGGGSGGGGSGGGGSDIVMTQAAPSPVPTPGESVSISCRSSKSLHNSNGNTYLWFLQRP
 QSPQRLIYMSNLASGVDPDRFSGRSGTDFTLRISRVEAEDVGYYCMQGLEDPYTFGGGTKEIKEP
 KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKN
 QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVSMHEAL
 55 HNHYTQKSLSLSPGKKDPKAAATTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI
 WAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFS

RSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDLGYQGLSTATKDTYDALHMQALPPR

ALK53SH-BBz (SEQ ID NO: 58):

5 LLVTSLLLCELPHPAFLIPDITDVQLQESGPVLVKTGASVKMSCTASGYTFTDHFMDWVKQSHGKSLE
WIGSLNPYSGGTSYNQKFKGKATLTVDKSSSTAYMELNSLTSVDSAVYYCARHNWGAYFDYWGQG
TTLTVSSGGGGSGGGSGGGGSDIVMTQAAPSPVPTPGESVSISCRSSKSLHNSGNTYLYWFLQRP
QSPQRLLIYMSNLASGVDPDRFSGRSGTDFTLRISRVEAEDVGYYCMQGLEDPYTFGGGTKEIKAA
ATTPAPRPPTPAPTASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITLYC
10 KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSADAPAYKQGQNQLYNELN
LGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDLGY
QGLSTATKDTYDALHMQALPPR

ALK53-28BBz (SEQ ID NO: 59):

15 LLVTSLLLCELPHPAFLIPDITDVQLQESGPVLVKTGASVKMSCTASGYTFTDHFMDWVKQSHGKSLE
WIGSLNPYSGGTSYNQKFKGKATLTVDKSSSTAYMELNSLTSVDSAVYYCARHNWGAYFDYWGQG
TTLTVSSGGGGSGGGSGGGGSDIVMTQAAPSPVPTPGESVSISCRSSKSLHNSGNTYLYWFLQRP
QSPQRLLIYMSNLASGVDPDRFSGRSGTDFTLRISRVEAEDVGYYCMQGLEDPYTFGGGTKEIKEP
KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
20 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSQSVMEAL
HNHYTQKSLSPGKKDPKAAAFVFPVFLPAKPTTTPAPRPPTPAPTASQPLSLRPEACRPAAGGAVHTR
GLDFACDIYWAPLAGTCGVLLLSLVITLYCNHRNRSKRSLHSDYMNMTPRRPGPTRKHYQPYAPP
RDFAAAYRSRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSADAPAY
25 QQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKG
ERRRGKGHDLGYQGLSTATKDTYDALHMQALPPR

ALK53SH-28BBz (SEQ ID NO: 60):

LLVTSLLLCELPHPAFLIPDITDVQLQESGPVLVKTGASVKMSCTASGYTFTDHFMDWVKQSHGKSLE
30 WIGSLNPYSGGTSYNQKFKGKATLTVDKSSSTAYMELNSLTSVDSAVYYCARHNWGAYFDYWGQG
TTLTVSSGGGGSGGGSGGGGSDIVMTQAAPSPVPTPGESVSISCRSSKSLHNSGNTYLYWFLQRP
QSPQRLLIYMSNLASGVDPDRFSGRSGTDFTLRISRVEAEDVGYYCMQGLEDPYTFGGGTKEIKAA
AFVFPVFLPAKPTTTPAPRPPTPAPTASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGV
LLSLVITLYCNHRNRSKRSLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAAYRSRFSVVKRGRKKL
35 LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSADAPAYQGGQNQLYNELNLGRREEYD
VLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDLGYQGLSTATK
DTYDALHMQALPPR

ALK58-28z (SEQ ID NO: 61):

40 LLVTSLLLCELPHPAFLIPDTALQLQSGAELVRPGASVTLSCKASGYTFTDYEMHWVKQTPVHGLE
WIGAIIDPETGGTAYNQKFEGKAILTADKSSSTAYMELRSLTSEDSPVYYCARRRYGSSSFYWGQGT
TLTVSSGGGGSGGGSGGGGSDVQMIQTPSSLSASLGDRVTISCRASQDIGNYLNWYQQKPDGTVKL
LIYYTSRLHSGVPSRFSGSGSGTEYSLTISNLEQEDIATYFCQQGSALPPTFGGGTKLEINRAEPKSCDKT
HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
45 QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQ
KSLSPGKKDPKAAAEVMPYPYLDNEKSNGTIIHVKGKHLCPSPFLPGPSKPFVVLVVVGGVLACY
SLLVTVAFIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAAYRSRVKFSRSADAPAYQ
QGGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGE
50 RRRRGKGHDLGYQGLSTATKDTYDALHMQALPPR

ALK58SH-28z (SEQ ID NO: 62):

LLVTSLLLCELPHPAFLIPDTALQLQSGAELVRPGASVTLSCKASGYTFTDYEMHWVKQTPVHGLE
WIGAIIDPETGGTAYNQKFEGKAILTADKSSSTAYMELRSLTSEDSPVYYCARRRYGSSSFYWGQGT
55 TLTVSSGGGGSGGGSGGGGSDVQMIQTPSSLSASLGDRVTISCRASQDIGNYLNWYQQKPDGTVKL

LIYYTSRLHSGVPSRFSGSGSGTEYSLTISNLEQEDIATYFCQQGSALPPTFGGGTKLEINRAAAIEVMY
 PPPYLDNEKSNGTIIHVKGKHLCPSPFLPGSPKPFVVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRL
 HSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYD
 5 VLDKRRGRDPEMGGKPRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRRGKGGHDGLYQGLSTATK
 DTYDALHMQALPPR

ALK58-BBz (SEQ ID NO: 63):

LLVTSLLLCELPHPAFLIPDTALQLQQSGAELVRPGASVTLSCKASGYTFTDYEMHWVKQTPVHGLE
 WIGAIIDPETGGTAYNQKFEGKAILTADKSSSTAYMELRSLTSEDSPVYYCARRRYYGSSSFYWGQGT
 10 TLTVSSGGGGSGGGSGGGSDVQMIQTPSSLSASLGDRVTISCRASQDIGNYLNWYQQKPDGTVKL
 LIYYTSRLHSGVPSRFSGSGSGTEYSLTISNLEQEDIATYFCQQGSALPPTFGGGTKLEINRAEPKSCDKT
 HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
 QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
 LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCVMHEALHNHYTQ
 15 KSLSLSPGKKDPKAAATTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAG
 TCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPA
 YKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQDKMAEAYSEIGMK
 GERRRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR

ALK58SH-BBz (SEQ ID NO: 64):

LLVTSLLLCELPHPAFLIPDTALQLQQSGAELVRPGASVTLSCKASGYTFTDYEMHWVKQTPVHGLE
 WIGAIIDPETGGTAYNQKFEGKAILTADKSSSTAYMELRSLTSEDSPVYYCARRRYYGSSSFYWGQGT
 TLTVSSGGGGSGGGSGGGSDVQMIQTPSSLSASLGDRVTISCRASQDIGNYLNWYQQKPDGTVKL
 LIYYTSRLHSGVPSRFSGSGSGTEYSLTISNLEQEDIATYFCQQGSALPPTFGGGTKLEINRAAATTPAP
 25 RPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKK
 LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEY
 DVLDKRRGRDPEMGGKPRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRRGKGGHDGLYQGLSTAT
 KDTYDALHMQALPPR

ALK58-28BBz (SEQ ID NO: 65):

LLVTSLLLCELPHPAFLIPDTALQLQQSGAELVRPGASVTLSCKASGYTFTDYEMHWVKQTPVHGLE
 WIGAIIDPETGGTAYNQKFEGKAILTADKSSSTAYMELRSLTSEDSPVYYCARRRYYGSSSFYWGQGT
 TLTVSSGGGGSGGGSGGGSDVQMIQTPSSLSASLGDRVTISCRASQDIGNYLNWYQQKPDGTVKL
 LIYYTSRLHSGVPSRFSGSGSGTEYSLTISNLEQEDIATYFCQQGSALPPTFGGGTKLEINRAEPKSCDKT
 35 HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
 QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
 LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCVMHEALHNHYTQ
 KSLSLSPGKKDPKAAAFVPVFLPAKPTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC
 DIYIWAPLAGTCGVLLLSLVITLYCNHRNRSKRSLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAY
 40 RSRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQ
 LYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRRGK
 GHDGLYQGLSTATKDTYDALHMQALPPR

ALK58SH-28BBz (SEQ ID NO: 66):

LLVTSLLLCELPHPAFLIPDTALQLQQSGAELVRPGASVTLSCKASGYTFTDYEMHWVKQTPVHGLE
 WIGAIIDPETGGTAYNQKFEGKAILTADKSSSTAYMELRSLTSEDSPVYYCARRRYYGSSSFYWGQGT
 TLTVSSGGGGSGGGSGGGSDVQMIQTPSSLSASLGDRVTISCRASQDIGNYLNWYQQKPDGTVKL
 LIYYTSRLHSGVPSRFSGSGSGTEYSLTISNLEQEDIATYFCQQGSALPPTFGGGTKLEINRAAFAVPVFL
 PAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVIT
 50 LYCNHRNRSKRSLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRFSVVKRGRKKLLYIFKQPF
 FMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRR
 GRDPEMGGKPRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRRGKGGHDGLYQGLSTATKDTYDAL
 HMQALPPR

hALK15-28z (SEQ ID NO: 67):

LLVTSLLLCELPHPAFLIPDTQVQLQQSGAEVKKPGSSVKVSCKASGFSLTSYAISWVRQAPGQGLE
 WMGGIIWSGGATNYAQKFQGRVTITADESTSTAYMELSSLRSEDNAVYYCAREHYYGSSAMDYWW
 GQGTMTVTVSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASQSIVHSYGNTRYAWYQQ
 5 KPGQAPRLLIYRVSRATGIPARFSGSGSGTDFTLTISSELPEDFAVYYCFQGTHTVPTTFGQGTKLEIKR
 EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
 AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
 KNQVSLTCLVKGFPYSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
 10 GGVLACYSLLTVAFIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRS
 ADAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYS
 EIGMKGERRRRGKGDGLYQGLSTATKDTYDALHMQALPPR

hALK15SH-28z (SEQ ID NO: 68):

LLVTSLLLCELPHPAFLIPDTQVQLQQSGAEVKKPGSSVKVSCKASGFSLTSYAISWVRQAPGQGLE
 WMGGIIWSGGATNYAQKFQGRVTITADESTSTAYMELSSLRSEDNAVYYCAREHYYGSSAMDYWW
 GQGTMTVTVSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASQSIVHSYGNTRYAWYQQ
 KPGQAPRLLIYRVSRATGIPARFSGSGSGTDFTLTISSELPEDFAVYYCFQGTHTVPTTFGQGTKLEIKR
 AAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKPFVVLVVGGVLACYSLLTVAFIIFWV
 20 RSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELN
 LGRREEYDVLDRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGDGLY
 QGLSTATKDTYDALHMQALPPR

hALK15-BBz (SEQ ID NO: 69):

LLVTSLLLCELPHPAFLIPDTQVQLQQSGAEVKKPGSSVKVSCKASGFSLTSYAISWVRQAPGQGLE
 WMGGIIWSGGATNYAQKFQGRVTITADESTSTAYMELSSLRSEDNAVYYCAREHYYGSSAMDYWW
 GQGTMTVTVSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASQSIVHSYGNTRYAWYQQ
 KPGQAPRLLIYRVSRATGIPARFSGSGSGTDFTLTISSELPEDFAVYYCFQGTHTVPTTFGQGTKLEIKR
 EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
 30 AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
 KNQVSLTCLVKGFPYSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
 ALHNHYTQKSLSPGKKDPKAAATTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDI
 YIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKF
 SRSADAPAYKQGQNQLYNELNLGRREEYDVLDRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE
 35 AYSEIGMKGERRRRGKGDGLYQGLSTATKDTYDALHMQALPPR

hALK15SH-BBz (SEQ ID NO: 70):

LLVTSLLLCELPHPAFLIPDTQVQLQQSGAEVKKPGSSVKVSCKASGFSLTSYAISWVRQAPGQGLE
 WMGGIIWSGGATNYAQKFQGRVTITADESTSTAYMELSSLRSEDNAVYYCAREHYYGSSAMDYWW
 40 GQGTMTVTVSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASQSIVHSYGNTRYAWYQQ
 KPGQAPRLLIYRVSRATGIPARFSGSGSGTDFTLTISSELPEDFAVYYCFQGTHTVPTTFGQGTKLEIKR
 AAATTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITL
 YCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYKQGQNQLYNE
 LNLGRREEYDVLDRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGDGLY
 45 LYQGLSTATKDTYDALHMQALPPR

hALK15-28BBz (SEQ ID NO: 71):

LLVTSLLLCELPHPAFLIPDTQVQLQQSGAEVKKPGSSVKVSCKASGFSLTSYAISWVRQAPGQGLE
 WMGGIIWSGGATNYAQKFQGRVTITADESTSTAYMELSSLRSEDNAVYYCAREHYYGSSAMDYWW
 50 GQGTMTVTVSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASQSIVHSYGNTRYAWYQQ
 KPGQAPRLLIYRVSRATGIPARFSGSGSGTDFTLTISSELPEDFAVYYCFQGTHTVPTTFGQGTKLEIKR
 EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
 AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
 KNQVSLTCLVKGFPYSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
 55 ALHNHYTQKSLSPGKKDPKAAAFVPVFLPAKPTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH

TRGLDFACDIYWAPLAGTCGVLLLSLVITLYCNHRNRSKRSRLLHSDYMNMTPRRPGPTRKHYPYA
 PPRDFAAYRSRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSADAP
 AYQQGQNQLYNELNLGRREEYDVL DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM
 KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

5

hALK15SH-28BBz (SEQ ID NO: 72):

LLVTSLLLCELPHPAFLIPDTQVQLQQSGAEVKKPGSSVKVSCKASGFSLSY AISWVRQAPGQGLE
 WMGGIWSGGATNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAREHYYGSSAMDYWW
 GQGTMTVTVSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASQSIVHSYGNTYAWYQQ
 10 KPGQAPRLLIYRVS RATGIPARFSGSGSGTDFTLTISSELEPEDFAVYYCFQGTHTVPTFFGQGTKLEIKR
 AA AFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTC
 GVLLLSLVITLYCNHRNRSKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRFSVVKRGR
 KKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRRE
 15 EYDVL DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLST
 ATKDTYDALHMQALPPR

hALK48-28z (SEQ ID NO: 73):

LLVTSLLLCELPHPAFLIPDTQVQLQQSGAEVKKPGSSVKVSCKASGYAFSSYISWVRQAPGQGLEW
 MGGQIYPGDGDTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCVRYYYGSSGYFDYWWG
 20 QGTMTVTVSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASESDNYGISFAWYQQKPG
 QAPRLLIYRASRATGIPARFSGSGSGTDFTLTISSELEPEDFAVYYCQNNKDPPTFFGQGTKLEIKRAEP
KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL
 25 *HNHYTQKSLSLSPGKKDPKAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKPFVWL VVVG*
 GVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSA
 DAPAYQQGQNQLYNELNLGRREEYDVL DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE
 IGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

hALK48SH-28z (SEQ ID NO: 74):

LLVTSLLLCELPHPAFLIPDTQVQLQQSGAEVKKPGSSVKVSCKASGYAFSSYISWVRQAPGQGLEW
 MGGQIYPGDGDTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCVRYYYGSSGYFDYWWG
 QGTMTVTVSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASESDNYGISFAWYQQKPG
 QAPRLLIYRASRATGIPARFSGSGSGTDFTLTISSELEPEDFAVYYCQNNKDPPTFFGQGTKLEIKRAAA
 35 IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKPFVWL VVVG GVLACYSLLVTVAFIIFWVRSK
 RSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGR
 REEYDVL DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGL
 STATKDTYDALHMQALPPR

hALK48-BBz (SEQ ID NO: 75):

LLVTSLLLCELPHPAFLIPDTQVQLQQSGAEVKKPGSSVKVSCKASGYAFSSYISWVRQAPGQGLEW
 MGGQIYPGDGDTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCVRYYYGSSGYFDYWWG
 QGTMTVTVSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASESDNYGISFAWYQQKPG
 QAPRLLIYRASRATGIPARFSGSGSGTDFTLTISSELEPEDFAVYYCQNNKDPPTFFGQGTKLEIKRAEP
 45 *KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK*
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL
HNHYTQKSLSLSPGKKDPKAAAATTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI
 WAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFS
 50 RSADAPAYKQGQNQLYNELNLGRREEYDVL DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEA
 YSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

hALK48SH-BBz (SEQ ID NO: 76):

LLVTSLLLCELPHPAFLIPDTQVQLQQSGAEVKKPGSSVKVSCKASGYAFSSYISWVRQAPGQGLEW
 MGGQIYPGDGDTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCVRYYYGSSGYFDYWWG

QGTMTVTSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASESDNYGISFAWYQQKPG
 QAPRLLIYRASRATGIPARFSGSGSGTDFTLTISLEPEDFAVYYCQNNKDPPTFFGQGTKEIKRAAA
 TTTAPRPPPTAPTASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITLYCK
 RGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNL
 5 GRREEYDVLDKRRGRDPPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
 GLSTATKDTYDALHMQALPPR

hALK48-28BBz (SEQ ID NO: 77):

LLVTSLLLCELPHPAFLIPDTQVQLQQSGAEVKKPGSSVKVSKASGYAFSSYISWVRQAPGQGLEW
 10 MGGQIYPGDGDTNYAQKFQGRVTITADESTSTAYMELSSLRSEDVAVYYCVRYYYGSSGYFDYWWG
 QGTMTVTSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASESDNYGISFAWYQQKPG
 QAPRLLIYRASRATGIPARFSGSGSGTDFTLTISLEPEDFAVYYCQNNKDPPTFFGQGTKEIKRAEP
KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKN
 15 *QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEAL*
HNHYTQKSLSLSPGKKDPKAAAFVPVFLPAKPTTTAPRPPPTAPTASQPLSLRPEACRPAAGGAVHTR
 GLDFACDIYWAPLAGTCGVLLLSLVITLYCNHRNRSKRSLHSDYMNMTPRRPGPTRKHYPYAPP
 RDFAAAYRSRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAY
 QQGQNQLYNELNLGRREEYDVLDKRRGRDPPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKG
 20 ERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

hALK48SH-28BBz (SEQ ID NO: 78):

LLVTSLLLCELPHPAFLIPDTQVQLQQSGAEVKKPGSSVKVSKASGYAFSSYISWVRQAPGQGLEW
 MGGQIYPGDGDTNYAQKFQGRVTITADESTSTAYMELSSLRSEDVAVYYCVRYYYGSSGYFDYWWG
 25 QGTMTVTSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASESDNYGISFAWYQQKPG
 QAPRLLIYRASRATGIPARFSGSGSGTDFTLTISLEPEDFAVYYCQNNKDPPTFFGQGTKEIKRAAA
 FVPVFLPAKPTTTAPRPPPTAPTASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLL
 LSLVITLYCNHRNRSKRSLHSDYMNMTPRRPGPTRKHYPYAPPDFAAAYRSRFSVVKRGRKKLL
 YIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYD
 30 VLDKRRGRDPPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK
 DTYDALHMQALPPR

hALK53-28z (SEQ ID NO: 79):

LLVTSLLLCELPHPAFLIPDTQVQLQQSGAEVKKPGSSVKVSKASGYTFTDHFISWVRQAPGQGLE
 35 WMGGLNPYSGGTNYAQKFQGRVTITADESTSTAYMELSSLRSEDVAVYYCARHNWGAYFDYWWG
 QGTMTVTSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASKSLLHSNGNTYAWYQQK
 PGQAPRLLIYMSRATGIPARFSGSGSGTDFTLTISLEPEDFAVYYCMQGLEDPYTFFGQGTKEIKRE
PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTTPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTK
 40 *NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEA*
LHNHYTQKSLSLSPGKKDPKAAAEVMPYPYLDNEKSNGTIIHVKGKHLCPSPFLPGPSKPFVWLVVV
 GGVLCYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPDFAAAYRSRVKFSRS
 ADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPPEMGGKPRRKNPQEGLYNELQKDKMAEAYS
 EIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
 45

hALK53SH-28z (SEQ ID NO: 80):

LLVTSLLLCELPHPAFLIPDTQVQLQQSGAEVKKPGSSVKVSKASGYTFTDHFISWVRQAPGQGLE
 WMGGLNPYSGGTNYAQKFQGRVTITADESTSTAYMELSSLRSEDVAVYYCARHNWGAYFDYWWG
 50 QGTMTVTSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASKSLLHSNGNTYAWYQQK
 PGQAPRLLIYMSRATGIPARFSGSGSGTDFTLTISLEPEDFAVYYCMQGLEDPYTFFGQGTKEIKRA
 AAIEVMPYPYLDNEKSNGTIIHVKGKHLCPSPFLPGPSKPFVWLVVVGGVLCYSLLVTVAFIIFWVR
 SKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPDFAAAYRSRVKFSRSADAPAYQQGQNQLYNELNL
 GRREEYDVLDKRRGRDPPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
 GLSTATKDTYDALHMQALPPR
 55

hALK53-BBz (SEQ ID NO: 81):

LLVTSLLLCELPHPAFLIPDTQVQLQQSGAEVKKPGSSVKVSCKASGYTFTDHFISWVRQAPGQGLE
 WMGGLNPYSGGTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARHNWGAYFDYWWG
 QGTMVTVSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASKSLLHSNGNTYAWYQQK
 5 PGQAPRLLIYYMSRATGIPARFSGSGSGTDFTLTISSELPEDFAVYYCMQGLEDPYTFGGQGTKLEIKRE
PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEA
 10 *LHNHYTQKSLSLSPGKKDPKAAATTTAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI*
 WAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFS
 RSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK
 YSEIGMKGERRRGKGHDLGYQGLSTATKDTYDALHMQALPPR

hALK53SH-BBz (SEQ ID NO: 82):

LLVTSLLLCELPHPAFLIPDTQVQLQQSGAEVKKPGSSVKVSCKASGYTFTDHFISWVRQAPGQGLE
 WMGGLNPYSGGTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARHNWGAYFDYWWG
 QGTMVTVSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASKSLLHSNGNTYAWYQQK
 PGQAPRLLIYYMSRATGIPARFSGSGSGTDFTLTISSELPEDFAVYYCMQGLEDPYTFGGQGTKLEIKRA
 AATTTAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLY
 20 CKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYKQGQNQLYNEL
 NLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDLGY
 YQGLSTATKDTYDALHMQALPPR

hALK53-28BBz (SEQ ID NO: 83):

LLVTSLLLCELPHPAFLIPDTQVQLQQSGAEVKKPGSSVKVSCKASGYTFTDHFISWVRQAPGQGLE
 WMGGLNPYSGGTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARHNWGAYFDYWWG
 QGTMVTVSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASKSLLHSNGNTYAWYQQK
 PGQAPRLLIYYMSRATGIPARFSGSGSGTDFTLTISSELPEDFAVYYCMQGLEDPYTFGGQGTKLEIKRE
 PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNA
 30 KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEA
LHNHYTQKSLSLSPGKKDPKAAAFVPVFLPAKPTTTAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT
 RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRSKRSRLHSDYMNMTPRRPGPTRKHYPYAP
 PRDFAAYRSRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPA
 35 YQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK
 GERRRGKGHDLGYQGLSTATKDTYDALHMQALPPR

hALK53SH-28BBz (SEQ ID NO: 84):

LLVTSLLLCELPHPAFLIPDTQVQLQQSGAEVKKPGSSVKVSCKASGYTFTDHFISWVRQAPGQGLE
 40 WMGGLNPYSGGTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARHNWGAYFDYWWG
 QGTMVTVSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASKSLLHSNGNTYAWYQQK
 PGQAPRLLIYYMSRATGIPARFSGSGSGTDFTLTISSELPEDFAVYYCMQGLEDPYTFGGQGTKLEIKRA
 AAFVPVFLPAKPTTTAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCG
 VLLLSLVITLYCNHRNRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRFSVVKRGRK
 45 KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREE
 YDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDLGYQGLSTA
 TKDTYDALHMQALPPR

hALK58-28z (SEQ ID NO: 85):

LLVTSLLLCELPHPAFLIPDTQVQLQQSGAEVKKPGSSVKVSCKASGYTFTDYEISWVRQAPGQGLE
 50 WMGGIDPETGGTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARRYYGSSSFYWWG
 QGTMVTVSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASQDIGNYAWYQQKPGQAP
 RLLIYYTSRATGIPARFSGSGSGTDFTLTISSELPEDFAVYYCQQGSALPPTFFGGQGTKLEIKRAEPKSCD
 KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR
 55 EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL

5 *TCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSEFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY*
TQKSLSLSPGKKDPKAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLPGPSKPFVVLVVVGGVLA
CYSLLVTVAFIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPA
YQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK
GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

hALK58SH-28z (SEQ ID NO: 86):

10 *LLVTSLLLCELPHPAFLIPDTQVQLQQSGAEVKKPGSSVKVSCKASGYTFTDYEISWVRQAPGQGLE*
WMGGIDPETGGTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARRRYYGSSSFYDWWG
QGTMTVTSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASQDIGNYAWYQQKPGQAP
RLLIYYTSRATGIPARFSGSGSGTDFTLTISSELEPEDFAVYYCQQGSALPPTFFGQGTKLEIKRAAAIEVM
YPPPYLDNEKSNGTIIHVKGKHLCPSPLPGPSKPFVVLVVVGGVLA
15 *CYSLLVTVAFIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEY*
DVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTAT
KDTYDALHMQALPPR

hALK58-BBz (SEQ ID NO: 87):

20 *LLVTSLLLCELPHPAFLIPDTQVQLQQSGAEVKKPGSSVKVSCKASGYTFTDYEISWVRQAPGQGLE*
WMGGIDPETGGTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARRRYYGSSSFYDWWG
QGTMTVTSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASQDIGNYAWYQQKPGQAP
RLLIYYTSRATGIPARFSGSGSGTDFTLTISSELEPEDFAVYYCQQGSALPPTFFGQGTKLEIKRAEPKSCD
KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR
EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL
25 *TCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSEFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY*
TQKSLSLSPGKKDPKAAAATTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPL
AGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSADA
PAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG
MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

30 **hALK58SH-BBz (SEQ ID NO: 88):**

LLVTSLLLCELPHPAFLIPDTQVQLQQSGAEVKKPGSSVKVSCKASGYTFTDYEISWVRQAPGQGLE
WMGGIDPETGGTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARRRYYGSSSFYDWWG
QGTMTVTSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASQDIGNYAWYQQKPGQAP
RLLIYYTSRATGIPARFSGSGSGTDFTLTISSELEPEDFAVYYCQQGSALPPTFFGQGTKLEIKRAAATTP
35 *APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITLYCKRGR*
KKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSADAPAYKQGQNQLYNELNLGRRE
EYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLST
ATKDTYDALHMQALPPR

40 **hALK58-28BBz (SEQ ID NO: 89):**

LLVTSLLLCELPHPAFLIPDTQVQLQQSGAEVKKPGSSVKVSCKASGYTFTDYEISWVRQAPGQGLE
WMGGIDPETGGTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARRRYYGSSSFYDWWG
QGTMTVTSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASQDIGNYAWYQQKPGQAP
RLLIYYTSRATGIPARFSGSGSGTDFTLTISSELEPEDFAVYYCQQGSALPPTFFGQGTKLEIKRAEPKSCD
45 *KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR*
EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSEFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGKKDPKAAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDF
50 *ACDIYWAPLAGTCGVLLLSLVITLYCNHRNRSKRSRLHSDYMNMTPRRPGPTRKHYQPYAPPRDFA*
AYRSRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSADAPAYQQGQ
NQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR
GKGHDGLYQGLSTATKDTYDALHMQALPPR

LLVTSLLLCELPHPAFLLPDTPVQLQQSGAEVKKPGSSVKVCSKASGYTFDTYEISWVRQAPGGGLE
WMGGIDPETGGTNYAQKFQGRVTTADESTSTAYMELSSLRSEDTAVYYCARRRYYGSSSFYWWG
QGTMTVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASQDIGNYAWYQQKPGQAP
RLLIYYTSRATGIPARFSGSGSGTDFTLTISLEPEDFAVYYCQQGSALPPTFFGGQGTKLEIKRAAAFVPV
FLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLV
ITLYCNHRNRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRFSVVKRGRKKLLYIFK
QPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDK
RRGRDPMEGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD
ALHMQALPPR

15 cccctgagccgccaccatggttctgctctgtgacaagcctgctgctgctgcgagctgccccaccctgcttctgtctgaccccgacaccgagctgaagctgcagga
atctggccctggactgtgtggcccttagccagagcctgagcatcactgtaccgtgtccggcttcagcctgaccagctacgccgtgtcttgggtgcccagcctcc
tggcaaaaggcctggaatggctgggcatcttggagcggcgagccaccaactacaacagcgccttgaagtcgccggtgagcatctcaaggacaacagcaa
gagccaggtgttctgaagatgaacggcctgcagaccgacgacaccgcccggtactattgcgcagagagcactactacggcagcagcgctatggactactg
gggcccagggcgccagcatcacagtgtctagcggagggcgaggatctggcgcgaggaaagtggcgaggggggtatggaatcgtgatgccagagccct
ctgagcctgcctgtgtccctgggagatcagcctccatcagctgcagatccagccagagcatctgtcacagctacggcaacacctactcttctgtatctgcag
20 aagcccgccagagccccaagctgtgtatctaccgggtgtccaaccgggttcagcggcggtgccgatatagatttcggcagcggctccggcaccacctacc
tgaagatcagccgggtggagccgaggacatggcggtgtactactgtttcaaggcaccacgtgccctacaccttcggaggcgccaccaagcttgaatcaa
agagccaagagctgcgacaagaccacacctgtlccccctgttcttgccttgaactgctggcgggacctagcgtgttctgtttcccccaagcccaaggaca
ccctgatgatctcccgacccccgaagtgcctgtggtgtgtggatgtgttccacgaggacctgaagtgaagttaattgtgtacgtggacggcggtggaaagt
cacaaccgaagaccaagcccagagaggaaacaglacacagcacctacagagtgtgttccgtgtgacctgtgtgcaccaggactgtgctgaacggcaaaaga
25 gtacaagtgaaaagtgtccaacaaggccctgcctgcccccatcgagaaaaccatcagcaaggccaagggccagcccccggaacccccaggtgtacacactgc
cccctagcagggacgagctgaccaagaatcaggtgtcctgacctgtctgtgaagggttctaccctccgatatcgccgtggaatgggagagcaacggcca
gcccgagaacaactacaagaccacccccctgtgtctggacagcgacggctcattcttctgtacagcaagctgacagtggacaagagccgtgtggcagcagg
caacgtgttcagctgtccgtgatgcacgaggccctgcacaaccactacaccagaagtcctgttccctgagccccggcaagaagacccccaaagcggccgc
aattgaagttaattatcctctctctacacatgacaatgagaagagcaatggaaccattatccatgtgaaagggaacaccttctccaagtccccctatttccggacct
30 tctaagcccttttgggtgtgtgtgtgtgtgtgggggagtcctggctgtctatagctgtctagtaacagtgtgcccttattattttctgggtgaggagtaagaggagcagg
ctcctgcagagtgactacatgaacatgactccccgcggccggccaccgccgaagcattaccagccctatgcccaaccacgcgacttcgagcctacgctc
cagagtgaagltcagcaggagcgcagacgcccccggtaccagcagggcgacaacagctataacgagctcaatcagcagcaagagaggatgtacgatg
ttttgacaagagacgttgccggggacacctgagatgggggaaagcgagaaggaaacacctcagaaagccctgtacaagtgaactgcagaagaataagatg
gcgaggccctacagtgaagtgtgatgaaggcgagcggcgaggggcaaggggcacgatggccctttaccagggtctcagtacagccaccaaggacacct
35 acgacgccccctacatgcaggccctgccccctgcctaa

40 cccctgagccgccaccatggcttctctcgtgacttcccttctgctgfcgagctcccacaccgcccttctgctcattctgataccgagtgaagctgcaagaatc
cgcccccgactggctcgcgcaagccaatcgtgagcattactgacgggtgtccggatttctgttgacctctacgctgtgtctctgggtcagacagccgccggg
taaaggactcgaatggcttggcatcatctgtgtcggggggagcgactaactacaactcagcgtgaaatcgcggctgtccatctcaaaggataattcaaaaagcca
45 ggtgtttctgaagatgaatggcctgcagactgacgacaccgctcgtactactgcgcccgagcattactacggatcatccgaatggactattggggcgagg
gctcatctatcaccgtcagcagcggggggcgagggtctgctgggaggggggttccggcgggggagggagcggaaatctgatgaccagctgccgcttctctg
cctgtcagcctgggagatcaggccagcatctatgtcggctgtccagagcatctgctcactctgtacggtaacacgtaccttctctgtgacctcaaaagcctggac
agtcaccaaaagctgtgatctatagggtgtccaatcgcttctcgggtgtgcccggaccggttctcgggctcgggatcaggaaaccaactttactctgaagatctccaga
45 gtggaagccgaggaatgggagtctactactgcttccaaggaaatcatgttccgtacaccttcggagagggaccaagctggaaatcaaggcggccgcaattg
aagttatgtatctctctcttacctagacaatgagaagagcaatggaaccattatcatgtgaaagggaacaccttctccaagtccccctatttccggaccttctaa
gcccccttgggtgctgtgtgtgtgtgtgggggagttctggctgtctagctgtgtagtaacagtggccttattatttcttgggtgaggagtaagaggagcaggctcct
gcacagtgtactacatgaacatgactccccgccgccgggccaccgccgaagcattaccagccctatgccccaccacgcgacttcgcagcctatctgtccaga
50 gtgaagttcagcaggagcgcagacgccccgcgtaccagcaggggccgaaccagctctataacgagctcaatctaggacgaagagaggagtacgatgttttg
gacaagagacgtgcccgggaccctgagatgtgggggaaagccgagaagggaagaacacctcaggaaggcctgtacaatgaactgcagaagaagataagatggcg
gaggccctacagtgagattggatgaaaggcgagcgcgggaggggcaaggggcacgatggcctttaccagggtgtctcgtacagccaccaaggacacctcaga
cgccccctcacatgcaggccctgccccctcgtctaa

55 ccctcgagccgccacatggtctgctcgtgacaagcctgctgctgctgcgagctgccccaccctgcttctgctgatccccgacaccgacgtgaagctgcaggga
atctggccctggactggtggcccttagccaagacgtgagcatcaccctgtaccgtgtccggcttcagcctgaccagctacgcgtgtctgttggtgcgccagcctcc
tggcaaaggcctggaattgctgggcacatcttgagcgcgggagccaccaactacacagcgcctgaagtcccgctgagcatctccaagacaacagcaa

20

ALK15SH-BBz (SEQ ID NO: 94):

25

ALK15-28BBz (SEQ ID NO: 95):

40

5

10

30

55

g c g g c c g c a a t t g a a g t t a t g t a t c t c t c t t a c c t a g a c a a t g a g a a g c a a t g g a a c c a t t a c c a t g t g a a g g g a a a c a c c t t t g c c a a g t c c c t a t t t
c c c g g a c c t t c t a a g c c c t t t g g g t g c t g t g g t g g t g g g g a g t c t g c t t g c t a t a g c t t g c t a g t a a c a g t g c c t t t a t t a t t t c t g g t g a g g a g t a a g a
g g a g c a g g c t c t g c a c a g t g a c t a c a t g a a c a t g a c t c c c c c c g c c c c g g c c c a c c c g c a a g c a t t a c c a g c c c t a t g c c c c a c c a g c g a c t t c g c a g
c c t a t c g t c c a g a g t g a a g t t c a g c a g g a g c g c a g a c g c c c c c g t a c c a g c a g g g c c a g a a c c a g c t c t a t a a c a g a g c t a a t c t a g g a c a a g a g a g
5 g a g t a c g a t g t t t g a c a a g a g a c g t g c c g g g a c c c t g a g a t g g g g g a a a g c c g a g a a g a a g a a c c c t c a g g a a g g c c t g t a c a a t g a a c t g c a g a a
a g a t a a g a t g c g g a g g c c t a c a g t g a g a t t g g a t g a a a g c g a g c c g g a g g g g c a a g g g c a c g a t g g c c t t a c c a g g g t c t c a g t a c a g c c a c c a
a g g a c a c t a c a c g c c c t t c a c a t g c a g g c c c t g c c c c t c g c t a a

ALK48-BBz (SEQ ID NO: 99):

10 c c c t c g a g c c g c c a c c a t g g t t c t c t g t g a c a a g c c t t c t c t g c g a a t t g c c c c a c c a g c c t t t t g c t t a t c c c g a c a c c c a a g t c c a g t g c a g c a a t c
a g g g g c c g a g t t g t c a a g c c t g g g c a t c g g t c a a a a t c t a t g t a a g c c t c g g g a t a t g c t t c t g c t a c t g g a t g a a t t g g t c a a g c a g c g g c c a g
g a a a g g a c t g g a a t g a t c g g c a a a t c t a c c a g g g a t g g a g a t a c a c a t a t a a c g g g a a g t t a a a g g a a a g c a a c t c t a c t g c g g a c a a g t c a t c
a t c g a c g g t a t a c a t g c a g c t t a a c t a t t g a c a a g c g a g g a c t c g c g g t c t a t t t c t g c t a c g g t a t t a c t a c g g a t c g t c g g g t a c t t c g a t t a t t g g g t c a
g g g a a c c a c g t g a c a g t g c c a g c g g a g g t g c g g g t c c g a g g c g g a g a t c c g g t g c g g t g g a a g c g a t g t g c a g a t a c c a g a c c g g a c t c a
15 c t c g c g g t g t c a c t c g g c a g c g g g c g a c g a t t c a t g c a g a c c t c g a g t c g t g g a c a a t t a c g g t a t c t c t c a t g c a t t g g t a t c a g c a g a a c c c g g g
c a g t c g c c a a g c t g t g a t c t a c a g a c g t c c a a c c t t g a g t c g g g a t t c c c g t a g g t t c c g g g t c a g g a t c c c g c a c g g a c t t c a c c t t g a c g a t t a a c c
c g g t g g a a a c t g a t g a c g t c c c a t t a c t a t g t c a g c a g a a a a t a a g a c c c t c c a c a t t t g c g g a g g t a c a a g c t t g a a t c a a g a g g c g g a g c c
g a a g a c t g c g a t a a a a c g c a c a c a t g c c c t c a t g c c c t g c a c c g g a g c t c t t g g c g g a c c t t c c g t t t c t g t t c c a c c g a a c c c a a g a c a c c t g a t
g a t t t c g c g c a c g c c g g a g g t a a c t t g t g t g t g t g a c g t a a g c c a t g a g g a c c c g a a g t a a a g t t a a c t g t a t g c a t g g c g t g g a g t c a c a a t g
20 c g a a a c c a a g c c g a g a g a g a a c a g t a t a a c t c c a c t a c c a g t a g t c g t a a g c g t g c t t a c a g t g c t t c a t a a g a t t g g t t a a g g t a a g a a t a c a a t g c a
a g g t g t c g a a c a a g c t c t g c c c g c a c a a t t g a g a a a c t a t t a g c a a g c g a a g g g g c a g c c c a g g g a a c c c a a g t g t a t a c t t t g c c c c t c g c g a
t g a a c t a c t a a g a a t a a g t c t c t g a c g t g t c t c t a a g g g g t t t a c c g a c g a c a t c g c g t g g a g t g g g a g t c a a c g t c a a c c g a g a a c a a t t a
c a a a c c a c a c c t c c c t g t c g a t t c g g a c g g a t c g t t t t c t c t a t t c c a a t t g a c c g t c g a t a a g t c g a t g g c a g c a g g g t a a t g t a t t t c g t t c g g t a
a t g c a c g a a g c c c t c c a c a c c a t t a t a c g c a g a a g t c g t g c c t g t c g c c c g a a a g a a g a c c c g a a g g c c g c a a c c a c g a c c c a g c g c c g c g
25 a c c a c c a a c c g g c g c c c a c c a t c g t c g c a g c c c t g t c c t g t c g c c c a g a g c g t g c c g c c a g c g c g g g g g c g c a g t g c a c a g a g g g g g t
g g a c t t c g c t g t g a t a t c a t a c t g g c g c c c t t g c c g g a c t t g t g g g t c c t t c t c t g t a c a t g g t a t c a c c t t a c t g c a a a c g g g c a g a a g a a c
t c c t g t a t a t t a c a a c a c c a t t a t a g a c c a g t a c a a c t a c t a a g a g a a g a t g c t g t a g c t g c g a t t t c c a g a a g a a g a a g a g a g a t g t a a c t
g a g a g t a a g t t c a g c a g g a c g c a g a c g c c c c c g c t a c a a g c a g g g c c a g a a c c a g c t c t a t a a c a g a t c a a t c t a g a c a a g a g a g a g t a c a t g
t t t t g a c a a g a g a c t g g c c g g a c c c t g a g a t g g g g g a a a g c c g a g a a g a a c c c t c a g a a g g c c t g t a c a a t g a a c t g c a g a a g a t a a g a t g
30 g c g g a g c c t a c a g t g a g a t t g g a t g a a a g c g a g c c c g a g g g g c a a g g g c a c g a t g c c t t a c c a g g t c t c a g t a c a g c c a a g g a c a c c t
a c g a c g c c c t t c a c a t g c a g g c c c t g c c c c t c g c t a a

ALK48SH-BBz (SEQ ID NO: 100):

35 c c c t c g a g c c g c c a c c a t g g t t c t c t g t g a c c t c c t g t c t c t g c g a a c t c c t a t c c g g c a t t c c t g c t a t c c c g a c a c c c a a g t c c a a c t c c a g c a g a
g c g g a g c c g a g t g t g a a g c c g g a g c g a g c g t c a a a a t c a g t g t a a g c c t c c g g t a c g c c t t a c t c a t a c t g g a t g a a c t g g t g a a g c a a a g a c
c g g g a a a g g g t t g a a t g g a t c g a c a a a t c a c c g g g a g a t g g a t a t a c t a c t a c a a t g g a a g t t a a a g g a a a g c c a c t c t g a c c g t g a t a a g t c
c t c g t c c a c g g t c a t a c a g c t a a c t a c t g a c t t c g a g a t a g c g c c g t g t a c t t c t g c g t c t a c t a c g g a t c a c a g g a t a c t c g a c t a c t g g g
g c c a a g t t a c c a c t c a c c g t g t c g t c g g a g g a g c g g c t c c g c g g t g g a g a t c c g a g g c g g a g g t c a g a c t g c a g a t g a t t c a g a c t c c c g a c
40 t c g t c g c g g t g t c c c t g g t c a g a g g g c c a c c a t t t c t g c c g g c t t c g a g t c a g t g a c a a t t a c g c a t c a g c t t a t g c a c t g g t a t c a g c a a a g c c a
g g c c a g t c c c a a a g t t g t g a t c a c c g c g a t c g a a t t g a g t c c g c a t c c a g c t c g t t c a g c g g a g c g g a t c g a g a a c t g a c t t a c t g t g a c c a t
c a a c c c g t c g a a a c c a t g a c t g c g a a c t t a t a c t c c a g c a c a a c a a g g a c c c t c c g a c c t c g g t g g a g g a c t a a g c t g g a a a t c a a a c g c g c g
g c g g c c g c a a c c a c a c g c c c g c g a c c a c a a c a c c g c g c c c a c c a t c g c t c g a g c c c t g t c c c t g c c c c a g a g g c g t c c c g c c a g c g
g c g g g g g c g c a g t g c a c a c a g g g g c t g g a c t t c c t g t a t a t c a t a c t g g c g c c c t t g c c g g a c t t g t g g g t c c t t c t c t g t a c t g g t a t c
a c c c t t a c t g c a a a c g g g c a g a a a g a a c t c t g t a t a t t a c a a c a c c a t t a t a g a c c a g t a c a a a c t a c t a a g a g a a g a g a g t g c t g a g t c c g a t t
45 t c c a g a a g a a g a a g a g a g a t g t a a c t g a g a g t a a g t t c a g c a g a g c g c a g a c g c c c c c g c t a c a a g c a g g g c c a g a a c c a g c t c t a a c a g
c t c a a t c t a g g a c a a g a g a g a g a t g t t t g a c a a g a g a c t g g c c g g a c c c t g a g a t g g g g a a a g c c g a g a a g a a a c c c t c a g g a a g
g c c t g t a c a a t g a a c t g c a g a a g a t a a g a t g c g g a g g c t a c a g t g a g a t t g g a t g a a a g c g a g c g c g g a g g g c a a g g g c a c a t g c c t t a c
c a g g g t c a g t a c a g c c a a g g a c a c t a c a c g c c c t t c a c a t g a g g c c c t g c c c c t c g c t a a

ALK48-28BBz (SEQ ID NO: 101):

55 c c c t c g a g c c g c c a c c a t g g t t c t c t g t g a c a a g c c t t c t c t g c g a a t t g c c c c a c c a g c c t t t t g c t t a t c c c g a c a c c c a a g t c c a g t g c a g c a a t c
a g g g g c c g a g t t g t c a a g c c t g g g c a t c g g t c a a a a t c a t g t a a g c c t c g g g a t a t g c t t c t g c t a c t g g a t g a a t t g g t c a a g c a g c g g c c a g
g a a a g g a c t g g a a t g a t c g g c a a a t c t a c c a g g g a t g g a g a t a c a c a t a t a a c g g g a a g t t a a a g g a a a g c a a c t c t a c t g c g g a c a a g t c a t c
a t c g a c g g t a t a c a t g c a g c t t a a c t a t t g a c a a g c g a g g a c t c g c g g t c t a t t t c t g c t a c g g t a t t a c t a c g g a t c g t c g g g t a c t t c g a t t a t t g g g t c a
g g g a a c c a c g t g a c a g t g c c a g c g g a g g t g c g g g t c c g a g g c g g a g a t c c g g t g c g g t g g a a g c g a t g t c a g a t a t c a g a c c g c c g a c t c a
c t c g c g g t g t c a c t c g g c a g c g g g c g a c g a t t c a t g c a g a c c t c g a g t c g t g g a c a a t t a c g g t a t c t c t c a t g c a t t g g t a t c a g c a g a a c c c g g g
c a g t c g c c a a g c t g t g a t c t a c a g a c g t c c a a c c t t g a g t c g g g a t t c c c g t a g g t t c c c g g t c a g g a t c c c g c a c g g a c t t c a c c t t g a c g a t t a a c c

ALK48SH-28BBz (SEQ ID NO: 102):

ALK53-28z (SEQ ID NO: 103):

-79-

ALK53SH-28z (SEQ ID NO: 104):

20

ccctcgagccgccaccatggctctgctcgtgacaagcctgtcgtgtgctgagctccccaccctgcctttctgtcgtatccccacaccgacgctgacgtgcaggagatctggacccgtgctcgtgaaaaccggcgccagcgtgaagatgagctgtaccgccagcgctacacctaccgaccacttcatgactgggtcaagcagagccacggcaagacgtggaaftggtcggcagcctgaaccctacagcggcgccaccagctacaaccagaagttaaggggcaaggccacctgaccgtggacaagagcagcagcaccgctacatggaaactgaacagcctgaccagcgtggacagcggcgtgtactactgcgccagacacaactggggcgctacttgcactattggggccagggcacaacctgacagtgtctagcggaggcggaggatctggcggcgagggaagtggcggagggggagtctgatatctgtgatgaccaggccgctcccagcgtgccagtacacctggcgagagcgtgtccatcagctgcagaagcagcaagtccctgtctgcacagcaacggcaatactacctgtactgttcttgcagaggcctggccagagccccagcggcgtgatctactacatgagcaacctggccagcggcgtgcccagacagattttctggcagaggcagcggcaccgacttcacctgagaatcagccgggtggaagccgaggacgtggcgctgtactattgcattgaggggcctggaagatccttacaccttcggcgaggcaccagctggaatcaagagcccaagagctgcgacaagaccacacctgtcccccttgccttgccttgaactgctggcggaacttagcgtgttctgttcccccaagcccaagggaacctgtgatgacagcaggacccccgaagtgaactgcgtgtgtgtggtggtggtggtgtgtccacaggagacctgaagtgaagttaattgtgactgtggacggcgtggaagtgcacaacgccaaagaccaagccccagagaggaaacgtataacagcacctaccgggtgtgtgtccgtctgtaccgtgtgtgcaccaggactgtgctgaacggcaagagtlacaagtgcgaaggtgttccaacaaggccctgcctgccccatcagaaaaacctacgaaggccaagggccagccccgcgaacccagggtgtacacctgccccctagcagggacgagctgaccaagaaccagggtgtccctgacctgtctcgtgaagggcttctacccctccgatatccgttggaaftgggagagcaacggccagcccagagaacaactacaagaccacccccctgtgtcgtgactccgacggctcatttctctgtacagcaagctgacagtggataagtccgggtggcagcagggaacgtgttcagctgtctccgtgatgcaggaagccctgcacaaccactacaccagaaaaagcctgtccctgagccctggcaagaaggacccccaaagcggccgcaaccacgacgccagcgccgcgaccaccaacaccggcgcccaccatcgcgtcgcagccctgtccctgcgcccagaggcgtccggccagcggcgggggcgcgagtgcacacgagggggctgacttgcctgtgatatctacatctggcgcccttggccgggacttgtgggttccttctctgtcactgtgtatccctttactgcaaacggggcgagaagaactctgttatataftcaacaacacctttatgagaccagatacaaaactactcaagagaagatgctgtagctgccgattttcagaagaagaagaggaggaggtgtgaactgagagttgaagttacgagcggcgagacggccccgcgtacaaagcagggcgcaagaaccagcttatacgaagctctagagagagcaggaagaggtacgattgttttgacaagagacgttggccgggacccttgagatgtgggggaaagccgagaagggaagaacctcaggaagcctgtacatgaaactgcagaagaataaagtgtgcggaggccctacagtgagttggatgaagggcgagcggcgagggggcaaggggcacgatggcctttaccagggtctcagtlacagccaccaaggacacctacgacgccttccatgcaggccctgccccctgcctaa

ccctcgagccgccacatggttctgctcgtgacaagcctgctgctgctgagctgcccacacctgcttctgctgatccccgacaccgacgtgcagctgcagga
atctggaccctgtgctcgtgaaaaccggcgccagcgtgaagatgagctgtaccgccagcgctacaccttcaccgaccacttcattgactgggtcaagcagagc
cacggcaagagcctggaatgatcggcagcctgaacccctacagcggcgccaccagctacaaccagaagttaaggggcaaggccacctgaccgtggaca
agagcagcagcaccgacctacatggaactgaacagcctgaccagcgtggacagcggcctgttactactgcgccagacacaactggggcgctacttcgactattg
50 gggccagggcacaacctgacagtgtctagcggaggcggaggatctggcgcgagggaagtgtggcgagggggactctgatatcgtgatgaccaggccgct
cccagcgtgccagtacacctggcagagcgtgtccatcagctgcagaagcagcaagtccctgctgcacagcaacggcaatattactctgtactggttctgtca
gaggcctgcccagagccccagcggctgatctactacatgagcaacctggccagcggcgtgcccagagatttctggcagaggcagcggcaccgacttcac
cctgagaatcagccgggtggaagccgaggacgtggggctgtactattgcatgcagggcctggagatccttacaccttcggcgaggcaccaagctggaatc
aaagcggccgcaaccacgacgcagcggccgcgaccaccaacaccggcgcccaccatcgcgtgcagccctgtccctgcgccagaggcgtgccggcca
55 gcggcggggggcgagtcgacacagggggcgctgacttcgctgtatattacatctatctggcgcccttggtggcggaactgtgggtctcttcctgtactgtgtt
atcaccctttactgcaaacggggcgagaaggaacctctgtatatattcaaacaaacctttagagcagactacaagactcaagggaagtggctgtactgtcc
gatttccagaagaagaagaggaggtgtaactgagagtgaagtgcagcagagcgcagagcccccgcgtacaagcagggccagaaaccagctctataac

5 **ALK53-28BBz (SEQ ID NO: 107):**

10

30

35

50

55

15

ALK58SH-28z (SEQ ID NO: 110):

20

ALK58-BBz (SEQ ID NO: 111):

35

ALK58SH-BBz (SEQ ID NO: 112):

ccctcgagccgccaccatggttctctgtgactcactctgctgtgcgaactgccgcatccagccttctgctgatccccgacactgcgctccaactgcagcaat
 ccggagctgaattgtggtcgccagggtgctccgtgactgtgagctgcaaggcatccggatacacctttaccgactacgagatgactgggtcaaacagactcct
 gtccacggcctcgaatggattggagcaatcgaccagaactgaggggaccgctacaaccagaagttgaaggaaagccattttgactgccgacaatcct
 5 cctcgaccgcctacatggaactgagatccctgacttcggaggattcgccgtgtactactgtgcacgccgagatactacgggagctcgtcgttcgactactggg
 gtccagggaaccactctgactgtctcatccggtggaggcggatcagcgggtggagggtcaggcggaggcggctccgacgtgcagatgatccagaccccgctct
 cgctctccgcttgcgttgagatcgggtcacgacagctgccgcttcacaagatctggaactatctcaactggtaccaacagaagccggacggaaactgtga
 agctgtctatctactacacctgcgcttcatagcggagtgcttcaagggttcaggcgtcggggtcgggaaccgaglacagcctgacctctcaatctggagc
 10 aggaagatacgcacttatttctgccagcaaggtagcgcctcctccgaccttcggaggcgggacgaagctggagatcaatcggcgcgccgccaacca
 cgacgccagcgcggcaccaccaaccggcgccaccatcgctgcagccccgttccctgcgcccagaggcgtgcggccagcggcgggggcgca
 gtgcacacgagggggctggacttcgctgtgatatctacatctggcgcccttggccgggactgtggggctcttctctgactggtatcacctttactgcaaa
 cggggcagaagaactcctgtatatactcaaacacatttatgagaccagtacaaactactcaagaggagatgctgtagctgccgatttcagaagaagaa
 gaaggaggtatgaactgagatgaagttcagcaggagcgcagacccccgcgtacaagcaggccagaaaccagctctataacgagctcaatctaggacg
 aagagaggagtacgatgttttgacaagagacgtggcgggaccctgagatgggggaaagccgagaaggagaaccctcaggaaaggcctgtacaatgaa
 15 ctgcagaagaataagatggcggaggcctacagtgagattggatgaaggcgcagcgccggaggggcaaggggcacgatggcctttaccagggtctcagta
 agccaccaaggacactacgacgccttcatagcaggccctgccttcgctaa

ALK58-28BBz (SEQ ID NO: 113):

ccctcgagccgccaccatggttctctgtgactagcctctgctgtgcgaactccacatccagcttctctgttatccagacaccgccccccaactccagcaaa
 20 gcgggtgctgaactgtgaggcctggcgcttctgtaccctgagctgcaaggcagcgggtatacttcaccgattacgaaatgattgggtgaagcagacccag
 tgcattgctgtgagtgattggagctatcgaccccgaactggagggactgcctacaaccagaagttgagggaagggccatcttactgccgacaagtcatcat
 ctaccgcatacatgagctgaggtcactgacctccgaggactccccgtgtactattgcgccagaaggagggtactacggttcatcttcttctgattattgggaca
 gggaaactactctgaccgtcagctcgtgggtgtgtgatcaggtggagcgggaagcggaggggagggttcagacgtcagatgattcagactccttcagcctt
 ctgcctcactcggggaccggtgacatctcatgtagagcctcccaagacatcggaattacctaattggtatcaaaaaaacctgatggcactgtgaagctct
 25 gatctactacactctcgttctactcagggtccccagcgggttctgtgctgtgttcaggggaccgaatactctctaccattagcaatctcgaacaaggagaca
 tcgcaacttacttctgccagcagggaagcgcactgcgcccacttcggaggaggaaccaagctggaaatcaatcggccgagccgaagagctgcgacaag
 actcatacttctctcttcttccagccccgaactgctcggcgaccctccgtgttctgttccgcccagcccaaggacactcttatgatcagccgacccccg
 aagtgaactgcgtcgtgtgacgtgagccacgaggacctgaagtgaagttcaactggtatgtgacggagtcgaagtgcataacgcaaaaaccaaacccg
 cgaggagcaataaactatcgcgtgtgagcgtgtcaccgtgtgtcaccaggactggttaacggttaaagagtacaagtgtaaagtgaagcaacaag
 30 ctctgcccgtctctattgagaaaaactatcagcaagggttaaggacagcctcgggaacctcaaggtatataccttccccctagccgggatgaactgaccaagaatc
 aagtcagccttactgtctgtgtaagggttctaccatccgacattgcagtggaatggagtcacaacgggcagcccagagaacaattacaagaccaccccgct
 gtgctggacagcgcagcatcttcttcttactcaagctgactgtgtataagtaagatggcagcagggtaacgtgttttcttgcagcgtcatgcagcagggccct
 gcacaaccattataccagaagagcctgtactgtctcgggaaggaagacctaaggcgccgcatctgtcgggtcttctctccagcgaagcccaccag
 acgcccagcggcgaccaccaacaccggcgcccaccatcgctgcagccttctcctgcgccagaggcgtgcccggcagcggcgggggcgagtg
 35 cacacgagggggtgacttcgctgtgatatctacatctggcgcccttggccgggactgtgtgggtccttctctgtcactggttatcacctttactgcaacca
 cagggaacaggagtaagaggagcagctcctgcacagtactacatgaacatgactccccgcgcccgggcccaccgcgaagcattaccagccctatcccc
 accacgcgacttcgcagcctatcgtcccgttctgtgtttaaagggggcagaagaagctcctgtatatactcaaacacatttatgagaccagtacaaactac
 tcaagagggaagatgctgtagctgccgatttcagaagaagaagaaggaggatgtgaactgagagtgaagttcagcaggagcgcagacgccccgcgtacca
 gcaggggcagaaccagctctataacgagctcaatctaggacgaagagaggagtagatgttttgacaagagacgtggcgggaccctgagatggggggaa
 40 agccgagaaggagaaccctcaggaaaggcctgtacaatgaactgcagaagaataagatggcggaggcctacagtgaattgggatgaaggcgagcgccg
 gaggggcaaggggcacgatggcctttaccagggtctcagtagcaccacaaggacacctacgacgccttcatatgcaggccctgccccctgcgtaa

ALK58SH-28BBz (SEQ ID NO: 114):

ccctcgagccgccaccatggttctctgtgactcactctgctgtgcgaactgccgcatccagccttctgctgatccccgacactgcgctccaactgcagcaat
 45 ccggagctgaattgtggtcgccagggtgctccgtgactgtgagctgcaaggcatccggatacacctttaccgactacgagatgactgggtcaaacagactcct
 gtccacggcctcgaatggattggagcaatcgaccagaactgaggggaccgctacaaccagaagttgaaggaaagccattttgactgccgacaatcct
 cctcgaccgcctacatggaactgagatccctgacttcggaggattcgccgtgtactactgtgcacgccgagatactacgggagctcgtcgttcgactactggg
 gtccagggaaccactctgactgtctcatccggtggaggcggatcagcgggtggagggtcaggcggaggcggctccgacgtgcagatgatccagaccccgctct
 cgctctccgcttgcgttgagatcgggtcacgacagctgccgcttcacaagatctggaactatctcaactggtaccaacagaagccggacggaaactgtga
 50 agctgtctatctactacacctgcgcttcatagcggagtgcttcaagggttcaggcgtcggggtcgggaaccgagtagacagcctgacctctcaaatctggagc
 aggaagatacgcacttatttctgccagcaaggtagcgcctcctccgaccttcggaggcgggacgaagctggagatcaatcggcgcgccgccaactgt
 gccgggttcttccgagcgaagcccaccacgacgccagcggcgaccaccaacaccggcgcccaccatcgctgcagccccgttccctgcgcccagag
 gcgtgcggccagcggcgggggcgagtgacacgagggggtggacttcgctgtgatatctacatctggcgcccttggccgggactgtgtgggtcctt
 ctctgtcactggttatcacctttactgcaaccacagggaacaggagtaagaggagcaggtcctgcacagtactacatgaacatgactccccgcccccgg
 55 gcccccgcgaagcattaccagccctatgccccaccacgcgacttcgcagcctatcgtccccgttctgtgtttaaagggggcagaagaagctcctgtatata
 tcaaacacatttatgagaccagtacaaactactcaagagggaagatgctgtagctgccgatttcagaagaagaagaaggaggatgtgaactgagagtgaag
 ttacagcaggagcgcagacgccccgcgtaccagcaggggcagaaccagctctataacgagctcaatctaggacgaagagaggagtagatgttttgacaag

agacgtggccgggaccctgagatgggggaaagccgagaaggaagaaccctcaggaaggcctgtacaatgaactgcagaaagataagatggcggaggcct
acagtgagattgggatgaaaggcgagcgcggaggggcaaggggcacgatggcctttaccagggtctcagtacagccaccaaggacacctacgacgccctt
cacatgcaggccctgccccctcgctaa

- 5 In view of the many possible embodiments to which the principles of the disclosed embodiments may be applied, it should be recognized that the illustrated embodiments are only preferred examples of the invention and should not be taken as limiting. All that comes within the scope and spirit of the following claims is claimed.

We claim:

1. A nucleic acid molecule encoding a chimeric antigen receptor, the chimeric antigen receptor comprising:

an antigen binding domain, a transmembrane domain, and at least one intracellular T-cell signaling domain, wherein the antigen binding domain comprises a heavy chain variable region and a light chain variable region comprising one of:

(a) a heavy chain complementarity determining region (H-CDR)1, a H-CDR2, and a H-CDR3 of the heavy chain variable region set forth as SEQ ID NO: 1, and a light chain complementarity determining region (L-CDR)1, a L-CDR2, and a L-CDR3 of the light chain variable region set forth as SEQ ID NO: 2 [ALK15];

(b) a H-CDR1, a H-CDR2, and a H-CDR3 of the heavy chain variable region sequence set forth as SEQ ID NO: 3, and a L-CDR1, a L-CDR2, and a L-CDR3 of the light chain variable region sequence set forth as SEQ ID NO: 4 [ALK48];

(c) a H-CDR1, a H-CDR2, and a H-CDR3 of the heavy chain variable region sequence set forth as SEQ ID NO: 5, and a L-CDR1, a L-CDR2, and a L-CDR3 of the light chain variable region sequence set forth as SEQ ID NO: 6 [ALK53]; or

(d) a H-CDR1, a H-CDR2, and a H-CDR3 of the heavy chain variable region sequence set forth as SEQ ID NO: 7, and a L-CDR1, a L-CDR2, and a L-CDR3 of the light chain variable sequence region set forth as SEQ ID NO: 8 [ALK58]; and

wherein the chimeric antigen receptor specifically binds to an extracellular domain of anaplastic lymphoma kinase.

2. The nucleic acid molecule of claim 1, wherein

(a) the H-CDR1, H-CDR2, and H-CDR3 comprise amino acids 26-33, 51-57, and 95-109 of SEQ ID NO: 1, respectively, and the L-CDR1, L-CDR2, and L-CDR3 comprise amino acids 27-37, 55-57, and 93-103 of SEQ ID NO: 2, respectively [ALK15];

(b) the H-CDR1, H-CDR2, and H-CDR3 comprise amino acids 26-33, 51-58, and 96-110 of SEQ ID NO: 3, respectively, and the L-CDR1, L-CDR2, and L-CDR3 comprise amino acids 27-36, 54-56, and 92-102 of SEQ ID NO: 4, respectively [ALK48];

(c) the H-CDR1, H-CDR2, and H-CDR3 comprise amino acids 26-33, 51-58, and 96-108 of SEQ ID NO: 5, respectively, and the L-CDR1, L-CDR2, and L-CDR3 comprise amino acids 27-37, 55-57, and 93-103 of SEQ ID NO: 6, respectively [ALK53]; or

(d) the H-CDR1, H-CDR2, and H-CDR3 comprise amino acids 26-33, 51-58, and 96-110 of SEQ ID NO: 7, respectively, and the L-CDR1, L-CDR2, and L-CDR3 comprise amino acids 27-32, 50-52, and 88-98 of SEQ ID NO: 8, respectively [ALK58].

3. The nucleic acid molecule of claim 1 or claim 2, wherein:

(a) the heavy chain variable region comprises or consists of the amino acid sequence set forth as SEQ ID NO: 1 or SEQ ID NO: 9;

(b) the heavy chain variable region comprises or consists of the amino acid sequence set forth as SEQ ID NO: 3 or SEQ ID NO: 11;

(c) the heavy chain variable region comprises or consists of the amino acid sequence set forth as SEQ ID NO: 5 or SEQ ID NO: 13; or

(d) the heavy chain variable region comprises or consists of the amino acid sequence set forth as SEQ ID NO: 7 or SEQ ID NO: 15.

5

4. The nucleic acid molecule of claim 1 or claim 2, wherein:

(a) the light chain variable region comprises or consists of the amino acid sequence set forth as SEQ ID NO: 2 or SEQ ID NO: 10;

10 (b) the light chain variable region comprises or consists of the amino acid sequence set forth as SEQ ID NO: 4 or SEQ ID NO: 12;

(c) the light chain variable region comprises or consists of the amino acid sequence set forth as SEQ ID NO: 6 or SEQ ID NO: 14; or

(d) the light chain variable region comprises or consists of the amino acid sequence set forth as SEQ ID NO: 8 or SEQ ID NO: 16.

15

5. The nucleic acid molecule of any of the preceding claims, wherein the heavy and light chain variable regions comprise or consist of the amino acid sequences set forth as

(a) SEQ ID NO: 1 and SEQ ID NO: 2, respectively;

(b) SEQ ID NO: 3 and SEQ ID NO: 4, respectively;

20 (c) SEQ ID NO: 5 and SEQ ID NO: 6, respectively;

(d) SEQ ID NO: 7 and SEQ ID NO: 8, respectively;

(e) SEQ ID NO: 9 and SEQ ID NO: 10, respectively;

(f) SEQ ID NO: 11 and SEQ ID NO: 12, respectively;

(g) SEQ ID NO: 13 and SEQ ID NO: 14, respectively; or

25 (h) SEQ ID NO: 15 and SEQ ID NO: 16, respectively.

6. The nucleic acid molecule of claim 1 or claim 2, wherein the heavy and light chain variable regions comprise human framework regions.

30 7. The nucleic acid molecule of any one of the preceding claims, wherein the antigen binding domain is a scFv.

8. The nucleic acid molecule of claim 7, wherein the scFv comprises or consists of the amino acid sequence set forth as SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, or SEQ ID NO: 24.

35

9. The nucleic acid molecule of any of the previous claims, wherein the transmembrane domain comprises or consists of the amino acid sequence set forth as SEQ ID NO: 27 or SEQ ID NO: 30

10. The nucleic acid molecule of any of the previous claims, wherein the T-cell signaling domain comprises or consists of a CD3 zeta signaling domain;

11. The nucleic acid molecule of any of the previous claims, wherein the chimeric antigen receptor comprises

- (a) a CD8 signaling domain;
- (b) a CD28 signaling domain;
- (c) a CD27 signaling domain
- (d) a CD154 signaling domain
- (e) a GITR (TNFRSF18) signaling domain
- (f) a OX40 (CD134) signaling domain;
- (g) a CD137 (4-1BB) signaling domain; or
- (h) a combination of two or more of (a) –(g)

12. The nucleic acid molecule of any of the previous claims, wherein the at least one T-cell signaling domain comprises, from N-terminus to C-terminus,

- (a) a CD3 zeta signaling domain;
 - (b) a CD28 signaling domain and a CD3 zeta signaling domain;
 - (c) a CD137 (4-1BB) signaling domain and a CD3 zeta signaling domain;
 - (d) an OX40 signaling domain and a CD3 zeta signaling domain;
 - (e) a CD28 signaling domain, a CD137 (4-1BB) signaling domain, and a CD3 zeta signaling domain;
- or
- (f) a CD28 signaling domain, an OX40 (CD134) signaling domain, and a CD3 zeta signaling domain.

13. The nucleic acid molecule of any of claims 10-12, wherein:

(a) the CD3 zeta signaling domain comprises or consists of the amino acid sequence set forth as SEQ ID NO: 34

(b) the CD8 signaling domain comprises or consists of the amino acid sequence set forth as SEQ ID NO: 31

(c) the CD28 signaling domain comprises or consists of the amino acid sequence set forth as SEQ ID NO: 28; or

(d) the CD137 signaling domain comprises or consists of the amino acid sequence set forth as SEQ ID NO: 32 or SEQ ID NO: 33.

14. The nucleic acid molecule of any of claims 1-13, wherein the transmembrane domain comprises or consists of the amino acid sequence set forth as SEQ ID NO: 27.

15. The nucleic acid molecule of any of claims 1-13, wherein the transmembrane domain comprises or consists of the amino acid sequence set forth as SEQ ID NO: 30.

16. The nucleic acid molecule of any of the prior claims, wherein the chimeric antigen receptor comprises, from N-terminus to C-terminus, the antigen binding domain, the transmembrane domain, and the at least one intracellular T-cell signaling domain.

5 17. The nucleic acid molecule of any of the preceding claims, wherein the chimeric antigen receptor further comprises a spacer domain C-terminal to the antigen binding domain and N-terminal to the transmembrane domain.

10 18. The nucleic acid molecule of claim 17, wherein the spacer domain comprises an immunoglobulin domain, optionally wherein the immunoglobulin domain comprises a CH2CH3 domain.

19. The nucleic acid molecule of claim 18, wherein the immunoglobulin domain comprises the amino acid sequence set forth as SEQ ID NO: 35.

15 20. The nucleic acid molecule of any of the prior claims, wherein the chimeric antigen receptor further comprises a signal peptide N-terminal to the antigen binding domain.

21. The nucleic acid molecule of claim 1, wherein the chimeric antigen receptor comprises or consists of the amino acid sequence set forth as SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, or SEQ ID NO: 90.

22. The nucleic acid molecule of any of the prior claims, codon optimized for expression in a human T cell.

23. The nucleic acid molecule of claim 1, comprising or consisting of the nucleic acid sequence set forth as SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, or SEQ ID NO: 114.

24. The nucleic acid molecule of any one of the prior claims, operably linked to an expression control sequence.

25. A vector comprising the nucleic acid molecule of any one of claims 1-24.

26. The vector of claim 25, wherein the vector is a recombinant DNA expression vector.

27. The vector of claim 25, wherein the vector is a viral vector.

28. The vector of claim 27, wherein the viral vector is a lentiviral vector

29. The vector of any one of claims 25-28, for use in making a chimeric antigen receptor T-cell.

30. A polypeptide comprising the chimeric antigen receptor encoded by the nucleic acid molecule of any one of claims 1-24.

31. A host cell, comprising the nucleic acid molecule or vector of any one of claims 1-29.

32. The host cell of claim 31, wherein the host cell is a T cell.

33. A composition, comprising an effective amount of the nucleic acid molecule, vector, polypeptide, or host cell of any one of claims 1-32, and a pharmaceutically acceptable carrier.

34. A method of making a chimeric antigen receptor T-cell comprising:
transducing a T cell with the vector of any one of claims 25-29, thereby making the chimeric antigen receptor T cell.

35. A method of treating a subject with a tumor, comprising:
administering to the subject a therapeutically effective amount of host cells expressing the chimeric antigen receptor encoded by the nucleic acid molecule of any of claims 1-24, under conditions sufficient to form an immune complex of the antigen binding domain on the chimeric antigen receptor and the extracellular domain of anaplastic lymphoma kinase in the subject.

36. The method of claim 35, wherein the host cells are T cells from the subject that have been transformed with the nucleic acid molecule encoding the chimeric antigen receptor or transduced with a vector comprising the nucleic acid molecule.

37. The method of claim 36, further comprising the steps of:
obtaining the T cells from the subject, and
transforming the T cells with the nucleic acid molecule encoding the chimeric antigen receptor or transducing the T cells with a vector comprising the nucleic acid molecule.

38. The method of any of claims 35-37, wherein the tumor comprises cell surface expression of anaplastic lymphoma kinase.

39. The method of any of claims 35-38, wherein the tumor is a neuroblastoma, a rhabdomyosarcoma, or a glioblastoma.

40. The method of any of claims 35-39, wherein the tumor does not comprise an anaplastic lymphoma kinase fusion protein.

41. The method of any of claims 35-40, further comprising administering to the subject a therapeutically effective amount of a chemotherapeutic agent.

42. The method of claim 41, wherein the chemotherapeutic agent comprises an anaplastic lymphoma kinase inhibitor, particularly wherein the anaplastic lymphoma kinase inhibitor comprises crizotinib.

43. The method of any of claims 35-42, wherein treating the tumor comprises a reduction in tumor burden.

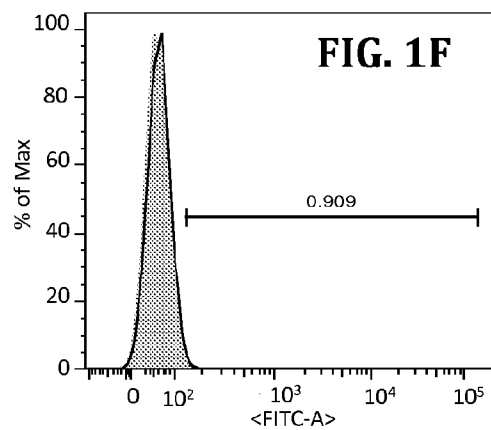
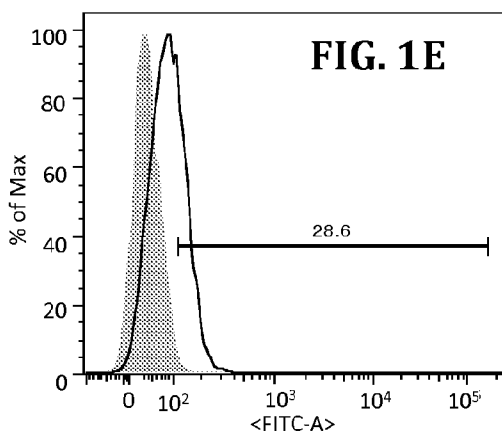
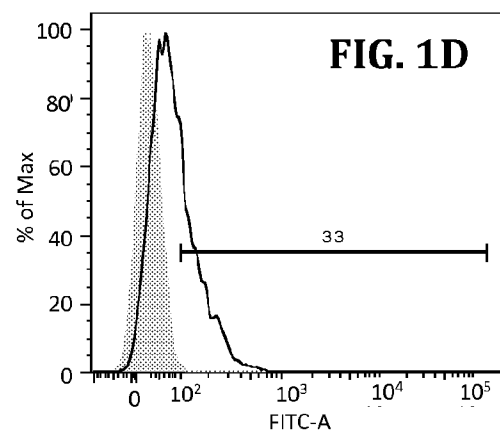
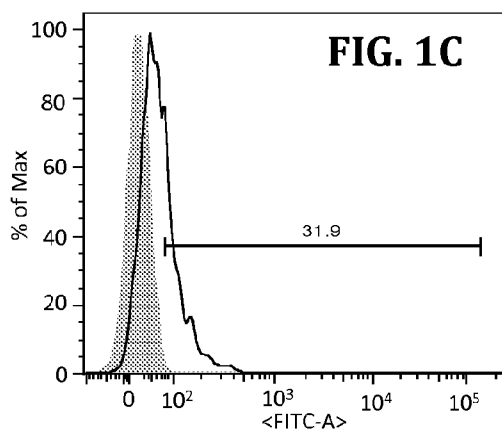
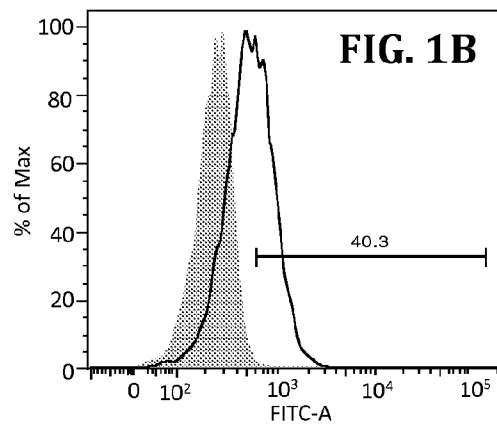
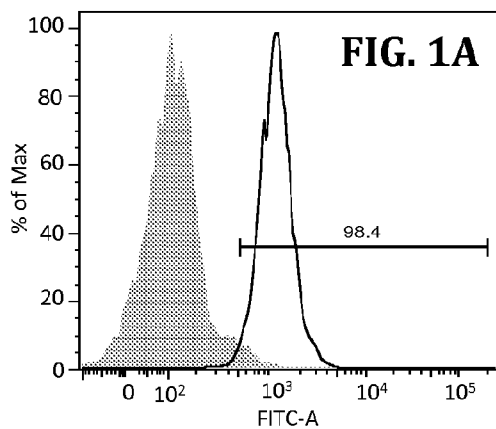
44. The method of any one of claims 35-43, further comprising selecting the subject for treatment.

45. The method of claim 44, wherein selecting the subject comprises detecting cell-surface expression of anaplastic lymphoma kinase on the tumor.

46. A kit for making a chimeric antigen receptor T-cell or treating a tumor in a subject, comprising a container comprising the nucleic acid molecule, vector, host cell, or composition of any of claims 1-29 or 31-33, and instructions for using the kit.

47. Use of the nucleic acid molecule, vector, host cell, or composition of any of claims 1-29 or 31-33, to treat a tumor in a subject.

48. Use of the nucleic acid molecule or vector of any of claims 1-29, to make a chimeric antigen receptor T cell.



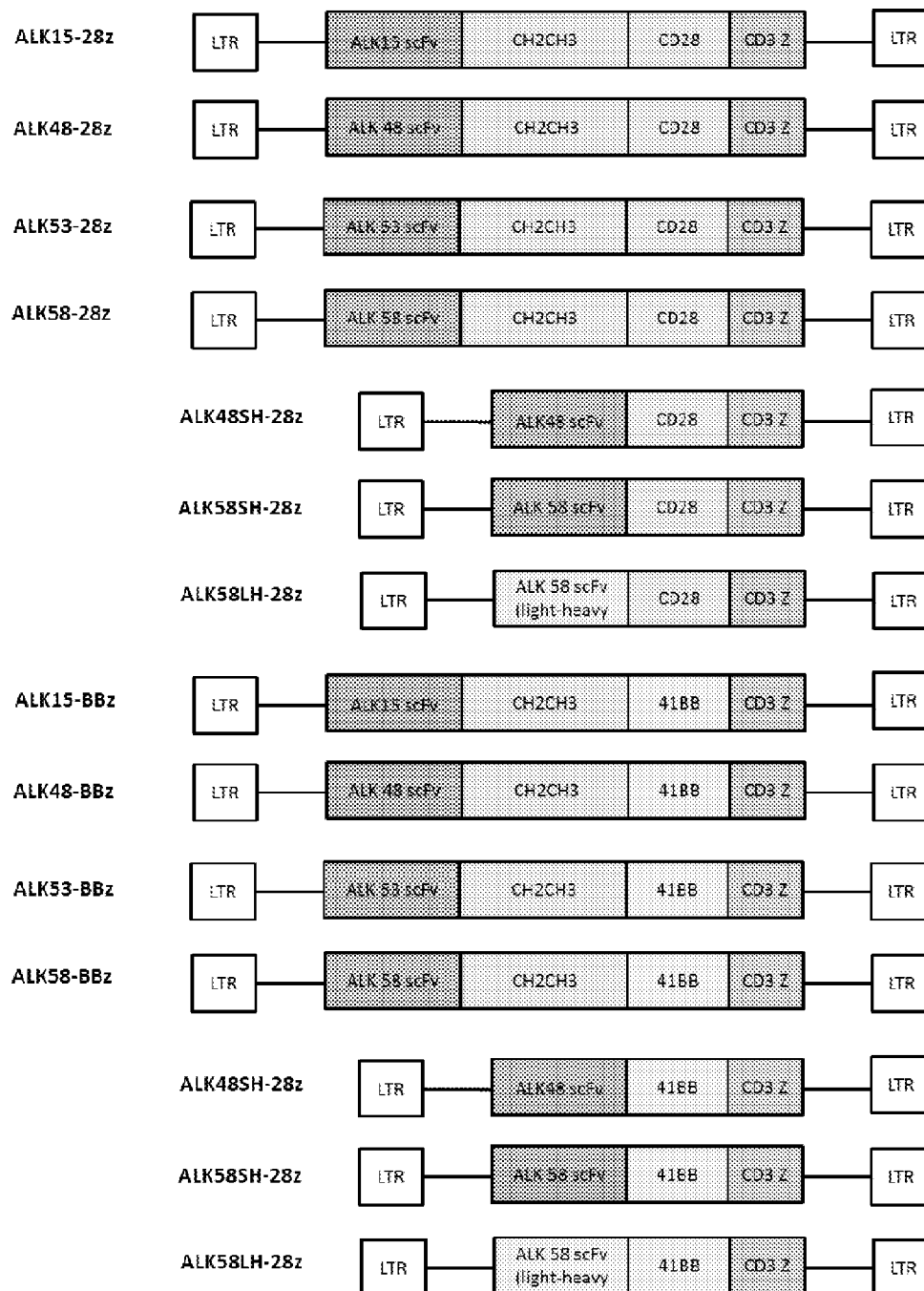


FIG. 2

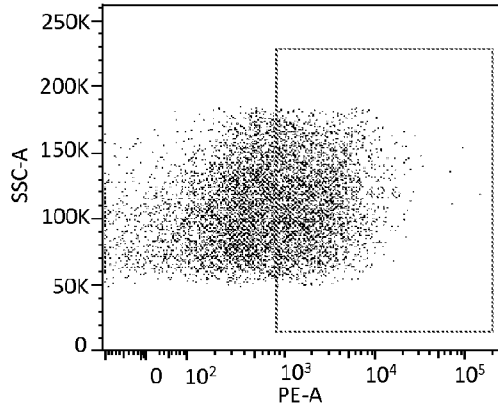
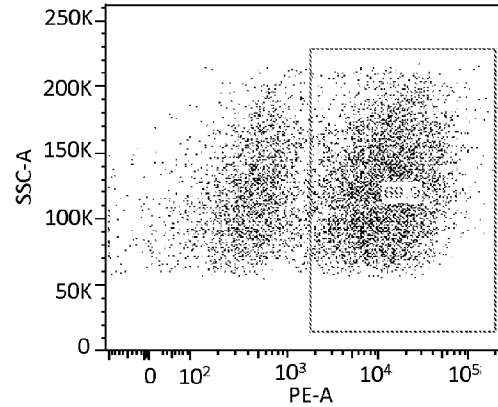
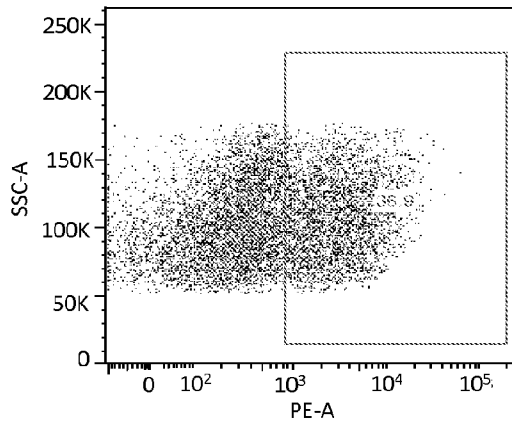
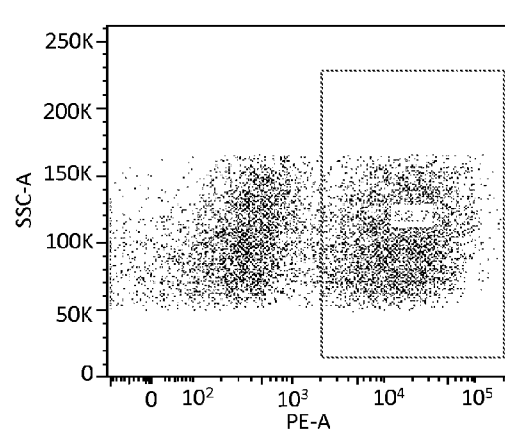
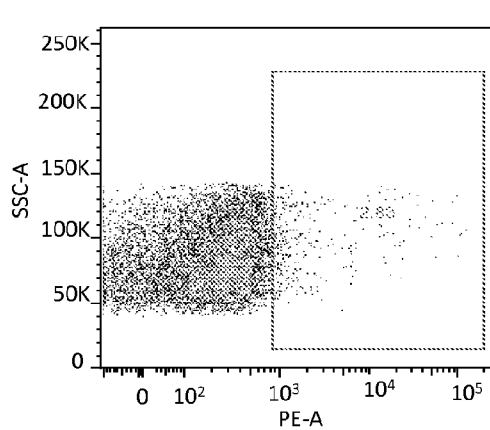
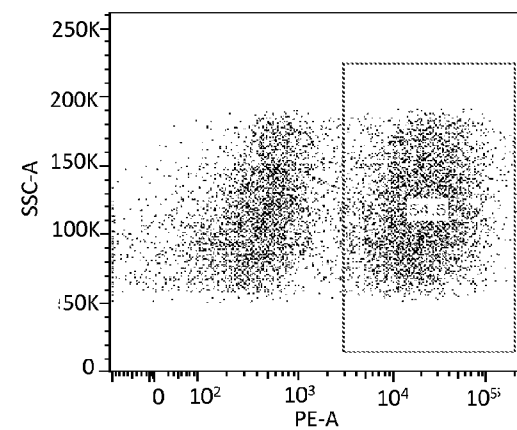
FIG. 3A. ALK48: 42.6%**FIG. 3D. ALK58: 69.9%****FIG. 3B. ALK48SH: 36.9%****FIG. 3E. ALK58SH: 52.7%****FIG. 3C. Mock: 2.83%****FIG. 3F. ALK58LH: 54.5%**

FIG. 4A LAN5

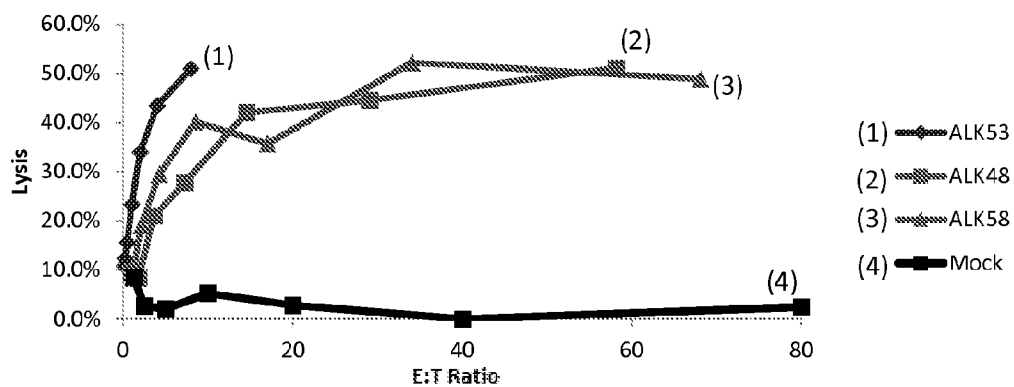


FIG. 4B Rh18

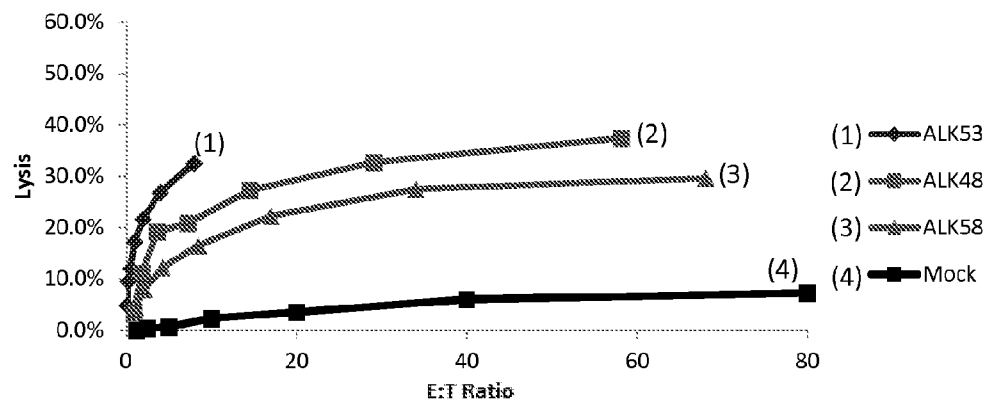


FIG. 4C K562

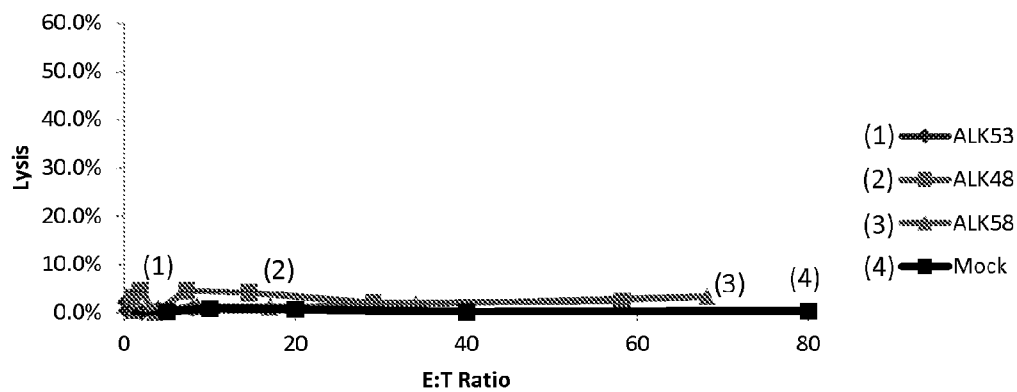


FIG. 5A LAN5

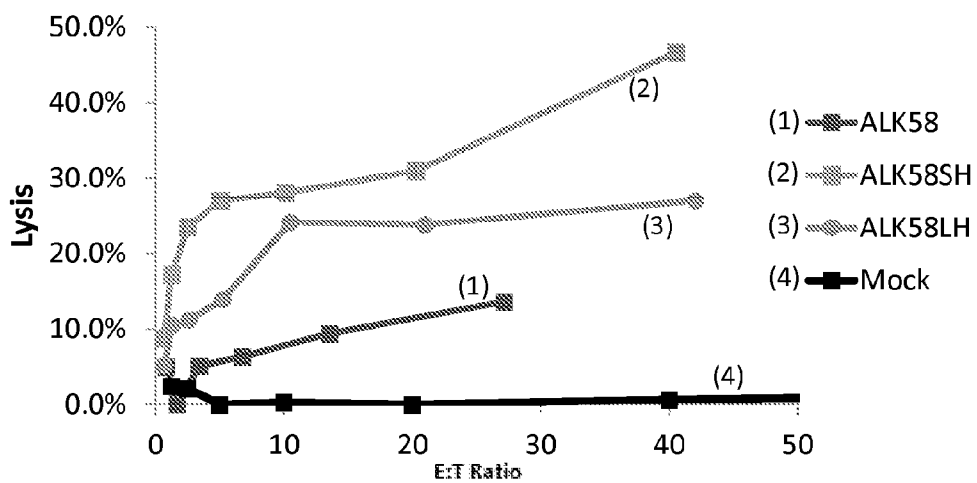
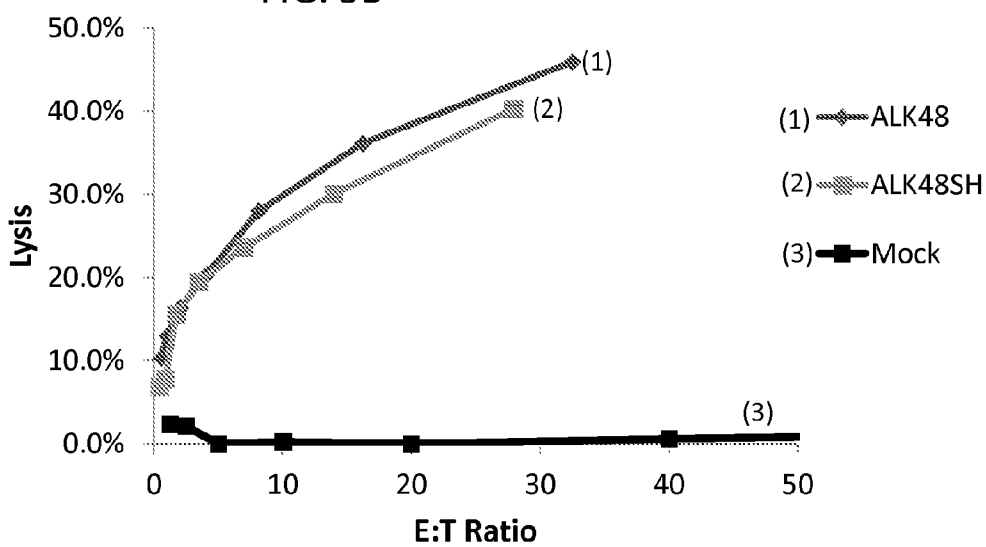
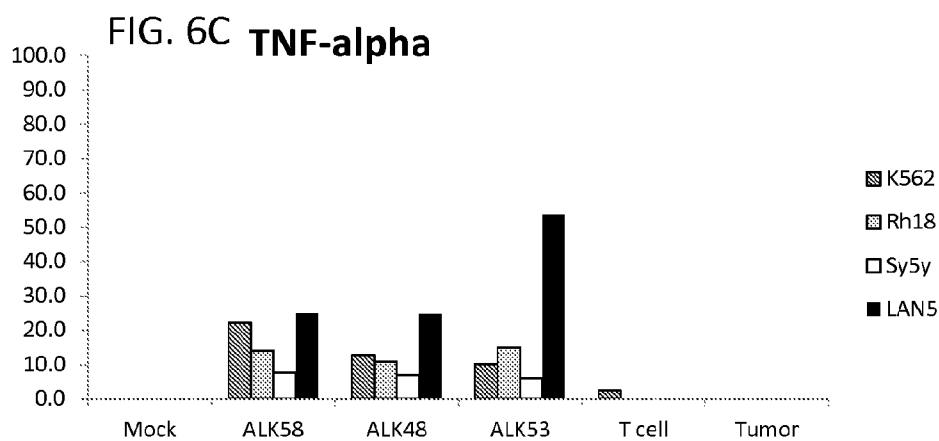
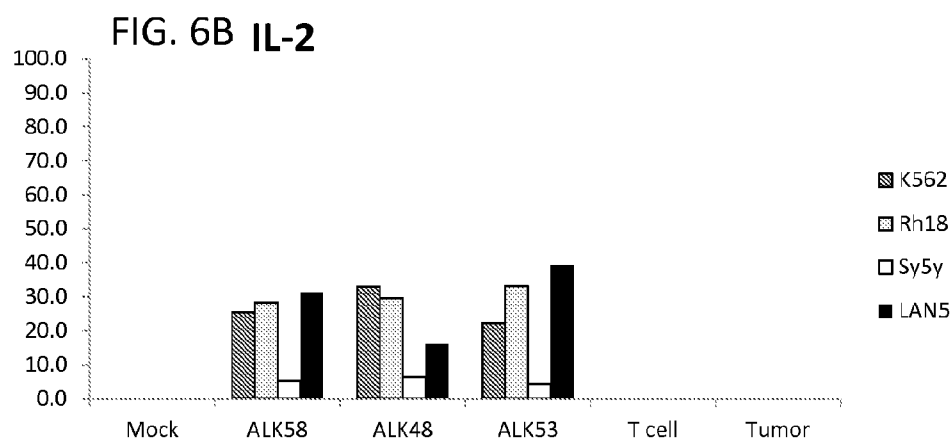
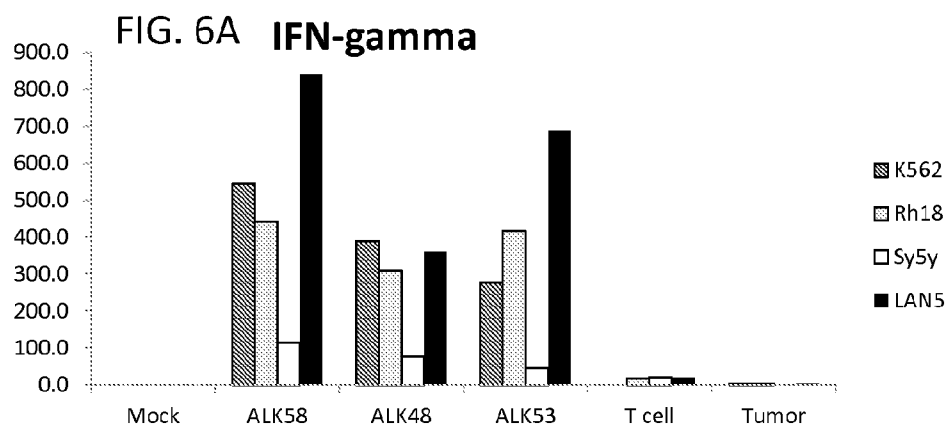
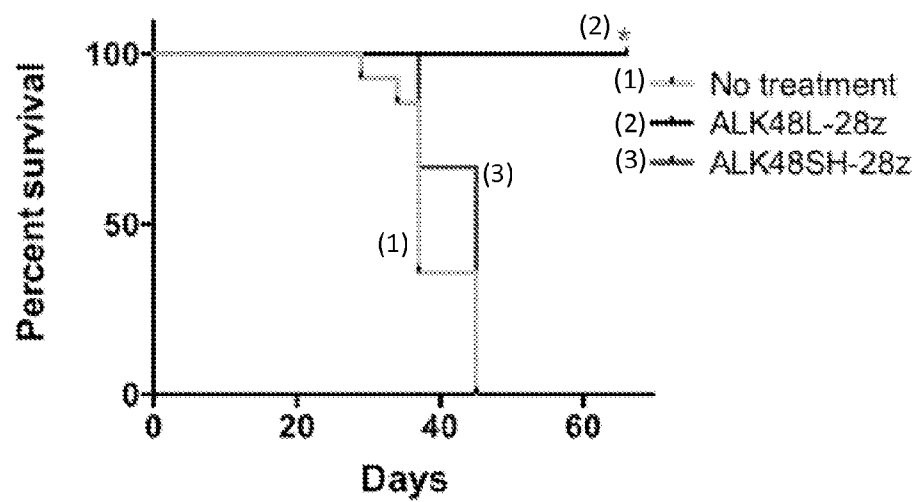


FIG. 5B LAN5







* mice euthanized due to strong GVHD symptoms
All mice recieved IL-7

FIG. 7