our anti-ALPPL2 human monoclonal antibody M25 and showed that M25 ADCs potently inhibited tumor cell proliferation in vitro and mesothelioma cell line xenograft growth in vivo (Su et al, 2020).

BRIEF SUMMARY

[0002a] In one aspect, the present disclosure refers to an antibody comprising a variable region that specifically binds to ALPPL2 and ALPP, wherein the antibody comprises a heavy chain variable region and a light chain variable region,

wherein complementarity determining region (CDR) 1, CDR2 and CDR3 of the heavy chain variable region are selected from the group consisting of:

SEQ ID NOs: 20, 21, and 22;
SEQ ID NOs: 23, 24, and 25;
SEQ ID NOs: 26, 27, and 28;
SEQ ID NOs: 29, 30, and 31;
SEQ ID NOs: 32, 33, and 34;
SEQ ID NOs: 35, 36, and 37;
SEQ ID NOs: 38, 39, and 40;
SEQ ID NOs: 41, 42, and 43;
SEQ ID NOs: 44, 45, and 46;
SEQ ID NOs: 47, 48, and 49; or
SEQ ID NOs: 50, 51, and 52; and/or

wherein CDR1, CDR2, and CDR3 of the light chain variable region are selected from the group consisting of:

SEQ ID NOs: 53, 54, and 55;
SEQ ID NOs: 56, 57, and 58;
SEQ ID NOs: 59, 60, and 61;
SEQ ID NOs: 62, 63, and 64;
SEQ ID NOs: 65, 66, and 67;
SEQ ID NOs: 68, 69, and 70;
SEQ ID NOs: 71, 72, and 73; or
SEQ ID NOs: 74, 75, and 76,

wherein the antibody does not have the heavy chain variable region of M25FYIA and the light chain variable region of M25FYIA.

[0003] In some examples, the disclosure provides an antibody comprising a variable region that specifically binds to ALPPL2 and ALPP. In some examples, the antibody comprises a heavy chain variable region and a light chain variable region, wherein complementarity determining region (CDR) 1, CDR2 and CDR3 of the heavy chain variable region are selected from the following sets:

SEQ ID NOs: 20, 21, and 22;

SEQ ID NOs: 23, 24, and 25;

SEQ ID NOs: 26, 27, and 28;

SEQ ID NOs: 29, 30, and 31;

SEQ ID NOs: 32, 33, and 34;

SEQ ID NOs: 35, 36, and 37;

SEQ ID NOs: 38, 39, and 40;

SEQ ID NOs: 41, 42, and 43;

SEQ ID NOs: 44, 45, and 46;

SEQ ID NOs: 47, 48, and 49; or

SEQ ID NOs: 50, 51, and 52;

CDR1, CDR2, and CDR3 of the light chain variable region are selected from the following sets:

SEQ ID NOs: 53, 54, and 55;

SEQ ID NOs: 56, 57, and 58;

SEQ ID NOs: 59, 60, and 61;

SEQ ID NOs: 62, 63, and 64;

SEQ ID NOs: 65, 66, and 67;

SEQ ID NOs: 68, 69, and 70;

SEQ ID NOs: 71, 72, and 73; or

SEQ ID NOs: 74, 75, and 76,

with the proviso that the antibody does not have the heavy chain variable region of M25FYIA and the light chain variable region of M25FYIA.

[0004] In some examples, CDR1, CDR2, and CDR3 of the heavy chain variable region comprise SEQ ID NO: 23, SEQ ID NO: 24, and SEQ ID NO: 25, respectively; and CDR1, CDR2, and CDR3 of the light chain variable region comprise SEQ ID NO: 56, SEQ ID NO: 57, and SEQ ID NO: 58, respectively; or

CDR1, CDR2, and CDR3 of the heavy chain variable region comprise SEQ ID NO: 26, SEQ ID NO: 27, and SEQ ID NO: 28, respectively; and CDR1, CDR2, and CDR3 of the light chain variable region comprise SEQ ID NO: 56, SEQ ID NO: 57, and SEQ ID NO: 58, respectively; or

CDR1, CDR2, and CDR3 of the heavy chain variable region comprise SEQ ID NO: 44, SEQ ID NO: 45, and SEQ ID NO: 46, respectively; and CDR1, CDR2, and CDR3 of the light chain variable region comprise SEQ ID NO: 71, SEQ ID NO: 72, and SEQ ID NO: 73, respectively; or

CDR1, CDR2, and CDR3 of the heavy chain variable region comprise SEQ ID NO: 38, SEQ ID NO: 39, and SEQ ID NO: 40, respectively; and CDR1, CDR2, and CDR3 of the light chain variable region comprise SEQ ID NO: 65, SEQ ID NO: 66, and SEQ ID NO: 67, respectively; or

CDR1, CDR2, and CDR3 of the heavy chain variable region comprise SEQ ID NO: 41, SEQ ID NO: 42, and SEQ ID NO: 43, respectively; and CDR1, CDR2, and CDR3 of the light chain variable region comprise SEQ ID NO: 53, SEQ ID NO: 54, and SEQ ID NO: 55, respectively; or

CDR1, CDR2, and CDR3 of the heavy chain variable region comprise SEQ ID NO: 35, SEQ ID NO: 36, and SEQ ID NO: 37, respectively; and CDR1, CDR2, and CDR3 of the light chain variable region comprise SEQ ID NO: 59, SEQ ID NO: 60, and SEQ ID NO: 61, respectively.

[0005] In some examples, the heavy chain variable region is selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11. In some examples, the light chain variable region is selected from the group consisting of SEQ ID NO: 12, 13, 14, 15, 16, 17, 18, and 19.

[0006] In some examples, the heavy chain variable region comprises SEQ ID NO: 1; and the light chain variable region comprises SEQ ID NO: 12; or

the heavy chain variable region comprises SEQ ID NO: 2; and the light chain variable region comprises SEQ ID NO: 13; or

the heavy chain variable region comprises SEQ ID NO: 3; and the light chain variable region comprises SEQ ID NO: 13; or

the heavy chain variable region comprises SEQ ID NO: 9; and the light chain variable region comprises SEQ ID NO: 18; or

the heavy chain variable region comprises SEQ ID NO: 1; and the light chain variable region comprises SEQ ID NO: 12; or

the heavy chain variable region comprises SEQ ID NO: 7; and the light chain variable region comprises SEQ ID NO: 16; or

the heavy chain variable region comprises SEQ ID NO: 8; and the light chain variable region comprises SEQ ID NO: 12; or

the heavy chain variable region comprises SEQ ID NO: 6; and the light chain variable region comprises SEQ ID NO: 14.

[0007] In some examples, the antibody is an IgG, IgA or IgE antibody. In some examples, the antibody is an IgG antibody. In some examples, the antibody is an IgG1, IgG2, IgG3, or IgG4 antibody.

[0008] In some examples, the antibody is a monospecific antibody. In some examples, the antibody is linked to a cytotoxic agent. In some examples, the cytotoxic agent is a radionucleotide.

[0009] In some examples, the antibody is a bi-specific antibody comprising a second variable region that specifically binds to a second target protein, wherein the antibody comprises a second heavy chain variable region and a second light chain variable region. In

some examples, the second target protein is expressed on the surface of a human immune effector cell. In some examples, the second target protein is human CD3. In some examples, the CDR1, CDR2, and CDR3 of the second heavy chain variable region comprise SEQ ID NO: 79, SEQ ID NO: 80 and SEQ ID NO: 81 and the CDR1, CDR2, and CDR3 of the second light chain variable region comprise SEQ ID NO: 82, SEQ ID NO: 83 and SEQ ID NO: 84. In some examples, the second heavy chain variable region comprises SEQ ID NO: 77 and the second light chain variable region comprises SEQ ID NO:78. In some examples, the bi-specific antibody comprises SEQ ID NO:85 and SEQ ID NO:86. In some examples, the bi-specific antibody comprises SEQ ID NO:87 and SEQ ID NO:88.

[0010] In another aspect, there is also provided a pharmaceutical composition comprising any antibody as described above or elsewhere herein.

[0010a] In another aspect, there is provided a nucleic acid encoding the antibody as defined above, or a vector comprising said nucleic acid sequence.

[0011] Also provided is a nucleic acid encoding any antibody as described above or elsewhere herein.

[0012] Also provided is a vector comprising the nucleic acid sequence as described above or elsewhere herein.

[0012a] In another aspect, there is provided a cell comprising the nucleic acid or vector as defined above, wherein the cell is optionally a mammalian cell.

[0013] Also provided is a cell comprising the nucleic acid as described above or elsewhere herein or the vector as described above or elsewhere herein. In some examples, the cell is a mammalian cell.

[0013a] In another aspect, there is provided a method for producing an antibody, the method comprising culturing a cell comprising a nucleic acid sequence encoding the antibody as defined above under conditions to allow for production of the antibody.

[0014] Also provided is a method for producing an antibody, the method comprising culturing the cell as described above or elsewhere herein under conditions to allow for production of the antibody.

[0014a] In another aspect, there is provided a method of killing a cancer cell, the method comprising, contacting the antibody as defined above to a cancer cell; (A) optionally wherein the antibody is a bi-specific antibody comprising a second variable region that specifically binds to human CD3, wherein the antibody comprises a second heavy chain variable region and a second light chain variable region, and wherein the cancer cell is brought in proximity to a peripheral blood mononuclear cell (PBMC) or T-cell expressing CD3 by binding of the antibody; optionally wherein the CDR1, CDR2, and CDR3 of the second heavy chain variable region comprise SEQ ID NO: 79, SEQ ID NO: 80 and SEQ ID NO: 81 and the CDR1, CDR2, and CDR3 of the second light chain variable region comprise SEQ ID NO: 82, SEQ ID NO: 83 and SEQ ID NO: 84, optionally wherein the second heavy chain variable region comprises SEQ ID NO: 77 and the second light chain variable region comprises SEQ ID NO:78, optionally wherein the antibody comprises SEQ ID NO:85 and SEQ ID NO:86, or optionally wherein the antibody comprises SEQ ID NO:87 and SEQ ID NO:88, or (B) optionally wherein the antibody is linked to a cytotoxic agent wherein the cytotoxic agent is optionally a radionucleotide, microtubule inhibitor, DNA-damaging agent, a polymerase inhibitor, auristatin, Dolastatin-10, maytansine or a maytansine derivative, Mertansine (DM1), DM3, or DM4, auristatin, auristatin E (AE), auristatin EB (AEB), auristatin EFP (AEFP), monomethyl auristatin D (MMAD) or monomethyl dolastatin 10, monomethyl auristatin F (MMAF), N-methylvaline-valine-dolaisoleucine-dolaproline-phenylalanine, monomethyl auristatin E (MMAE), N-methylvaline-valine-dolaisoleucine-dolaproline-norephedrine, 5-benzoyl valeric acid-AE ester (AEVB), vcMMAE, or vcMMAF.

[0015] Also provided is a method of killing a cancer cell, the method comprising, contacting the antibody as described above or elsewhere herein to a cancer cell. In some examples, the antibody is a bi-specific antibody comprising a second variable region that specifically binds to a second target protein, wherein the second target protein is human CD3, wherein the antibody comprises a second heavy chain variable region and a second light chain variable region, and wherein the cancer cell is brought in proximity to a peripheral blood mononuclear cell (PBMC) expressing CD3 by binding of the antibody. In some examples, the PBMC is a T-cell. In some examples, the cancer cell is a mesothelioma cell, a testicular cancer cell, an endometrial cancer cell, a pancreatic cancer

cell, an ovarian cancer cell, a non-small cell lung cancer cell, a gastric cancer cell, or a colon cancer cell.

[0016] In some examples, the CDR1, CDR2, and CDR3 of the second heavy chain variable region comprise SEQ ID NO: 79, SEQ ID NO: 80 and SEQ ID NO: 81 and the CDR1, CDR2, and CDR3 of the second light chain variable region comprise SEQ ID NO: 82, SEQ ID NO: 83 and SEQ ID NO: 84. In some examples, the second heavy chain variable region comprises SEQ ID NO: 77 and the second light chain variable region comprises SEQ ID NO:78 In some examples, the antibody comprises SEQ ID NO:85 and SEQ ID NO:86. In some examples, the antibody comprises SEQ ID NO:87 and SEQ ID NO:88.

[0017] In some examples, the antibody is linked to a cytotoxic agent. In some embodiments, the cytotoxic agent is a radionucleotide.

[0018] In some examples, the cancer cell is in a human having the cancer cell and the antibody is administered to the human, thereby killing the cancer cell.

[0018a] In another aspect, there is provided a chimeric antigen receptor (CAR)-expressing human cell, optionally a T-cell, natural killer cell or a macrophage, wherein the CAR comprises the heavy chain variable region and the light chain variable region of the antibody as defined above.

[0019] Also provided is a chimeric antigen receptor (CAR)-expressing human cell, wherein the CAR comprises the heavy chain variable region and the light chain variable region as described above or elsewhere herein. In some examples, the human cell is a T-cell, natural killer cell or a macrophage.

[0020] Also provided is a method of detecting a tumor cell in a sample, the method comprising contacting an antibody as described above or elsewhere herein to the sample; and detecting specific binding of the antibody to the sample.

[0020a] In another aspect, the present disclosure refers to a method of detecting a tumor cell in a sample, the method comprising contacting the antibody as defined above to the sample; and detecting specific binding of the antibody to the tumor cell.

[0020b] In another aspect, there is provided the use of antibody as defined above, the pharmaceutical composition as defined above, or the CAR-expressing human cell as defined above in the manufacture of a medicament for the treatment of cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] FIG. 1. Biolayer interferometry (BLI) measurements of the affinity of FYIA, FYIA_germ, FYIA_germ_6-6, and FYIA_germopt (aka SYLY) Fabs for human ALPPL2. Tips were loaded with Fabs, followed by an association step with 5 nM human ALPPL2-Fc then a dissociation step. Calculated affinities by curve fitting are 8.6 nM for FYIA, 13.0 nM for FYIA_germ, 0.88 nM for FYIA_germ_6-6, and 0.19 nM for FYIA_germopt.

[0022] FIG. 2. Binding of FYIA, FYIA_germ, and FYIA_germopt Fabs to living M28 cells. M28 cells were incubated with Fabs for 1h at RT and binding was analyzed by flow cytometry. Calculated affinities by curve fitting are 30.55 nM for FYIA, 69.41 nM for FYIA_germ, and 0.97 nM for FYIA_germopt.

bispecific T cell engager. The original M25 antibody is fully human in sequence and binds to ALPPL2 expressing cells with sub nM to low nM apparent binding affinity in the IgG1 form (Su et al, 2020). The monovalent binding of M25 single chain fragment variable (scFv) or Fab to ALPPL2 is moderate/low. We have previously performed affinity maturation study and identify a high affinity variant of M25, M25FYIA (aka FYIA), with over 100-fold improvement in monovalent binding to ALPPL2 over the parental M25 (Su et al, 2020; Liu et al, 2017). Most importantly, FYIA retains high specificity, binding only to ALPPL2/ALPP but not the closely related ALPI (Su, Y., *et al*. *Cancer Res*., 2020 Aug 31; WO2017095823).

[0250] In this study, in the context of bispecific T cell engager development, FYIA was further optimized to increase monovalent binding affinity to ALPPL2 while improving developability characteristics (avoiding charged or hydrophobic patches and removal of deamidation and isomerization motifs). The framework regions of FYIA were modified to match germline sequences (IGHV3-23*04 and IGLV2-14*01) to create FYIA_germ. Using FYIA_germ as a starting point, a combination of site-directed and error-prone PCR mutagenesis was used to generate clones that were selected or screened for improved binding to human ALPPL2 and no binding to human ALPI. A panel of affinity- and developability-optimized human antibodies was identified (VH and VL sequences are shown in Tables 1 and 2, respectively). The lead antibody in this panel, FYIAgermopt aka SYLY, was selected for bispecific antibody construction.

Table 1: Heavy chain variable sequences

SEQ ID NO:	Name	VH sequence	CDR SEQ ID NO:	CDR (1, 2, 3, respectively)
1	FYIA germ_HC	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKEGDSSRWSYDLWGRGTLVTVSS	20 21 22	AASGFTFSSYAMH VISYDGSNKY AKEGDSSRWSYDL

2	FYIA germ _SYL Y	EVQLVESGGGLVQPGGSLRLSCAASGFTF SAYAMHWVRQAPGKGLEWVAVISYEGYNA YYADSVKGRFTISRDNSKNTLYLQMNSLR AEDTAVYYCAKEGDYSRWAYDYWGRGTLV TVSS	23 24 25	AASGFTFSAYAMH VISYEGYNAY AKEGDYSRWAYDY
3	FYIA germ _SY	EVQLVESGGGLVQPGGSLRLSCAASGFTF SAYAMHWVRQAPGKGLEWVAVISYEGYNA YYADSVKGRFTISRDNSKNTLYLQMNSLR AEDTAVYYCAKEGDYSRWAYDLWGRGTLV	26 27 28	AASGFTFSAYAMH VISYEGYNAY AKEGDYSRWAYDL

Table 2: Light chain variable sequences

SEQ ID NOS:	Name	VL sequence	CDR SEQ ID NO:	CDR (1, 2, 3, respectively)
12	FYIAgerm_LC	QSALTQPASVSGSPGQSITISCTG TSSDVGGYNYVSWYQQHPGKAPKL MIYDVTNRPSGVSNRFSGSKSGNT ASLTISGLQAEDEADYYCSSYTIA STLVVFGGGTKLTVL	53 54 55	TGTSSDVGGYNYVS YDVTNRPS SSYTIASTLVV
13	FYIAgerm_SYLY_LC	QSALTQPASVSGSPGQSITISCTG TSSDVGGYNYVSWYQQHPGKAPKL MIYDVTNYPSGVSNRFSGSKSGNT ASLTISGLQAEDEADYYCSSYTVA TTLVVFGGGTKLTVL	56 57 58	TGTSSDVGGYNYVS YDVTNYPS SSYTVATTLVV
14	FYIAgerm_LC2	QSALTQPASVSGSPGQSITISCTG TSSDVGGYNYVSWYQQHPGKAPKL MIYDVTNRPSGVSNRFSGSKSGNT ASLTISGLQAEDEADYYCSSYTIA STPVVFGGGTKLTVL	59 60 61	TGTSSDVGGYNYVS YDVTNRPS SSYTIASTPVV
15	FYIAgerm_LC3	QSALTQPASVSGSPGQSITISCTG TSSDVGGYNYVSWYQQHPGKAPKL MIYDVTNRPSGVSNRFSGSKSGNT ASLTISGLQAEDEADYYCSSYTIA STFVVFGGGTKLTVL	62 63 64	TGTSSDVGGYNYVS YDVTNRPS SSYTIASTFVV
16	FYIAgerm_LC4	QSALTQPASVSGSPGQSITISCTG TSSDVGGYNYVSWYQQHPGKAPKL MIYDVTNRPSGVSNRFSGSKSGNT ASLTISGLQAEDEADYYCSSYAIA STLVVFGGGTKLTVL	65 66 67	TGTSSDVGGYNYVS YDVTNRPS SSYAIASTLVV
17	FYIAgerm_LC5	QSALTQPASVSGSPGQSITISCTG TSSDVGGYNYVSWYQQHPGKAPKL MIYDVTDRPSGVSNRFSGSKSGNT ASLTISGLQAEDEADYYCSSYTIA STLVVFGGGTKLTVL	68 69 70	TGTSSDVGGYNYVS YDVTDRPS SSYTIASTLVV
18	FYIAgerm_LC6	QSALTQPASVSGSPGQSITISCTG TSSDVGGYNYVSWYQQHPGKAPKL MIYDVTDRPSGVSNRFSGSKSGNT ASLTISGLQAEDEADYYCSSYAIA STLVVFGGGTKLTVL	71 72 73	TGTSSDVGGYNYVS YDVTDRPS SSYAIASTLVV

19	FYIAgerm_LC7	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVTDRPSGVSNRFSGSKSGNTASLTISGLQAEDEADYYCSSYAIASTPVVFGGGTKLTVL	74 75 76	TGTSSDVGGYNYVS YDVTDRPS SSYAIASTPVV

	TAC-G4S-antiCD3VL-CL (CL underlined)	SLTACDTAVYYCAKEGDYSRWAYDYWGR GTLVTVSSGGGGSDIQMTQSPSSLSASV GDRVTITCRSSTGAVTTSNYANWVQQKP GKAPKGLIGGTNKRAPGVPSRFSGSLIG DKATLTISSLQPEDFATYYCALWYSNLW VFGQGTKVEIKRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLT LSKADYEKHKVYACEVTHQGLSSPVTKS FNRGEC
87	SYLY-based diabody-CH1/CL (without disulfide stabilization): Chain 1: antiCD3VH-G4S-FYIA_germoptVL-CH1 (CH1 underlined)	EVQLVESGGGLVQPGGSLRLSCAASGFT FNTYAMNWVRQAPGKGLEWVGRIRSKYN NYATYYADSVKDRFTISRDDSKNSLYLQ MNSLKTEDTAVYYCVRHGNFGNSYVSWF AYWGQGTLVTVSSGGGGSQSALTQPASV SGSPGQSITISCTGTSSDVGGYNYVSWY QQHPGKAPKLMIYDVTYRPSGVSNRFSG SKSGNTASLTISGLQAEDEADYYCSSYT VATTLVVFGGGTKLTVLASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVD KKVEPKSC
88	SYLY-based diabody-CH1/CL (without disulfide stabilization): Chain 2: FYIA_germoptVH-G4S-antiCD3VL-CL (CL underlined)	MGWSCIILFLVATATGVHSEVQLVESGG GLVQPGGSLRLSCAASGFTFSAYAMHWV RQAPGKGLEWVAVISYEGYNAYYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAV YYCAKEGDYSRWAYDYWGRGTLVTVSSG GGGSDIQMTQSPSSLSASVGDRVTITCR SSTGAVTTSNYANWVQQKPGKAPKGLIG GTNKRAPGVPSRFSGSLIGDKATLTISS LQPEDFATYYCALWYSNLWVFGQGTKVE IKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC

A hexahistidine tag (SEQ ID NO: 102) was added to the C terminal of CH1 for purification by metal affinity chromatography using nickel-charged affinity resin (Ni-NTA agarose). DSDbody was produced in HEK293A or ExpiCHO cells by transient transfection, purified by NI-NTA and analyzed by reducing SDS-PAGE (FIG. 11). The bispecific can also be constructed from a variant of DSDbody without the interchain disulfide bond (sequences shown in Table 6).

[0254] Biolayer interferometry analysis showed that the SYLY-based bispecific ALPPL2 x CD3 DSDbody binds to human ALPPL2 with high affinity (KD ~ 0.2 nM) and specificity (no binding to human ALPI) (FIG. 12). The DSDbody was also evaluated by biolayer

cells in the presence of human PBMC at E:T ratio = 10:1 for 72h, followed by cell viability assessment by Calcein AM. As shown in FIG. 20, the calculated EC50 for the SYLY DSDbody is 8.5 pM, and > 100 nM for the control bispecific YSC10 x CD3 DSDbody. No killing was observed on HEK293 cells that do not express the target antigen (ALPP or ALPPL2, FIG. 21).

[0259] The DSDbody showed excellent thermal stability. In the thermal induced aggregation assay, the FYIA-based ALPPl2 x CD3 DSDbody showed an aggregation temperature (Tagg) of 65 ^{o}C (FIG. 22), and the SYLY-based ALPPL2 x CD3 DSDbody showed an even higher Tagg of 70 ^{o}C (FIG. 23).

Example 2

[0260] This example shows the effect of antibodies described herein on a reporter-expressing pancreatic cancer line, AsPC1.

[0261] The bispecific (ALPPL2 x CD3) SYLY DSDBody was incubated with AsPC1 cells at room temperature for 1h, washed and further incubated with anti-tag (hexahistidine (SEQ ID NO: 102)) antibodies for detection by flow cytometry. MFI values were curve-fitted with the K_D estimated as 4.35 ± 0.68 nM. See, FIG. 24.

[0262] An *in vitro* cyto tox assay using a reporter expressing cancer cell line was performed as follows. Firefly luciferase expressing AsPC1-luc cells were plated into 96-well tissue culture plate at 3,000 cell/well and cultured overnight. 30,000 cell/well of PBMC were added to target cells (E:T ratio = 10:1). Antibodies were serially diluted and incubated for 72 hours. Cell viability was measured by bioluminescence using a microtiter plate reader. The percent of cell viability was derived from normalized luciferase signal, and curve-fitted to generate an estimated EC50 (less than 1 pM, about 0.245 pM). See, FIG. 25.

[0263] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

WHAT IS CLAIMED IS:

1. An antibody comprising a variable region that specifically binds to ALPPL2 and ALPP, wherein the antibody comprises a heavy chain variable region and a light chain variable region,

wherein complementarity determining region (CDR) 1, CDR2 and CDR3 of the heavy chain variable region are selected from the group consisting of:

SEQ ID NOs: 20, 21, and 22;

SEQ ID NOs: 23, 24, and 25;

SEQ ID NOs: 26, 27, and 28;

SEQ ID NOs: 29, 30, and 31;

SEQ ID NOs: 32, 33, and 34;

SEQ ID NOs: 35, 36, and 37;

SEQ ID NOs: 38, 39, and 40;

SEQ ID NOs: 41, 42, and 43;

SEQ ID NOs: 44, 45, and 46;

SEQ ID NOs: 47, 48, and 49; or

SEQ ID NOs: 50, 51, and 52; and/or

wherein CDR1, CDR2, and CDR3 of the light chain variable region are selected from the group consisting of:

SEQ ID NOs: 53, 54, and 55;

SEQ ID NOs: 56, 57, and 58;

SEQ ID NOs: 59, 60, and 61;

SEQ ID NOs: 62, 63, and 64;

SEQ ID NOs: 65, 66, and 67;

SEQ ID NOs: 68, 69, and 70;

SEQ ID NOs: 71, 72, and 73; or

SEQ ID NOs: 74, 75, and 76,

wherein the antibody does not have the heavy chain variable region of M25FYIA and the light chain variable region of M25FYIA.

2. The antibody of claim 1, wherein

CDR1, CDR2, and CDR3 of the heavy chain variable region comprise SEQ ID NO: 23, SEQ ID NO: 24, and SEQ ID NO: 25, respectively; and

CDR1, CDR2, and CDR3 of the light chain variable region comprise SEQ ID NO: 56, SEQ ID NO: 57, and SEQ ID NO: 58, respectively; or

CDR1, CDR2, and CDR3 of the heavy chain variable region comprise SEQ ID NO: 26, SEQ ID NO: 27, and SEQ ID NO: 28, respectively; and

CDR1, CDR2, and CDR3 of the light chain variable region comprise SEQ ID NO: 56, SEQ ID NO: 57, and SEQ ID NO: 58, respectively; or

CDR1, CDR2, and CDR3 of the heavy chain variable region comprise SEQ ID NO: 44, SEQ ID NO: 45, and SEQ ID NO: 46, respectively; and

CDR1, CDR2, and CDR3 of the light chain variable region comprise SEQ ID NO: 71, SEQ ID NO: 72, and SEQ ID NO: 73, respectively; or

CDR1, CDR2, and CDR3 of the heavy chain variable region comprise SEQ ID NO: 38, SEQ ID NO: 39, and SEQ ID NO: 40, respectively; and

CDR1, CDR2, and CDR3 of the light chain variable region comprise SEQ ID NO: 65, SEQ ID NO: 66, and SEQ ID NO: 67, respectively; or

CDR1, CDR2, and CDR3 of the heavy chain variable region comprise SEQ ID NO: 41, SEQ ID NO: 42, and SEQ ID NO: 43, respectively; and

CDR1, CDR2, and CDR3 of the light chain variable region comprise SEQ ID NO: 53, SEQ ID NO: 54, and SEQ ID NO: 55, respectively; or

CDR1, CDR2, and CDR3 of the heavy chain variable region comprise SEQ ID NO: 35, SEQ ID NO: 36, and SEQ ID NO: 37, respectively; and

CDR1, CDR2, and CDR3 of the light chain variable region comprise SEQ ID NO: 59, SEQ ID NO: 60, and SEQ ID NO: 61, respectively.

3. The antibody of any one of claim 1-2, wherein

the heavy chain variable region is selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 and/or

the light chain variable region is selected from the group consisting of SEQ ID NOs: 12, 13, 14, 15, 16, 17, 18, and 19, and/or

optionally wherein the heavy chain variable region comprises SEQ ID NO: 1; and

the light chain variable region comprises SEQ ID NO: 12; or

the heavy chain variable region comprises SEQ ID NO: 2; and

the light chain variable region comprises SEQ ID NO: 13; or

the heavy chain variable region comprises SEQ ID NO: 3; and

the light chain variable region comprises SEQ ID NO: 13; or
the heavy chain variable region comprises SEQ ID NO: 9; and
the light chain variable region comprises SEQ ID NO: 18; or
the heavy chain variable region comprises SEQ ID NO: 1; and
the light chain variable region comprises SEQ ID NO: 12; or
the heavy chain variable region comprises SEQ ID NO: 7; and
the light chain variable region comprises SEQ ID NO: 16; or
the heavy chain variable region comprises SEQ ID NO: 8; and
the light chain variable region comprises SEQ ID NO: 12; or
the heavy chain variable region comprises SEQ ID NO: 6; and
the light chain variable region comprises SEQ ID NO: 14.

4. The antibody of any one of claims 1-3, wherein the antibody is an IgG, IgA or IgE antibody, wherein IgG the antibody is optionally an IgG1, IgG2, IgG3, or IgG4 antibody, and/or the antibody is a monospecific antibody.

5. The antibody of any one of claims 1-4, wherein the antibody is linked to a cytotoxic agent, wherein the cytotoxic agent is a radionucleotide, microtubule inhibitor, DNA-damaging agent, a polymerase inhibitor, auristatin, Dolastatin-10, maytansine or a maytansine derivative, Mertansine (DM1), DM3, or DM4, auristatin, auristatin E (AE), auristatin EB (AEB), auristatin EFP (AEFP), monomethyl auristatin D (MMAD) or monomethyl dolastatin 10, monomethyl auristatin F (MMAF), N-methylvaline-valine-dolaisoleucine-dolaproline-phenylalanine, monomethyl auristatin E (MMAE), N-methylvaline-valine-dolaisoleucine-dolaproline-norephedrine, 5-benzoyl valeric acid-AE ester (AEVB), vcMMAE, or vcMMAF.

6. The antibody of any one of claims 1-4, wherein the antibody is a bi-specific antibody comprising a second variable region that specifically binds to a second target protein, wherein the antibody comprises a second heavy chain variable region and a second light chain variable region, optionally wherein the second target protein is expressed on the surface of a human immune effector cell, or optionally wherein the second target protein is human CD3.

7. The antibody of claim 6, wherein the CDR1, CDR2, and CDR3 of the second heavy chain variable region comprise SEQ ID NO: 79, SEQ ID NO: 80 and SEQ ID NO: 81 and the CDR1, CDR2, and CDR3 of the second light chain variable region comprise SEQ ID NO: 82, SEQ ID NO: 83 and SEQ ID NO: 84, optionally wherein (a) the second heavy chain variable region comprises SEQ ID NO: 77 and the second light chain variable region comprises SEQ ID NO:78 (b) the antibody comprises SEQ ID NO:85 and SEQ ID NO:86, or (c) the antibody comprises SEQ ID NO:87 and SEQ ID NO:88.

8. A pharmaceutical composition comprising the antibody of any one of claims 1-7.

9. A nucleic acid encoding the antibody of any one of claims 1-3 or 6-7, or a vector comprising said nucleic acid sequence.

10. A cell comprising the nucleic acid or vector of claim 9, wherein the cell is optionally a mammalian cell.

11. A method for producing an antibody, the method comprising culturing a cell comprising a nucleic acid sequence encoding the antibody of any of claims 1-3 or 6-7 under conditions to allow for production of the antibody.

12. A method of killing a cancer cell, the method comprising, contacting the antibody of any one of claims 1-7 to a cancer cell; (A) optionally wherein the antibody is a bi-specific antibody comprising a second variable region that specifically binds to human CD3, wherein the antibody comprises a second heavy chain variable region and a second light chain variable region, and wherein the cancer cell is brought in proximity to a peripheral blood mononuclear cell (PBMC) or T-cell expressing CD3 by binding of the antibody; optionally wherein the CDR1, CDR2, and CDR3 of the second heavy chain variable region comprise SEQ ID NO: 79, SEQ ID NO: 80 and SEQ ID NO: 81 and the CDR1, CDR2, and CDR3 of the second light chain variable region comprise SEQ ID NO: 82, SEQ ID NO: 83 and SEQ ID NO: 84, optionally wherein the second heavy chain variable region comprises SEQ ID NO: 77 and the second light chain variable region comprises SEQ ID NO:78, optionally wherein the antibody comprises SEQ ID NO:85 and SEQ ID NO:86, or optionally wherein the antibody comprises SEQ ID NO:87 and SEQ ID NO:88, or (B) optionally

wherein the antibody is linked to a cytotoxic agent wherein the cytotoxic agent is optionally a radionucleotide, microtubule inhibitor, DNA-damaging agent, a polymerase inhibitor, auristatin, Dolastatin-10, maytansine or a maytansine derivative, Mertansine (DM1), DM3, or DM4, auristatin, auristatin E (AE), auristatin EB (AEB), auristatin EFP (AEFP), monomethyl auristatin D (MMAD) or monomethyl dolastatin 10, monomethyl auristatin F (MMAF), N-methylvaline-valine-dolaisoleucine-dolaproline-phenylalanine, monomethyl auristatin E (MMAE), N-methylvaline-valine-dolaisoleucine-dolaproline-norephedrine, 5-benzoyl valeric acid-AE ester (AEVB), vcMMAE, or vcMMAF.

13. A chimeric antigen receptor (CAR)-expressing human cell, optionally a T-cell, natural killer cell or a macrophage, wherein the CAR comprises the heavy chain variable region and the light chain variable region of the antibody any one of claims 1-3.

14. A method of detecting a tumor cell in a sample, the method comprising contacting the antibody of any one of claims 1-3 to the sample; and

detecting specific binding of the antibody to the tumor cell.

15. Use of antibody of any of claims 1-7, the pharmaceutical composition of claim 8, or the CAR-expressing human cell of claim 13 in the manufacture of a medicament for the treatment of cancer.