1.38 million deaths (18.2% of the total) (GLOBOCAN 2008). Non-small-cell lung cancer (NSCLC) represents approximately 80% to 85% of all lung cancers. With recent progress in immunotherapies (programmed death ligand 1 antibodies) and targeted therapies (such as epidermal growth factor receptor, anaplastic lymphoma kinase inhibitors, etc.), still only a limited portion of patients with NSCLC benefit from the therapies.

[0010] Soft tissue sarcomas (STSs) are mesenchymal-derived cancers which have more than 100 histological subtypes according to the most recent World Health Organization (WHO) classification (WHO 2013). The management of STSs is also a challenging problem, as treatment is essentially palliative and the potential for cure decreases drastically. The reported median overall survival period is approximately 12 to 18 months (Cioffi 2012; Martin-Liberal 2014). Unfortunately, despite progress in the treatment of cancer, there continues to be an unmet medical need for more effective and less toxic therapies, especially for those patients with advanced disease that do not respond to, or have become resistant to, existing therapies.

SUMMARY

[0011] In one example, the present disclosure provides an isolated polypeptide that specifically binds to the Axl protein and uses of the polypeptide in methods of treating an Axl-expressing tumor that involve administering the polypeptide to a human in need of such treatment.

[0012] The polypeptide of the present disclosure includes six complementarity determining regions H1, H2, H3, L1, L2 and L3, wherein:

the H1 sequence is $X_1GX_2X_3MX_4$ (SEQ ID NO: 1) (X_1, X_2, X_3 and X_4 each independently represent an amino acid): wherein

X_1 is T or A or W,

X_2 is H or A,

X_3 is T or I, and

X_4 is N or I;

the H2 sequence is $LIKX_5SNGGTX_6YNQKFKG$ (SEQ ID NO: 2) (X_5 and X_6 each independently respresent an amino acid): wherein
X_5 is P or N, and
X_6 is S or I or T; and

the H3 sequence is $GX_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}DYX_{15}X_{16}$ (SEQ ID NO: 3) (X_7, X_8, X_9, X_{10}, X_{11}, X_{12}, X_{13}, X_{14}, X_{15}, and X_{16}, each independently represent an amino acid): wherein
X_7 is H or D or E or P or R or W,

X_8 is Y or N,

X_9 is E or A or D or F or G or H or I or L or M or N or R or V or Y,

X_{10} is S or D or M or N or Q,

X_{11} is Y or C or E or P,

X_{12} is F or E or N or S or T or V,

X_{13} is A or D or G or L or Y,

X_{14} is M or E or F,

X_{15} is W or A or D or H or L or N or P or R or T, and

X_{16} is G or H,

the L1 sequence is KASQDX$_{17}$X$_{18}$SX$_{19}$VX$_{20}$ (SEQ ID NO: 4) (X_{17}, X_{18}, X_{19}, and X_{20} each independently represent an amino acid): wherein

X_{17} is V or D or G or N or W,

X_{18} is S or V,

X_{19} is A or L or M, and

X_{20} is A or D or N or Q;

the L2 sequence is $X_{21}X_{22}X_{23}$TRX$_{24}$T (SEQ ID NO: 5) (X_{21}, X_{22}, X_{23}, and X_{24}, each independently represent an amino acid): wherein

X_{21} is W or F,

X_{22} is A or I or N or P or Q,

X_{23} is S or D, and

X_{24} is H or D; and

the L3 sequence is QEX$_{25}$X$_{26}$SX$_{27}$X$_{28}$X$_{29}$X$_{30}$ (SEQ ID NO: 6) (X_{25}, X_{26}, X_{27}, X_{28}, X_{29}, and X_{30}, each independently represent an amino acid): wherein

X_{25} is H or C or F or I or L or Q or S or T or V or Y,

X_{26} is F or C or D or E or G or N or S,

X_{27} is T or C or P,

X_{28} is P or A or C or D or E or H or K or S or T or V or W,

X_{29} is L or G or R, and

X_{30} is T or I or R, and

the polypeptide of the present disclosure excludes the parent polypeptide which has the following six CDRs:

H1 = TGHTMN,

H2 = LIKPSNGGTSYNQKFKG,

H3 = GHYESYFAMDYWG,

L1 = KASQDVSSAVA,

L2 = WASTRHT, and

L3 = QEHFSTPLT.

[0013] In another example, the present disclosure provides an isolated polypeptide as described above which has up to one substitution in CDRs H1, H2 and H3, relative to the parent polypeptide and up to one substitution in CDRs L1, L2 and Le, relative to the parent polypeptide. This includes isolated polypeptides with one substitution in CDRs H1, H2 and H3, relative to the parent polypeptide, isolated polypeptides with one substitution in CDRs L1, L2 and L3, relative to the parent polypeptide, and isolated polypeptides with one substitution in CDRs H1, H2 and H3, and one substitution in CDRs L1, L2 and L3, relative to the parent polypeptide, relative to the parent polypeptide. The possible combinations of CDRs H1, H2 and H3 are shown in Fig. 1A and the possible combinations of CDRs L1, L2 and L3 are shown in Fig. 1B.

[0014] In another example, the present invent provides an isolated polypeptide that specifically binds to the Axl protein and uses of the polypeptide in methods of treating an Axl-expressing tumor that involve administering the polypeptide to a human in need of such treatment wherein the isolated polypeptide comprises a heavy chain variable region selected from SEQ ID NO: 20 and a light chain variable region selected from SEQ ID NO: 21.

[0015] In yet another example, the present disclosure provides anti-Axl antibodies or antibody fragments, or immunoconjugates that include at least one said isolated polypeptide of the disclosure.

[0016] In another example, the present disclosure provides a method of using the above-described anti-Axl antibodies or antibody fragments, or immunoconjugates for treatment of an Axl-expressing tumor.

[0017] In yet another example, the present disclosure provides an immunoconjugates that include the antibodies or antibody fragments of the disclosure, optionally conjugated to an agent selected from a chemotherapeutic agent, a radioactive atom, a cytostatic agent and a cytotoxic agent, as well as provides methods of treating an Axl-expressing tumor that involve administering such immunoconjugates.

[0018] In one example, the immunoconjugate is an antibody-drug conjugate (ADC) in which a Conditionally Active Biologic (CAB) anti-Axl antibody is conjugated to one or more heterologous molecule(s) via a cleavable linker (CAB-Axl-ADC). The CAB-Axl-ADC may bemAbBA3011-cleavable linker-$MMAE_{(n)}$, in which the heterologous molecule is monomethyl auristatin E (MMAE), and (n) is an integer between 1 and 4, inclusive.

[0019] In yet another example, the present disclosure provides methods of treating an Axl-expressing tumor that includes administering to a human subject in need of such treatment, a mAbBA301-cleavable linker-$MMAE_{(n)}$, where the mAbBA301 is an antibody or antibody fragment having a heavy chain variable region that includes a hcCDR1 of SEQ ID NO. 14, a hcCDR2 of SEQ ID NO. 15 and a hcCDR3 of SEQ ID NO. 16; and a light chain variable region that includes a lcCDR1 of SEQ ID NO. 17, a lcCDR2 of SEQ ID NO. 18, and a lcCDR3 of SEQ ID NO. 19; MMAE is monomethyl auristatin E (MMAE), and (n) is an integer between 1 and 4, inclusive.

[0020] In yet another example, the present disclosure provides a pharmaceutical composition that includes the polypeptide, the antibody or antibody fragment, or the immunoconjugate of the disclosure, together with a pharmaceutically acceptable carrier.

[0021] In yet another example, the present disclosure provides a kit for diagnosis or treatment including the polypeptide, the antibody or antibody fragment, or the immunoconjugate of the present disclosure, with instructions for use to diagnose or treat Axl-expressing tumors.

[0022] In another example, the present disclosure provides a method of treating an Axl-expressing tumor that includes administering to a human subject in need of such treatment, a pharmaceutical composition containing a mAbBA301-cleavable linker-$MMAE_{(n)}$ and a pharmaceutically acceptable carrier, in which the pharmaceutical composition is administered at a dose of 1.8 mg/kg of the human subject weight on days 1 and 8 every 21 days by intravenous infusion. The mAbBA301 is an antibody or antibody fragment having a heavy chain variable region that includes a hcCDR1 of SEQ ID NO. 14, a hcCDR2 of SEQ ID NO. 15 and a hcCDR3 of SEQ ID NO. 16; and a light chain variable region that includes a lcCDR1 of SEQ ID NO. 17, a lcCDR2 of SEQ ID NO. 18, and a lcCDR3 of SEQ ID NO. 19; and (n) is an integer between 1 and 4, inclusive, preferably, (n) equals 4.

[0022a] In one aspect, the present disclosure refers to use of a pharmaceutical composition comprising mAbBA301-cleavable linker-$MMAE_n$ and a pharmaceutically acceptable carrier, in the manufacture of a medicament for treating an Axl expressing tumor in a human subject in need of such treatment, wherein the pharmaceutical composition is to be administered at a dose of 1.8 mg/kg of the human subject weight on days 1 and 8 every 21 days by intravenous infusion; wherein mAbBA301 is an antibody or antibody fragment having a heavy chain variable region comprising a hcCDR1 of SEQ ID NO. 14, a hcCDR2 of SEQ ID NO. 15 and a hcCDR3 of SEQ ID NO. 16; and a light chain variable region comprising a lcCDR1 of SEQ ID NO. 17, a lcCDR2 of SEQ ID NO. 18, and a lcCDR3 of SEQ ID NO. 19; and wherein n is an integer between 1 and 4, inclusive.

[0022b] In one aspect, the present disclosure refers to use of a pharmaceutical composition comprising mAbBA301-cleavable linker-$MMAE_n$ and a pharmaceutically acceptable carrier, in the manufacture of a medicament for treating an Axl expressing tumor in a human subject in need of such treatment, wherein the pharmaceutical composition is to be administered at a dose of 1.8 mg/kg of the human subject weight every 21 days by intravenous infusion; wherein mAbBA301 is an antibody or antibody fragment having a heavy chain variable region comprising a hcCDR1 of SEQ ID NO. 14, a hcCDR2 of SEQ ID NO. 15 and a hcCDR3 of SEQ ID NO. 16; and a light chain variable region comprising a lcCDR1 of SEQ ID NO. 17, a lcCDR2 of SEQ ID NO. 18 and a lcCDR3 of SEQ ID NO. 19; and wherein n is an integer between 1 and 4, inclusive.

[0023] In one example, the heavy chain variable region of the mAbBA301 includes SEQ ID NO. 20 and the light chain variable region of the mAbBA301 includes SEQ ID NO. 21.

[0024] In a further example, the cleavable linker is mc-vc-PAB.

[0025] In a further example, the Axl-expressing tumor is a sarcoma, an adenocarcinoma, or a non-small lung cell cancer, preferably, the Axl-expressing tumor is a sarcoma.

[0026] In a further example, the methods further include administering a programmed death receptor-1 (PD-1) blocking antibody.

[0027] In a further example, the Axl-expressing tumor has a tumor membrane P score of at least 70.

[0028] In a further example, the methods further include administering a granulocyte colony stimulating factor or an analog thereof.

[0029] In a further example, the pharmaceutically acceptable carrier has a pH of 6.0 and comprises 20 mM histidine-HCl, 70 mg/mL sucrose and 0.5 mg/mL polysorbate 80.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] FIGS. 1A-1B show sequence alignments of the heavy chain variable regions and the light chain variable regions, respectively, of anti-Axl antibodies of the present invention.

[0031] FIG. 2 shows binding (OD450) of various conditionally active antibodies of the invention to Axl's extracellular domain at pH 6.0 and pH 7.4. These conditionally active antibodies were more active at pH 6.0 than at pH 7.4.

[0032] FIG. 3 shows the selectivity of various conditionally active antibodies of the invention to Axl's extracellular domain. The selectivity was measured as the ratio of the binding affinity to a binding partner at pH6.0 to the binding affinity to the same binding partner at pH 7.4.

[0033] FIG. 4 shows by size exclusion chromatograph indicating that conditionally active antibodies of the invention do not aggregate, as described in Example 1.

[0034] FIG. 5 shows thermostability of conditionally active antibodies of the invention before and after heat shock as measured by an ELISA assay, as described in Example 1.

[0035] FIGS. 6A-6B show selectivity of conditionally active antibodies of the invention as measured by SPR assay in Example 1.

[0036] FIG. 7 shows pH dependent binding profiles for binding of anti-Axl antibodies of the present invention to Axl in KREBS buffer.

[0037] FIGS. 8A-8E show results of another cell killing study using A549 cells wherein anti-Axl antibodies of the present invention were employed for cell killing at pH 6.0 and pH 7.4 and at different antibody concentrations.

[0038] FIGS. 9A-9D show binding affinity to human Axl and cynomolgus Axl for anti-Axl antibodies of the present invention in different buffers and at different pH levels.

[0039] FIGS. 10A-10H show cell killing of different cell lines at different pH levels by anti-Axl antibodies of the present invention that were conjugated to duomycin.

[0040] FIG. 11 shows cell killing of A549 cells at different pH levels by anti-Axl antibodies of the present invention that were conjugated to gemcitabine.