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(19) **United States**(12) **Patent Application Publication****Van Berkel et al.**(10) **Pub. No.: US 2020/0129637 A1**(43) **Pub. Date: Apr. 30, 2020**(54) **COMBINATION THERAPY WITH AN
ANTI-AXL ANTIBODY-DRUG CONJUGATE****Publication Classification**(71) Applicants: **ADC Therapeutics SA**, Epalinges
(CH); **Medimmune Limited**,
Cambridge, Cambridgeshire (GB)(72) Inventors: **Patricius Hendrikus Cornelis Van
Berkel**, Epalinges (CH); **Jens
Wuerthner**, Epalinges (CH); **John
Hartley**, London, Greater London (GB);
Francesca Zammarchi, Epalinges (CH)(21) Appl. No.: **16/605,692**(22) PCT Filed: **Apr. 20, 2018**(86) PCT No.: **PCT/EP2018/060209**

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(57)

ABSTRACT

The present disclosure relates to combination therapies for the treatment of pathological conditions, such as cancer. In particular, the present disclosure relates to combination therapies comprising treatment with an Antibody Drug Conjugate (ADC) and a secondary agent.

Specification includes a Sequence Listing.

SEQUENCESSEQ ID NO.1 [1H12 VH, CDR underline]

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMSWVRQAPGKGLEWVATISSGGSY
TYYPDSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCARHPIYYTYDDTMDYWGQG
TTVTVSS

SEQ ID NO.2 [1H12 VL, CDR underline]

EIVLTQSPGTLSPGERATLSCSASSSVSSGNFHWYQQKPLAPRLLIYRTSNLASGIP
ARFSGSGSGTDFTLTISSELPEDFAVYYCQQWSGYPWTFGGGTKLEIK

SEQ ID NO.3 [1H12 Heavy Chain]

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMSWVRQAPGKGLEWVATISSGGSY
TYYPDSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCARHPIYYTYDDTMDYWGQG
TTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYN*STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFF
LYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG

N* indicates Asn297 (numbering according to Kabat)

SEQ ID NO.4 [1H12 Light Chain]

EIVLTQSPGTLSPGERATLSCSASSSVSSGNFHWYQQKPLAPRLLIYRTSNLASGIP
ARFSGSGSGTDFTLTISSELPEDFAVYYCQQWSGYPWTFGGGTKLEIKRTVAAPSVFIFP
PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS
SLTSLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO.5 [1H12 VH CDR1]

SYGMS

SEQ ID NO.6 [1H12 VH CDR2]

TISSGGSYTYYPDSVKG

SEQ ID NO.7 [1H12 VH CDR3]

HPIYYTYDDTMDY

SEQ ID NO.8 [1H12 VL CDR1]

SASSSVSSGNFH

SEQ ID NO.9 [1H12 VL CDR2]

RTSNLAS

Figure 1A

SEQ ID NO.10 [1H12 VL CDR3]

QQWSGYPWT

SEQ ID NO.11 [murine 5F11 VH, CDR underline]

EVKLLESGGGLVQPGGSLKLSCAASGFD~~FS~~RYWMSWVRQAPGKGLEWIG~~E~~INPDSSTI
NYTPSLKDKFIISRDNANTLYLQMSKVRSEDTALYYCAS~~P~~YYYGPFAYWGQGT~~L~~TVS
S

SEQ ID NO.12 [murine 5F11 VL, CDR underline]

DIVLTQSPASLAVSLGQRAIISCKASQSVSFAGTSLMH~~WY~~QKPGQPKLLIYRASNLEA
GFPT~~R~~FSGSGSRTDFTLNHPVEEEDAATYYCQ~~Q~~SREYPRTFGGGTKLEVK

SEQ ID NO.13 [5F11 VH CDR1]

RYWMS

SEQ ID NO.14 [5F11 VH CDR2]

EINPDSSTINYTPSLKD

SEQ ID NO.15 [5F11 VH CDR3]

PYYYGPFAY

SEQ ID NO.16 [5F11 VL CDR1]

KASQSVSFAGTSLMH

SEQ ID NO.17 [5F11 VL CDR2]

RASNLEA

SEQ ID NO.18 [5F11 VL CDR3]

QQSREYPRT

SEQ ID NO.19 [5F11 RHA]

QVQLVESGGGVVQPG~~R~~SLRLSCAASGFTFSRYWMSWVRQAPGKGLEWVAEINPDSST
IN~~Y~~TPSLKDRFAISRDN~~S~~KNTLYLQMNSLRAEDTAVYYCAS~~P~~YYYGPFAYWGQGT~~L~~TVT
S

SEQ ID NO.20 [5F11 RHB]

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWMSWVRQAPGKGLEWVAEINPDSSTI
NYTPSLKDRFTISRDN~~A~~KNSLYLQMNSLRAEDTAVYYCAS~~P~~YYYGPFAYWGQGT~~L~~TVS

SEQ ID NO.21 [5F11 RHC]

EVQLLES~~G~~GGGLVQPGGSLRLSCAASGFTFSRYWMSWVRQAPGKGLEWVSEINPDSSTI
NYTPSLKDRFTISRDN~~S~~KNTLYLQMNSLRAEDTAVYYCAS~~P~~YYYGPFAYWGQGT~~L~~TVS

Figure 1B

SEQ ID NO.22 [5F11 RKA]

EIVLTQSPLSLPVTPGEPASISCKASQSVSFAGTSLMHWYLQKPGQSPQLLIYRASNLEA
GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCQQSREYPRTFGQGTKVEIK

SEQ ID NO.23 [Human Axl]

MAWRCPRMGRVPLAWCLALCGWACMAPRGTAEESEPFVGNPGNITGARGLTGTLRC
QLQVQGEPPEVHWLRDGGQILELADSTQTQVPLGEDEQDDWIVVSQLRITSLQLSDTGQY
QCLVFLGHQTFVSQPGYVGLEGLPYFLEEPEDRTVAANTPFNLSCQAQGPPEPVDLLW
LQDAVPLATAPGHGPQRSLHVPGLNKTSSFSCEAHNAKGVTTSRTATITVLPQQPRNLH
LVSRQPTLEVAWTPGLSGIYPLTHCTLQAVLSDDGMGIQAGEPDPEEPLTSQASVPP
HQLRLGSLHPHTPYHIRVACTSSQGPSSWTHWLPVETPEGVPLGPPENISATRNGSQAF
VHWQEPRAPLQGTLLGYRLAYQQQDTPEVLMDIGLRQEVTLQLQGDGSVSNLTVCVAA
YTAAGDGPWSLPVPLEAWRPGQAQPVHQLLVKEPSTPAFSWPWWYVLLGAVVAAACVL
ILALFLVHRRKKETRYGEVFEPTVERGELVVRVYRVRKSYSRRTEATLNSLGISEELKEKL
RDVMVDRHKVALGKTLGEGEFGAVMEGQLNQDDSILKVAVKTMKIAICTRSELEDFLSE
AVCMKEFDHPNVMRLIGVCFQGSERESFPAPVVILPFMKHGDLSFLLYSRLGDQPVYL
PTQMLVKFMADIASGMEYLSTKRFIHRDLAARNCMLNENMSVCVADFGLSKKIYNGDYY
RQGRIAKMPVKWIAIESLADRVYTSKSDVVSFVGVTMWEIATRGQTPYPGVENSEIYDYL
RRGNRLKQPADCLDGLYALMSRCWELNPQDRPSFTELREDLENTLKALPPAQEPDEILY
VNMDEGGGYPEPPGAAGGADPPTQPDPKDSCSCLTAAEVHPAGRYVLCPSTTPSPAQ
PADRGSPAAPGQEDGA

Figure 1C

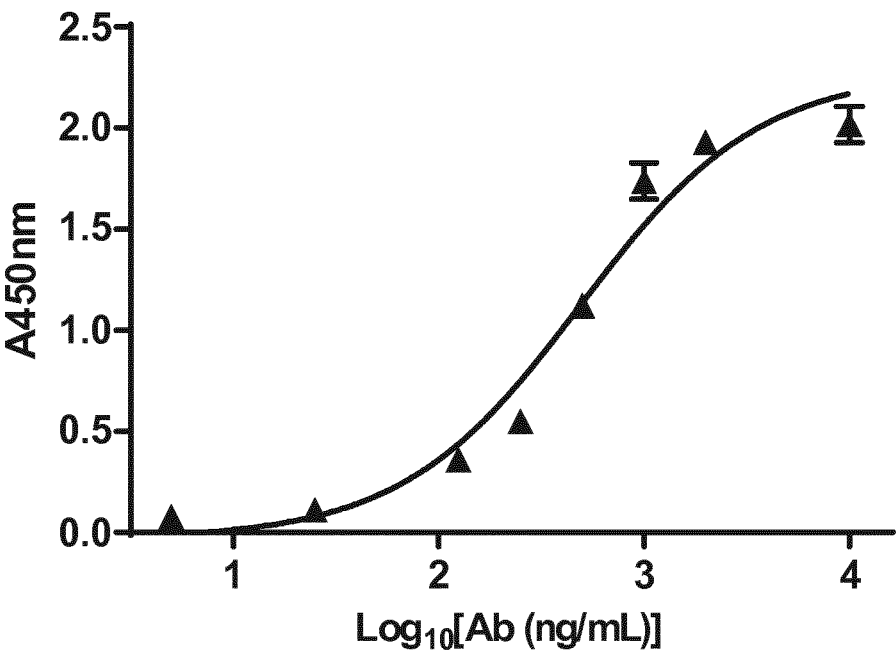


Figure 2

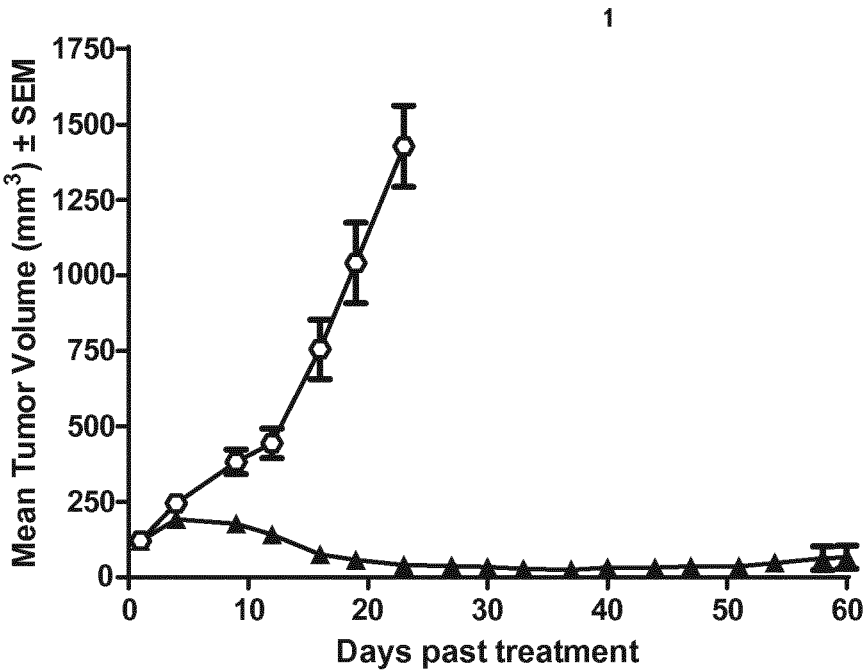


Figure 3

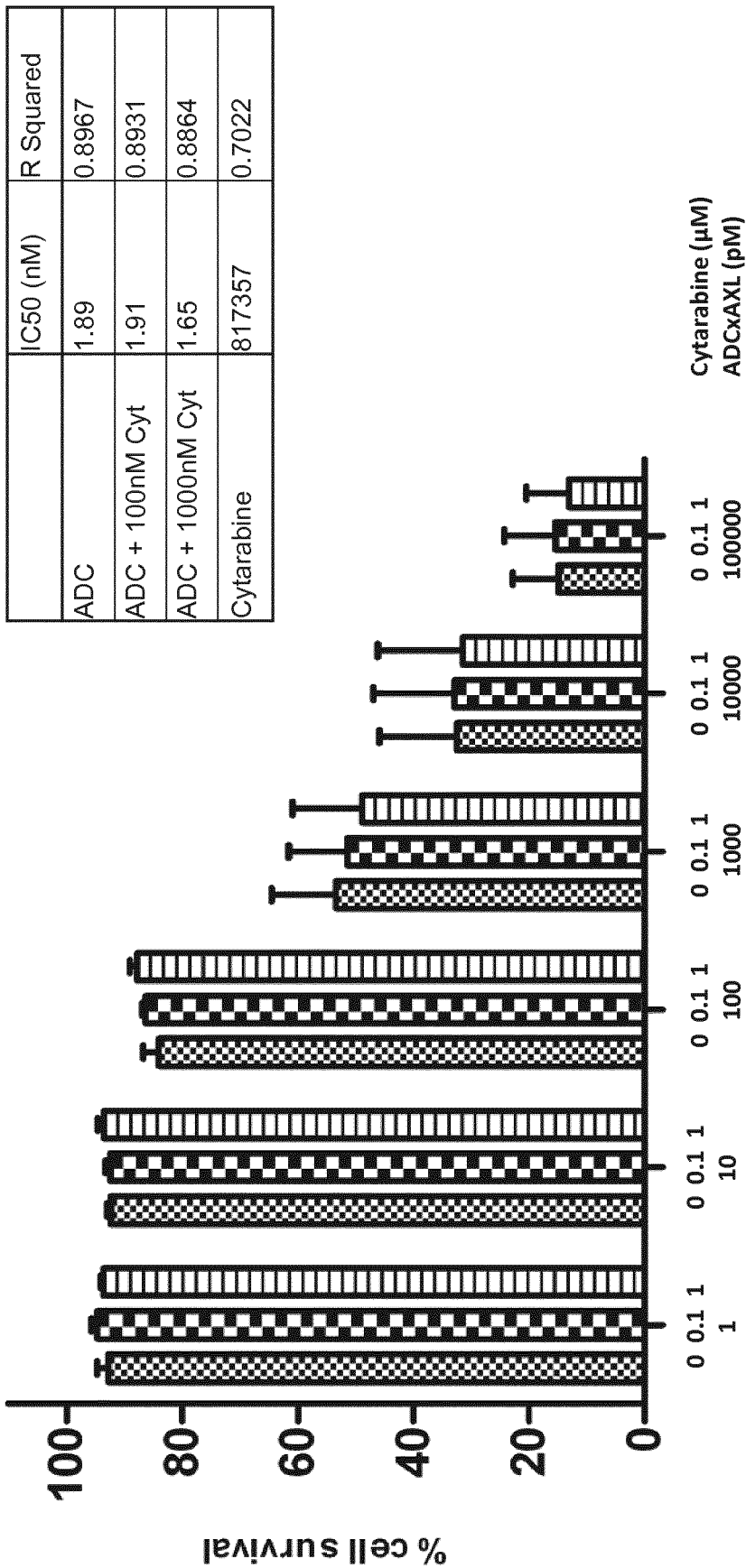


Figure 4

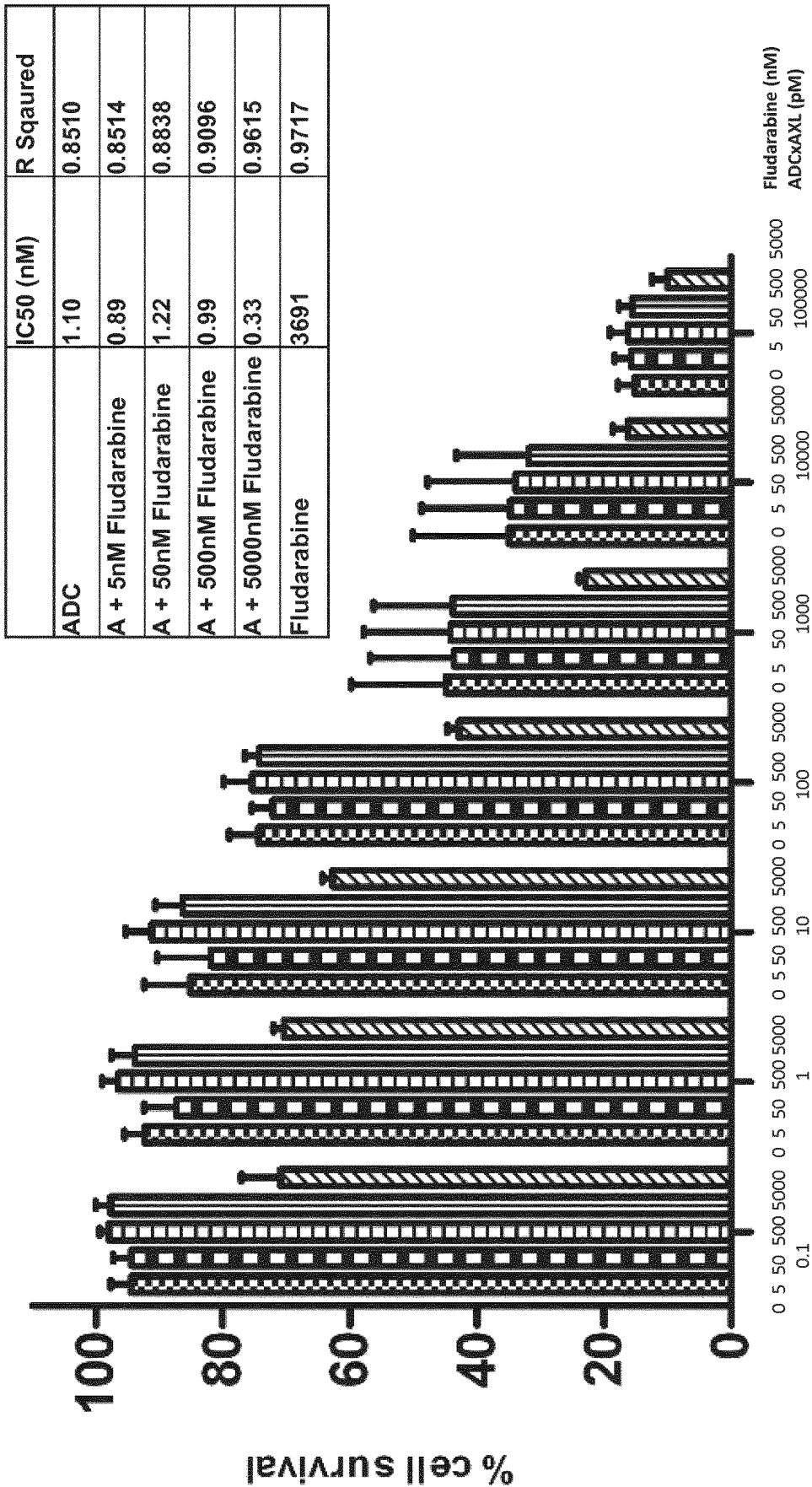


Figure 5

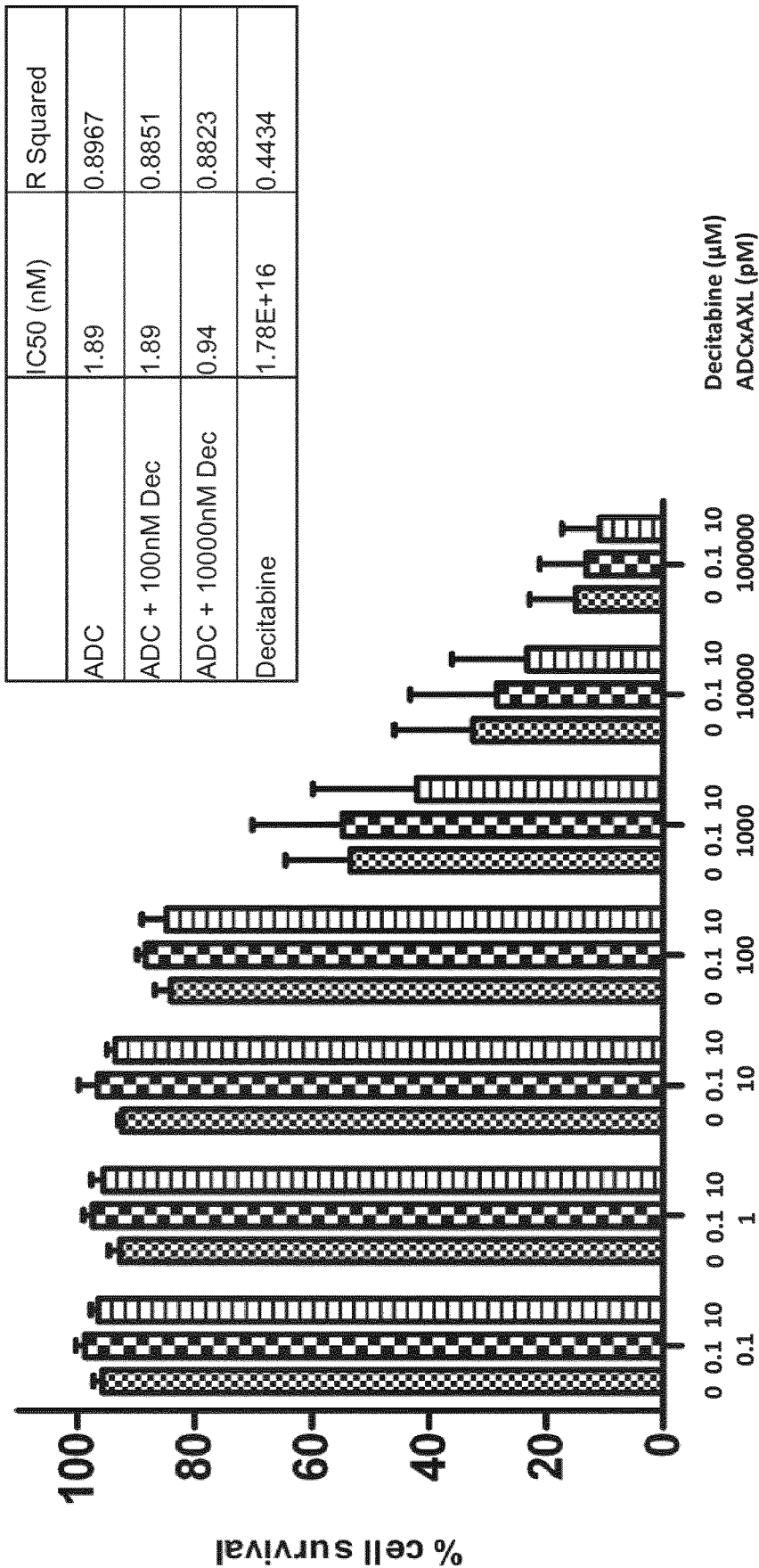


Figure 6

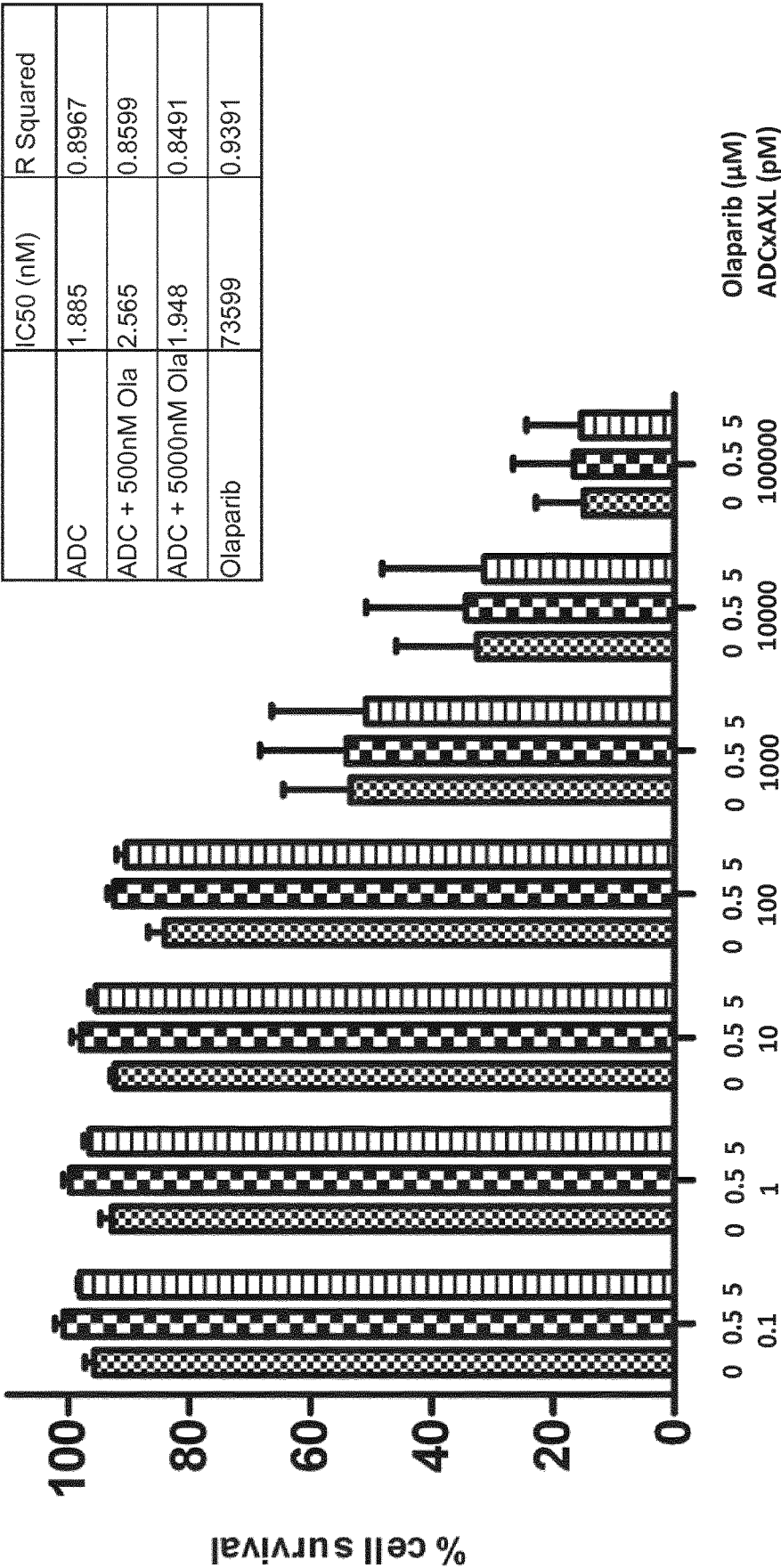


Figure 7

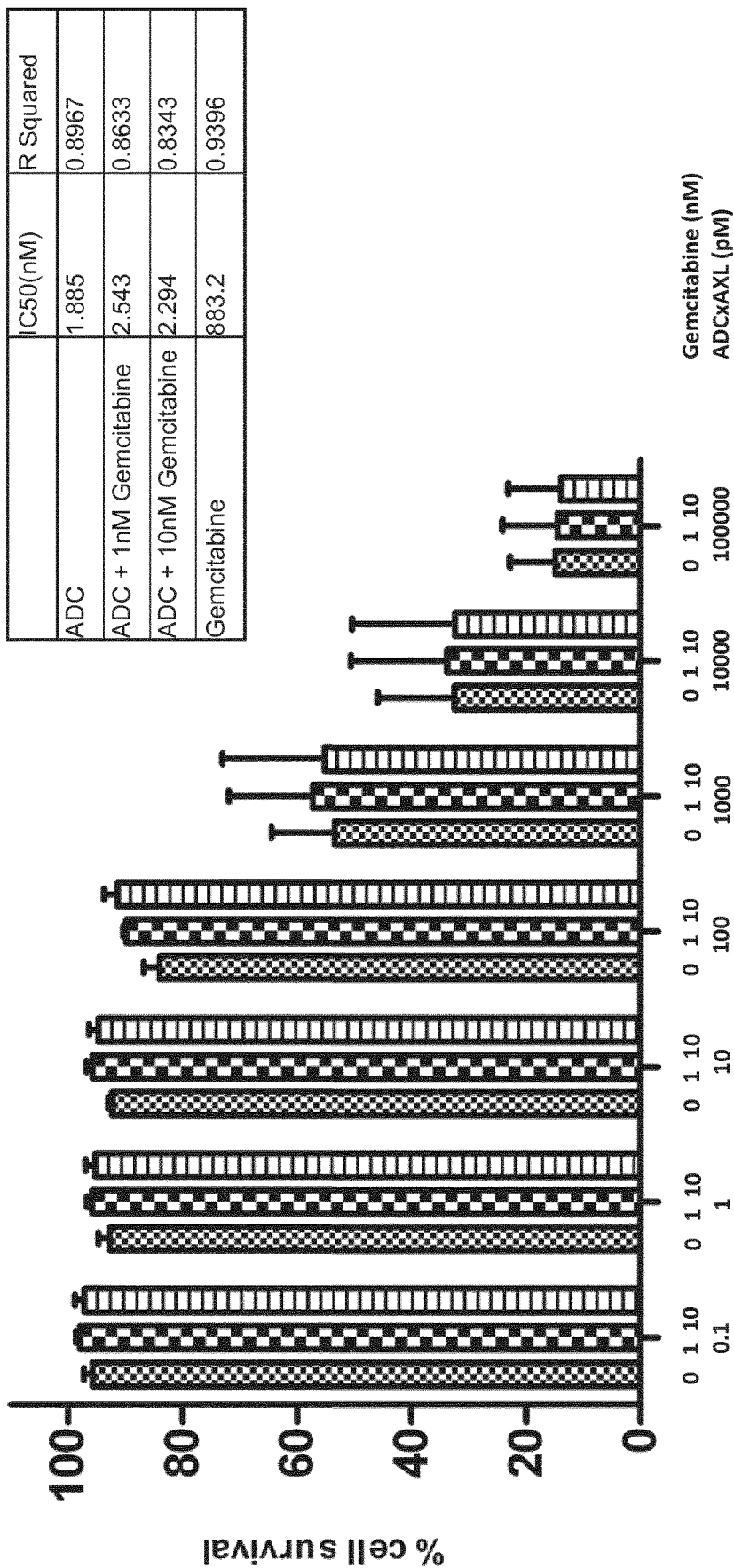


Figure 8

COMBINATION THERAPY WITH AN ANTI-AXL ANTIBODY-DRUG CONJUGATE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of GB1706231.6, GB1706230.8, GB1706229.0, GB1706228.2, GB1706227.4, GB1706226.6, GB1706225.8, and GB1706224.1, GB1706223.3, all filed 20 Apr. 2017.

FIELD

[0002] The present disclosure relates to combination therapies for the treatment of pathological conditions, such as cancer. In particular, the present disclosure relates to combination therapies comprising treatment with an Anti-body Drug Conjugate (ADC) and a secondary agent.

BACKGROUND

Antibody Therapy

[0003] Antibody therapy has been established for the targeted treatment of subjects with cancer, immunological and angiogenic disorders (Carter, P. (2006) *Nature Reviews Immunology* 6:343-357). The use of antibody-drug conjugates (ADC), i.e. immunoconjugates, for the local delivery of cytotoxic or cytostatic agents, i.e. drugs to kill or inhibit tumour cells in the treatment of cancer, targets delivery of the drug moiety to tumours, and intracellular accumulation therein, whereas systemic administration of these unconjugated drug agents may result in unacceptable levels of toxicity to normal cells (Xie et al (2006) *Expert. Opin. Biol. Ther.* 6(3):281-291; Kovtun et al (2006) *Cancer Res.* 66(6):3214-3121; Law et al (2006) *Cancer Res.* 66(4):2328-2337; Wu et al (2005) *Nature Biotech.* 23(9):1137-1145; Lambert J. (2005) *Current Opin. in Pharmacol.* 5:543-549; Hamann P. (2005) *Expert Opin. Ther. Patents* 15(9):1087-1103; Payne, G. (2003) *Cancer Cell* 3:207-212; Trail et al (2003) *Cancer Immunol. Immunother.* 52:328-337; Syrigos and Epenetos (1999) *Anticancer Research* 19:605-614).

AXL

[0004] Axl is a member of the receptor tyrosine kinase sub-family. Although similar to other receptor tyrosine kinases, the Axl protein represents a unique structure of the extracellular region that juxtaposes IgL and FNIII repeats, and has an intracellular region containing an intracellular domain, part of which is the kinase domain. Axl transduces signals from the extracellular matrix into the cytoplasm by binding growth factors like vitamin K-dependent protein growth-arrest-specific gene 6 (Gas6). The extracellular domain of Axl can be cleaved and a soluble extracellular domain of 65 kDa can be released. Cleavage enhances receptor turnover and generates a partially activated kinase (O'Bryan J P, et al (1995) *J Bioi Chem.* 270 (2): 551-557).

[0005] Structural information relating to the human Axl gene and gene product is described in WO2003/068983. The following patent publications also relate to Axl or other tyrosine kinase receptors: U.S. Pat. Nos. 5,468,634; 6,087,144; 5,538,861; 5,968,508; 6,211,142; 6,235,769; WO1999/49894; WO02000/76309; WO2001/16181 and WO2001/32926.

[0006] Axl is involved in the stimulation of cell proliferation. Specifically, Axl is a chronic myelogenous leukaemia-

associated oncogene, that is also associated with colon cancer and melanoma. It is in close vicinity to the bcl3 oncogene which is at 19q13.1-q13.2. The Axl gene is evolutionarily conserved among vertebrate species, and is expressed during development in the mesenchyme.

[0007] Upon interaction with the Gas6 ligand, Axl becomes autophosphorylated, and a cascade of signal transduction events takes place. PI3K, AKT, src, Bad, 14-3-3, PLC, ERK, S6K (mitogen-regulated kinase) and STAT are each known to be involved in this cascade. Gas6 has a region rich with γ -carboxyglutamic acid (GLA domain) that allows for Ca^{++} -dependent binding to membrane phospholipids. Gas6 is a weak mitogen and has an anti-apoptotic effect in NIH3T3 fibroblasts subjected to stress by TNF-induced cytotoxicity, or growth factor withdrawal. In NIH3T3 the binding of Gas6 to Axl results in activation of P13K, AKT, src and Bad.

[0008] Studies have shown that Axl plays a number of different roles in tumour formation. Axl is a key regulator of angiogenic behaviours including endothelial cell migration, proliferation and tube formation. Axl is also required for human breast carcinoma cells to form a tumour in vivo, indicating that Axl regulates processes that are vital for both neovascularisation and tumorigenesis (Holland S. et al, *Cancer Res* 2005; 65 (20), Oct. 15, 2005).

[0009] The activity of Axl receptor tyrosine kinase is positively correlated with tumour metastasis. More specifically, studies have shown that Axl enhances expression of MMP-9, which is required for Axl-mediated invasion. Axl promotes cell invasion by inducing MMP-9 activity through activation of NF-BK and Brg-1 (Tai, K-Y et al, *Oncogene* (2008), 27, 4044-4055). Axl is overexpressed in human glioma cells and can be used to predict poor prognosis in patients with Glioblastoma Multiforme (GBM) (Vajkoczy P. et al, *PNAS*, Apr. 11, 2006, vol 103, no. 15, 5799-5804; Hutterer M. et al, *Clinical Cancer Res* 2008; 14 (1) Jan. 1, 2008). Axl is also relatively overexpressed in highly invasive lung cancer cell lines compared to their minimally invasive counterparts (Shieh, Y-S et al, *Neoplasia*, vol 7, no. 12, Dec. 2005, 1058-1064). Axl is therefore believed to play an important role in tumour invasion and progression.

[0010] Likewise, Axl is expressed in highly invasive breast cancer cells, but not in breast cancer cells of low invasivity. More specifically, inhibition of Axl signalling (by dominant-negative Axl mutant, an antibody against the extracellular domain of Axl, or by short hairpin RNA knockdown of Axl) decreased the mobility and invasivity of highly invasive breast cancer cells. Small molecule Axl inhibitors interfered with motility and invasivity of breast cancer cells. Thus, Axl is understood to be a critical element in the signalling network that governs the motility/invasivity of breast cancer cells (Zhang, Y-X et al., *Cancer Res* 2008; 68 (6), Mar. 15, 2008).

[0011] In mesangial cells, Gas6 was found to have a mitogenic effect, indicative of a possible role in the progression of glomerulosclerosis. Evidence has suggested that the Gas6/Axl pathway also plays a role in glomerulonephritis (Yanagita M. et al, *The Journal of Clinical Investigation*; 2002, 110 (2) 239-246). Further studies have shown that Gas6 promotes the survival of endothelial cells in a model for arterial injury. Angiotensin II, via its AT1 receptor, was shown to increase Axl mRNA and protein receptor in vascular smooth muscle cells (Melaragno M. G. et al, *Circ Res.*, 1998, 83 (7): 697-704). Axl has also been shown to be

involved in cellular adhesion, cell proliferation and regulation of homeostasis in the immune system (Lu Q., 2001) Science 293 (5528): 306-311). Following Axl activation, the following phenomena have been observed: inhibition of apoptosis, increase in “normal” cell (non-transformed) survival of fibroblasts and endothelial cells, migration of Vascular Smooth Muscle Cell (VSMC) (inactivation of the Axl kinase blocks migration), enhancement of neointima formation in blood vessel wall (Melaragno M. G. et al, Trends Cardiovasc Med., 1999, (Review) 9 (8): 250-253) and involvement in lesion formation and the progression of atherosclerosis.

Therapeutic Uses of Anti-AXL ADCs

[0012] The efficacy of an Antibody Drug Conjugate comprising an anti-AXL antibody (an anti-AXL-ADC) in the treatment of, for example, cancer has been established—see, for example, WO2016/166297, WO2016/166302, GB1702029.8, GB1719906.8, and PCT/EP2018/053163.

[0013] Research continues to further improve the efficacy, tolerability, and clinical utility of anti-AXL ADCs. To this end, the present authors have identified clinically advantageous combination therapies in which an anti-AXL ADC is administered in combination with at least one secondary agent.

SUMMARY

[0014] The present authors have determined that the administration of a combination of an ADC and secondary agent to an individual leads to unexpected clinical advantages.

[0015] Accordingly, in one aspect the disclosure provides a method for treating a disorder in an individual, the method comprising administering to the individual an effective amount of an ADC and secondary agent.

[0016] The disorder may be a proliferative disease, for example a cancer. Cancers include metastatic cancers and metastatic cancer cells, such as circulating tumour cells, which may be found circulating in body fluids such as blood or lymph. Cancers of particular interest include, but are not limited to, breast, lung, gastric, head and neck, colorectal, renal, pancreatic, uterine, hepatic, bladder, endometrial and prostate cancers as well as lymphomas (e.g., non-Hodgkin's lymphoma, NHL) and leukemia (particularly acute myeloid leukemia, AML).

[0017] Other disorders of interest include any condition in which Axl is overexpressed, or wherein Axl antagonism will provide a clinical benefit. These include immune disorders, cardiovascular disorders, thrombosis, diabetes, immune checkpoint disorders, or fibrotic disorders (fibrosis) such as strabismus, scleroderma, keloid, Nephrogenic systemic fibrosis, pulmonary fibrosis, idiopathic pulmonary fibrosis (IPF), cystic fibrosis (CF), systemic sclerosis, cardiac fibrosis, non-alcoholic steatohepatitis (NASH), other types of liver fibrosis, primary biliary cirrhosis, renal fibrosis, cancer, and atherosclerosis.

[0018] The proliferative disease may be characterised by the presence of a neoplasm comprising both AXL+ve and AXL−ve cells.

[0019] The proliferative disease may be characterised by the presence of a neoplasm composed of AXL−ve neoplastic cells, optionally wherein the AXL−ve neoplastic cells are associated with AXL+ve non-neoplastic cells.

[0020] The target neoplasm or neoplastic cells may be all or part of a solid tumour.

[0021] “Solid tumor” herein will be understood to include solid haematological cancers such as lymphomas (Hodgkin's lymphoma or non-Hodgkin's lymphoma) which are discussed in more detail herein.

[0022] Solid tumors may be neoplasms, including non-haematological cancers, comprising or composed of AXL+ve neoplastic cells. Solid tumors may be neoplasms, including non-haematological cancers, infiltrated with AXL+ve cells, such as AXL+ve immune suppressive dendritic cells, NK cells, or macrophages; such solid tumours may lack expression of AXL (that is, comprise or be composed of AXL−ve neoplastic cells).

[0023] For example, the solid tumour may be a tumour with high levels of infiltrating AXL+ve cells, such as infiltrating dendritic cells, NK cells, or macrophages (Paolino, M., et al., Cancers 2016, 8, 97; doi:10.3390/cancers8100097). Accordingly, the solid tumour may be pancreatic cancer, breast cancer, colorectal cancer, gastric and oesophageal cancer, leukemia and lymphoma, melanoma, non-small cell lung cancer, ovarian cancer, hepatocellular carcinoma, renal cell carcinoma, and head and neck cancer.

[0024] The ADC may be anti-AXL-ADC, such as ADCx-AXL described herein.

[0025] The secondary agent may be a PD1 antagonist, a PD-L1 antagonist, a GITR agonist, an OX40 agonist, a CTLA-4 antagonist, Fludarabine or Cytarabine, a hypomethylating agent, a PARP inhibitor (PARPi), an agent that upregulates HER2 expression, an AXL inhibitor (AXLi), a BRAF inhibitor (BRAFi), or a MEK inhibitor (MEKi).

[0026] The individual may be human. The individual may have cancer, or may have been determined to have cancer. The individual may have, or have been determined to have, a AXL+ cancer or AXL+ tumour-associated non-tumour cells. The individual may have, or have been determined to have, a AXL+ cancer or AXL+ tumour-associated non-tumour cells.

[0027] The individual may have, or have been determined to have, a PD-L1+ cancer.

[0028] In the disclosed methods the ADC may be administered before the secondary agent, simultaneous with the secondary agent, or after the secondary agent. The disclosed methods may comprise administering a further chemotherapeutic agent to the individual.

[0029] In another aspect, the disclosure provides a first composition comprising an ADC for use in a method of treating a disorder in an individual, wherein the treatment comprises administration of the first composition in combination with a second composition comprising a secondary agent.

[0030] Also provided by this aspect is a first composition comprising a secondary agent for use in a method of treating a disorder in an individual, wherein the treatment comprises administration of the first composition in combination with a second composition comprising an ADC.

[0031] The disorder may be a proliferative disease, for example a cancer. Cancers include metastatic cancers and metastatic cancer cells, such as circulating tumour cells, which may be found circulating in body fluids such as blood or lymph. Cancers of particular interest include, but are not limited to, breast, lung, gastric, head and neck, colorectal, renal, pancreatic, uterine, hepatic, bladder, endometrial and

prostate cancers as well as lymphomas (e.g., non-Hodgkin's lymphoma, NHL) and leukemia (particularly acute myeloid leukemia, AML).

[0032] Other disorders of interest include any condition in which Axl is overexpressed, or wherein Axl antagonism will provide a clinical benefit. These include immune disorders, cardiovascular disorders, thrombosis, diabetes, immune checkpoint disorders, or fibrotic disorders (fibrosis) such as strabismus, scleroderma, keloid, Nephrogenic systemic fibrosis, pulmonary fibrosis, idiopathic pulmonary fibrosis (IPF), cystic fibrosis (CF), systemic sclerosis, cardiac fibrosis, non-alcoholic steatohepatitis (NASH), other types of liver fibrosis, primary biliary cirrhosis, renal fibrosis, cancer, and atherosclerosis.

[0033] The proliferative disease may be characterised by the presence of a neoplasm comprising both AXL+ve and AXL-ve cells.

[0034] The proliferative disease may be characterised by the presence of a neoplasm composed of AXL-ve neoplastic cells, optionally wherein the AXL-ve neoplastic cells are associated with AXL+ve non-neoplastic cells.

[0035] The target neoplasm or neoplastic cells may be all or part of a solid tumour.

[0036] "Solid tumor" herein will be understood to include solid haematological cancers such as lymphomas (Hodgkin's lymphoma or non-Hodgkin's lymphoma) which are discussed in more detail herein.

[0037] Solid tumors may be neoplasms, including non-haematological cancers, comprising or composed of AXL+ve neoplastic cells. Solid tumors may be neoplasms, including non-haematological cancers, infiltrated with AXL+ve cells, such as AXL+ve immune suppressive dendritic cells, NK cells, or macrophages; such solid tumours may lack expression of AXL (that is, comprise or be composed of AXL-ve neoplastic cells).

[0038] The ADC may be anti-AXL-ADC, such as ADCx-AXL described herein.

[0039] The secondary agent may be a PD1 antagonist, a PD-L1 antagonist, a GITR agonist, an OX40 agonist, a CTLA-4 antagonist, Fludarabine or Cytarabine, a hypomethylating agent, a PARP inhibitor (PARPi), an agent that upregulates HER2 expression, an AXL inhibitor (AXLi), a BRAF inhibitor (BRAFi), or a MEK inhibitor (MEKi).

[0040] The individual may be human. The individual may have cancer, or may have been determined to have cancer. The individual may have, or have been determined to have, a AXL+ cancer or AXL+ tumour-associated non-tumour cells. The individual may have, or have been determined to have, a AXL+ cancer or AXL+ tumour-associated non-tumour cells.

[0041] The individual may have, or have been determined to have, a PD-L1+ cancer.

[0042] The first composition may be administered before the second composition, simultaneous with the second composition, or after the second composition. The treatment may comprise administering a further chemotherapeutic agent to the individual.

[0043] In a further aspect, the disclosure provides the use of n ADC in the manufacture of a medicament for treating a disorder in an individual, wherein the medicament comprises an ADC, and wherein the treatment comprises administration of the medicament in combination with a composition comprising secondary agent.

[0044] Also provided by this aspect is the use of secondary agent in the manufacture of a medicament for treating a disorder in an individual, wherein the medicament comprises a secondary agent, and wherein the treatment comprises administration of the medicament in combination with a composition comprising an ADC.

[0045] The disorder may be a proliferative disease, for example a cancer. Cancers include metastatic cancers and metastatic cancer cells, such as circulating tumour cells, which may be found circulating in body fluids such as blood or lymph. Cancers of particular interest include, but are not limited to, breast, lung, gastric, head and neck, colorectal, renal, pancreatic, uterine, hepatic, bladder, endometrial and prostate cancers as well as lymphomas (e.g., non-Hodgkin's lymphoma, NHL) and leukemia (particularly acute myeloid leukemia, AML).

[0046] Other disorders of interest include any condition in which Axl is overexpressed, or wherein Axl antagonism will provide a clinical benefit. These include immune disorders, cardiovascular disorders, thrombosis, diabetes, immune checkpoint disorders, or fibrotic disorders (fibrosis) such as strabismus, scleroderma, keloid, Nephrogenic systemic fibrosis, pulmonary fibrosis, idiopathic pulmonary fibrosis (IPF), cystic fibrosis (CF), systemic sclerosis, cardiac fibrosis, non-alcoholic steatohepatitis (NASH), other types of liver fibrosis, primary biliary cirrhosis, renal fibrosis, cancer, and atherosclerosis.

[0047] The proliferative disease may be characterised by the presence of a neoplasm comprising both AXL+ve and AXL-ve cells.

[0048] The proliferative disease may be characterised by the presence of a neoplasm composed of AXL-ve neoplastic cells, optionally wherein the AXL-ve neoplastic cells are associated with AXL+ve non-neoplastic cells.

[0049] The target neoplasm or neoplastic cells may be all or part of a solid tumour.

[0050] "Solid tumor" herein will be understood to include solid haematological cancers such as lymphomas (Hodgkin's lymphoma or non-Hodgkin's lymphoma) which are discussed in more detail herein.

[0051] Solid tumors may be neoplasms, including non-haematological cancers, comprising or composed of AXL+ve neoplastic cells. Solid tumors may be neoplasms, including non-haematological cancers, infiltrated with AXL+ve cells, such as AXL+ve immune suppressive dendritic cells, NK cells, or macrophages; such solid tumours may lack expression of AXL (that is, comprise or be composed of AXL-ve neoplastic cells).

[0052] The ADC may be anti-AXL-ADC, such as ADCx-AXL described herein.

[0053] The secondary agent may be a PD1 antagonist, a PD-L1 antagonist, a GITR agonist, an OX40 agonist, a CTLA-4 antagonist, Fludarabine or Cytarabine, a hypomethylating agent, a PARP inhibitor (PARPi), an agent that upregulates HER2 expression, an AXL inhibitor (AXLi), a BRAF inhibitor (BRAFi), or a MEK inhibitor (MEKi).

[0054] The individual may be human. The individual may have cancer, or may have been determined to have cancer. The individual may have, or have been determined to have, a AXL+ cancer or AXL+ tumour-associated non-tumour cells. The individual may have, or have been determined to have, a AXL+ cancer or AXL+ tumour-associated non-tumour cells.

[0055] The individual may have, or have been determined to have, a PD-L1+ cancer.

[0056] The medicament may be administered before the composition, simultaneous with the composition, or after the composition. The treatment may comprise administering a further chemotherapeutic agent to the individual.

[0057] Another aspect of the disclosure provides a kit comprising:

[0058] a first medicament comprising an ADC;

[0059] a second medicament comprising a secondary agent; and, optionally,

[0060] a package insert comprising instructions for administration of the first medicament to an individual in combination with the second medicament for the treatment of a disorder.

[0061] Also provided by this aspect is a kit comprising a medicament comprising an ADC and a package insert comprising instructions for administration of the medicament to an individual in combination with a composition comprising a secondary agent for the treatment of a disorder.

[0062] Further provided by this aspect is a kit comprising a medicament comprising a secondary agent and a package insert comprising instructions for administration of the medicament to an individual in combination with a composition comprising an ADC for the treatment of a disorder.

[0063] The disorder may be a proliferative disease, for example a cancer. Cancers include metastatic cancers and metastatic cancer cells, such as circulating tumour cells, which may be found circulating in body fluids such as blood or lymph. Cancers of particular interest include, but are not limited to, breast, lung, gastric, head and neck, colorectal, renal, pancreatic, uterine, hepatic, bladder, endometrial and prostate cancers as well as lymphomas (e.g., non-Hodgkin's lymphoma, NHL) and leukemia (particularly acute myeloid leukemia, AML).

[0064] Other disorders of interest include any condition in which Axl is overexpressed, or wherein Axl antagonism will provide a clinical benefit. These include immune disorders, cardiovascular disorders, thrombosis, diabetes, immune checkpoint disorders, or fibrotic disorders (fibrosis) such as strabismus, scleroderma, keloid, Nephrogenic systemic fibrosis, pulmonary fibrosis, idiopathic pulmonary fibrosis (IPF), cystic fibrosis (CF), systemic sclerosis, cardiac fibrosis, non-alcoholic steatohepatitis (NASH), other types of liver fibrosis, primary biliary cirrhosis, renal fibrosis, cancer, and atherosclerosis.

[0065] The proliferative disease may be characterised by the presence of a neoplasm comprising both AXL+ve and AXL-ve cells.

[0066] The proliferative disease may be characterised by the presence of a neoplasm composed of AXL-ve neoplastic cells, optionally wherein the AXL-ve neoplastic cells are associated with AXL+ve non-neoplastic cells.

[0067] The target neoplasm or neoplastic cells may be all or part of a solid tumour.

[0068] "Solid tumor" herein will be understood to include solid haematological cancers such as lymphomas (Hodgkin's lymphoma or non-Hodgkin's lymphoma) which are discussed in more detail herein.

[0069] Solid tumors may be neoplasms, including non-haematological cancers, comprising or composed of AXL+ve neoplastic cells. Solid tumors may be neoplasms, including non-haematological cancers, infiltrated with AXL+ve cells, such as AXL+ve immune suppressive dendritic cells,

NK cells, or macrophages; such solid tumours may lack expression of AXL (that is, comprise or be composed of AXL-ve neoplastic cells).

[0070] The ADC may be anti-AXL-ADC, such as ADCx-AXL described herein.

[0071] The secondary agent may be a PD1 antagonist, a PD-L1 antagonist, a GITR agonist, an OX40 agonist, a CTLA-4 antagonist, Fludarabine or Cytarabine, a hypomethylating agent, a PARP inhibitor (PARPi), an agent that upregulates HER2 expression, an AXL inhibitor (AXLi), a BRAF inhibitor (BRAFi), or a MEK inhibitor (MEKi).

[0072] The individual may be human. The individual may have cancer, or may have been determined to have cancer. The individual may have, or have been determined to have, a AXL+ cancer or AXL+ tumour-associated non-tumour cells. The individual may have, or have been determined to have, a AXL+ cancer or AXL+ tumour-associated non-tumour cells.

[0073] The individual may have, or have been determined to have, a PD-L1+ cancer.

[0074] The medicament or composition comprising the ADC may be administered before the medicament or composition comprising the secondary agent, simultaneous with the medicament or composition comprising the secondary agent, or after the medicament or composition comprising the secondary agent. The treatment may comprise administering a further chemotherapeutic agent to the individual.

[0075] In a yet further aspect, the disclosure provides a composition comprising an ADC and a secondary agent.

[0076] Also provided in this aspect of the disclosure is a method of treating a disorder in an individual, the method comprising administering to the individual an effective amount of the composition comprising an ADC and a secondary agent.

[0077] Also provided in this aspect of the disclosure is a composition comprising an ADC and a secondary agent for use in a method of treating a disorder in an individual.

[0078] Also provided in this aspect of the disclosure is the use of a composition comprising an ADC and a secondary agent in the manufacture of a medicament for treating a disorder in an individual.

[0079] Also provided in this aspect of the disclosure is a kit comprising composition comprising an ADC and a secondary agent and a set of instructions for administration of the medicament to an individual for the treatment of a disorder.

[0080] The disorder may be a proliferative disease, for example a cancer. Cancers include metastatic cancers and metastatic cancer cells, such as circulating tumour cells, which may be found circulating in body fluids such as blood or lymph. Cancers of particular interest include, but are not limited to, breast, lung, gastric, head and neck, colorectal, renal, pancreatic, uterine, hepatic, bladder, endometrial and prostate cancers as well as lymphomas (e.g., non-Hodgkin's lymphoma, NHL) and leukemia (particularly acute myeloid leukemia, AML).

[0081] Other disorders of interest include any condition in which Axl is overexpressed, or wherein Axl antagonism will provide a clinical benefit. These include immune disorders, cardiovascular disorders, thrombosis, diabetes, immune checkpoint disorders, or fibrotic disorders (fibrosis) such as strabismus, scleroderma, keloid, Nephrogenic systemic fibrosis, pulmonary fibrosis, idiopathic pulmonary fibrosis (IPF), cystic fibrosis (CF), systemic sclerosis, cardiac fibro-

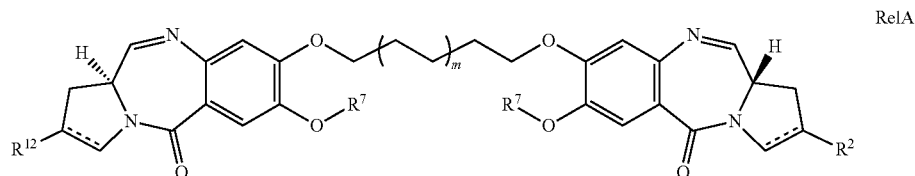
sis, non-alcoholic steatohepatitis (NASH), other types of liver fibrosis, primary biliary cirrhosis, renal fibrosis, cancer, and atherosclerosis.

[0082] The proliferative disease may be characterised by the presence of a neoplasm comprising both AXL+ve and AXL-ve cells.

[0083] The proliferative disease may be characterised by the presence of a neoplasm composed of AXL-ve neoplastic

one of the PBD moieties conjugated to an antibody as defined below.

[0094] The present disclosure is suitable for use in providing a PBD compound to a preferred site in a subject. The conjugate allows the release of an active PBD compound that does not retain any part of the linker. There is no stub present that could affect the reactivity of the PBD compound. Thus the conjugate of formula (I) would release the compound RelA:



cells, optionally wherein the AXL-ve neoplastic cells are associated with AXL+ve non-neoplastic cells.

[0084] The target neoplasm or neoplastic cells may be all or part of a solid tumour.

[0085] “Solid tumor” herein will be understood to include solid haematological cancers such as lymphomas (Hodgkin’s lymphoma or non-Hodgkin’s lymphoma) which are discussed in more detail herein.

[0086] Solid tumors may be neoplasms, including non-haematological cancers, comprising or composed of AXL+ve neoplastic cells. Solid tumors may be neoplasms, including non-haematological cancers, infiltrated with AXL+ve cells, such as AXL+ve immune suppressive dendritic cells, NK cells, or macrophages; such solid tumours may lack expression of AXL (that is, comprise or be composed of AXL-ve neoplastic cells).

[0087] The ADC may be anti-AXL-ADC, such as ADCx-AXL described herein.

[0088] The secondary agent may be a PD1 antagonist, a PD-L1 antagonist, a GITR agonist, an OX40 agonist, a CTLA-4 antagonist, Fludarabine or Cytarabine, a hypomethylating agent, a PARP inhibitor (PARPi), an agent that upregulates HER2 expression, an AXL inhibitor (AXLi), a BRAF inhibitor (BRAFi), or a MEK inhibitor (MEKi).

[0089] The individual may be human. The individual may have cancer, or may have been determined to have cancer. The individual may have, or have been determined to have, a AXL+ cancer or AXL+ tumour-associated non-tumour cells. The individual may have, or have been determined to have, a AXL+ cancer or AXL+ tumour-associated non-tumour cells.

[0090] The individual may have, or have been determined to have, a PD-L1+ cancer.

[0091] The treatment may comprise administering a further chemotherapeutic agent to the individual.

DETAILED DESCRIPTION

Antibody Drug Conjugates (ADCs)

[0092] The present disclosure relates to the improved efficacy of combinations of an ADC and a secondary agent.

[0093] The ADC of present disclosure provides a PBD dimer with a linker connected through the N10 position on

[0095] The specified link between the PBD dimer and the antibody in the present invention is preferably stable extracellularly. Before transport or delivery into a cell, the antibody-drug conjugate (ADC) is preferably stable and remains intact, i.e. the antibody remains linked to the drug moiety. The linkers are stable outside the target cell and may be cleaved at some efficacious rate inside the cell. An effective linker will: (i) maintain the specific binding properties of the antibody; (ii) allow intracellular delivery of the conjugate or drug moiety; (iii) remain stable and intact, i.e. not cleaved, until the conjugate has been delivered or transported to its targeted site; and (iv) maintain a cytotoxic, cell-killing effect or a cytostatic effect of the PBD drug moiety. Stability of the ADC may be measured by standard analytical techniques such as mass spectroscopy, HPLC, and the separation/analysis technique LC/MS.

[0096] Delivery of the compounds of formulae RelA is achieved at the desired activation site of the conjugate of formula (I) by the action of an enzyme, such as cathepsin, on the linking group, and in particular on the valine-alanine dipeptide moiety.

[0097] The disclosure also particularly relates treatment with an anti-AXL ADC disclosed in GB1702029.8, GB1719906.8, PCT/EP2018/053163, and as herein described.

Anti-AXL ADCs

[0098] As used herein, the term “AXL-ADC” refers to an ADC in which the antibody component is an anti-AXL antibody. The term “PBD-ADC” refers to an ADC in which the drug component is a pyrrolobenzodiazepine (PBD) warhead. The term “anti-AXL-ADC” refers to an ADC in which the antibody component is an anti-AXL antibody, and the drug component is a PBD warhead.

[0099] The ADC may comprise a conjugate of formula (I):

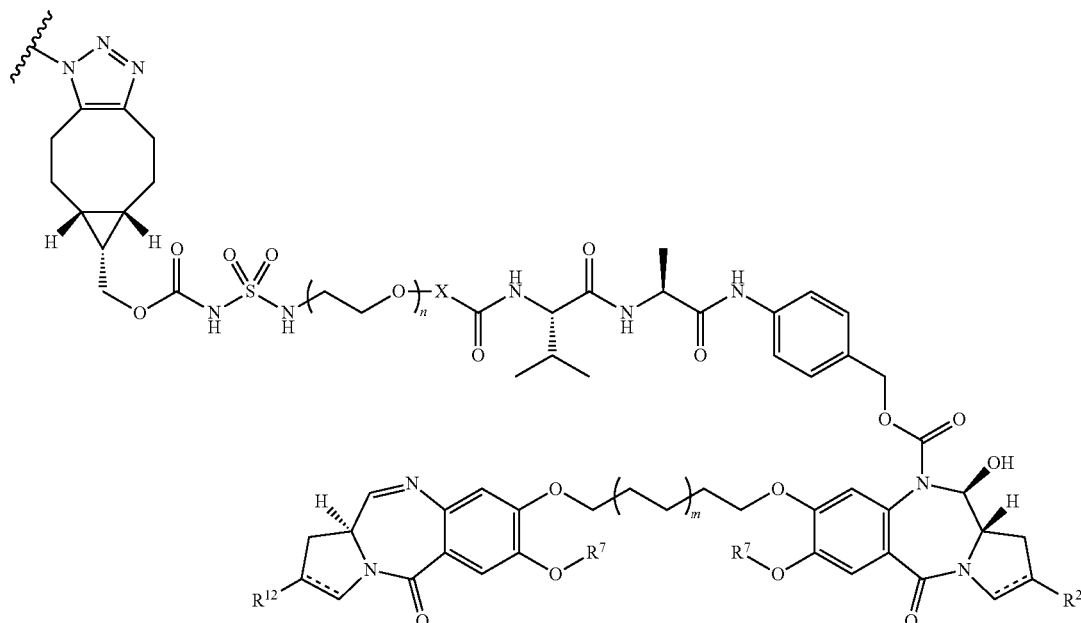


wherein:

Ab is an antibody that binds to AXL;

DL is

[0100]

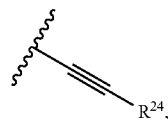


wherein:

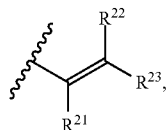
X is selected from the group comprising: a single bond, —CH₂— and —C₂H₄—;

n is from 1 to 8;

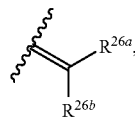
m is 0 or 1;

R⁷ is either methyl or phenyl;when there is a double bond between C2 and C3, R² is selected from the group consisting of:(ia) C₅₋₁₀ aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, carboxy, ester, C₁₋₇ alkyl, C₃₋₇ heterocyclyl and bis-oxy-C₁₋₃ alkylene;(ib) C₁₋₅ saturated aliphatic alkyl;(ic) C₃₋₆ saturated cycloalkyl;wherein one of R^{25a} and R^{25b} is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl; and

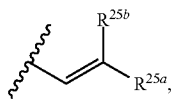
(if)



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where R²⁴ is selected from: H; C₁₋₃ saturated alkyl; C₂₋₃ alkenyl; C₂₋₃ alkynyl; cyclopropyl; phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl;when there is a single bond between C2 and C3, R² is

(ie)

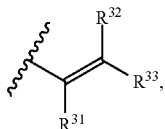
where R^{26a} and R^{26b} are independently selected from H, F, C₁₋₄ saturated alkyl, C₂₋₃ alkenyl, which alkyl and alkenyl groups are optionally substituted by a group selected from C₁₋₄ alkyl amido and C₁₋₄ alkyl ester; or, when one of R^{26a} and R^{26b} is H, the other is selected from nitrile and a C₁₋₄ alkyl ester;wherein each of R²¹, R²² and R²³ are independently selected from H, C₁₋₃ saturated alkyl, C₂₋₃ alkenyl, C₂₋₃ alkynyl and cyclopropyl, where the total number of carbon atoms in the R¹² group is no more than 5;

when there is a double bond between C2' and C3', R¹² is selected the group consisting of:

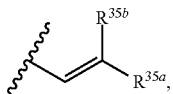
(ia) C₅₋₁₀ aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, carboxy, ester, C₁₋₇ alkyl, C₃₋₇ heterocyclyl and bis-oxy-C₁₋₃ alkylene;

(ib) C₁₋₅ saturated aliphatic alkyl;

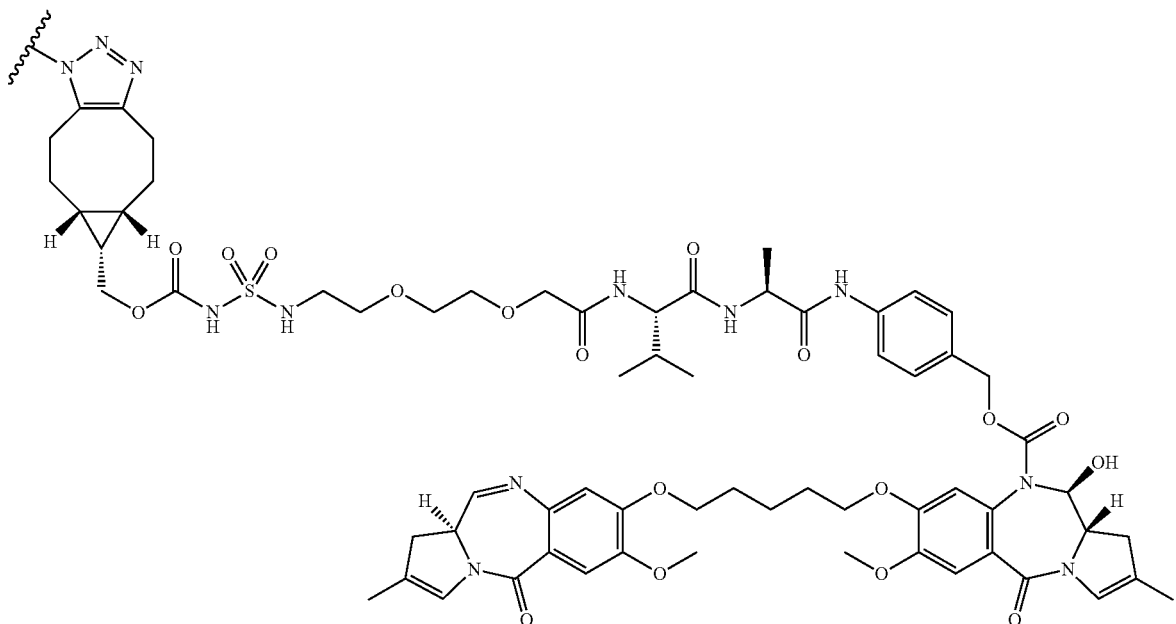
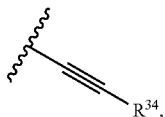
(ic) C₃₋₆ saturated cycloalkyl;



wherein each of R^{31} , R^{32} and R^{33} are independently selected from H, C_{1-3} saturated alkyl, C_{2-3} alkenyl, C_{2-3} alkynyl and cyclopropyl, where the total number of carbon atoms in the R^{12} group is no more than 5;

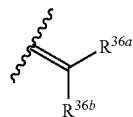


wherein one of R^{35a} and R^{35b} is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl; and



where R²⁴ is selected from: H; C₁₋₃ saturated alkyl; C₂₋₃ alkenyl; C₂₋₃ alkynyl; cyclopropyl; phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl;

when there is a single bond between C2' and C3', R¹² is



where R^{36a} and R^{36b} are independently selected from H, F, C₁₋₄ saturated alkyl, C₂₋₃ alkenyl, which alkyl and alkenyl groups are optionally substituted by a group selected from C₁₋₄ alkyl amido and C₁₋₄ alkyl ester; or, when one of R^{36a} and R^{36b} is H, the other is selected from nitrile and a C₁₋₄ alkyl ester;

and p is from 1 to 8.

[0101] It has previously been shown that such ADCs are useful in the treatment of AXL expressing cancers (see, for example, GB1702029.8, GB1719906.8, and PCT/EP2018/053163, which are incorporated by reference herein in their entirety).

[10102] The term anti-AXL-ADC may include any embodiment described in GB1702029.8. In particular, in preferred embodiments the ADC may have the chemical structure:



wherein:

Ab is an antibody that binds to AXL;

DL is:

[0103]

wherein the Ab is an anti-AXL antibody.

[0104] DL may be conjugated to the antibody through the sidechain of an antibody asparagine residue, for example Asn297 according to the numbering system of Kabat. The structure of the linkage to the antibody may be N-[sugar]-DL, wherein N is the asparagine residue, and [sugar] represents a sugar residue, such as a GlcNAc residue. p may be 1 to 4, preferably 2.

[0105] In some embodiments Ab is an antibody that binds to AXL, the antibody comprising:

[0106] (a) a heavy chain having the sequence according to SEQ ID NO. 3, wherein DL is conjugated to the antibody through the asparagine at position 302 of SEQ ID NO.3; and

[0107] (b) a light chain having the sequence according to SEQ ID NO. 4.

DL Embodiments

X

[0108] In some embodiments, X is a single bond.

[0109] In other embodiments, X is $-\text{CH}_2-$.

[0110] In further embodiments, X is $-\text{C}_2\text{H}_4-$.

[0111] In some embodiments, n is 1 to 4.

[0112] In some of these embodiments, n is 1.

[0113] In other of these embodiments, n is 2.

[0114] In further of these embodiments, n is 4.

R^7

[0115] In one embodiment, R^7 is methyl.

[0116] In another embodiment, R^7 is phenyl.

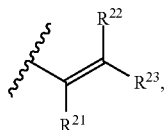
R^2

[0117] When there is a double bond present between C2 and C3, R^2 is selected from:

(a) C_{5-10} aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, C_{1-7} alkyl, C_{3-7} heterocyclyl and bis-oxy- C_{1-3} alkylene;

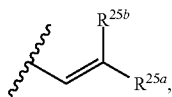
(b) C_{1-5} saturated aliphatic alkyl;

(c) C_{3-6} saturated cycloalkyl;



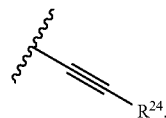
(d)

wherein each of R^{21} , R^{22} and R^{23} are independently selected from H, C_{1-3} saturated alkyl, C_{2-3} alkenyl, C_{2-3} alkynyl and cyclopropyl, where the total number of carbon atoms in the R^2 group is no more than 5;



(e)

wherein one of R^{25a} and R^{25b} is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo methyl, methoxy; pyridyl; and thiophenyl; and



(f)

where R^{24} is selected from: H; C_{1-3} saturated alkyl; C_{2-3} alkenyl; C_{2-3} alkynyl; cyclopropyl; phenyl, which phenyl is optionally substituted by a group selected from halo methyl, methoxy; pyridyl; and thiophenyl.

[0118] When R^2 is a C_{5-10} aryl group, it may be a C_{5-7} aryl group. A C_{5-7} aryl group may be a phenyl group or a C_{5-7} heteroaryl group, for example furanyl, thiophenyl and pyridyl. In some embodiments, R^2 is preferably phenyl. In other embodiments, R^{12} is preferably thiophenyl, for example, thiophen-2-yl and thiophen-3-yl.

[0119] When R^2 is a C_{5-10} aryl group, it may be a C_{8-10} aryl, for example a quinolinyl or isoquinolinyl group. The quinolinyl or isoquinolinyl group may be bound to the PBD core through any available ring position. For example, the quinolinyl may be quinolin-2-yl, quinolin-3-yl, quinolin-4-yl, quinolin-5-yl, quinolin-6-yl, quinolin-7-yl and quinolin-8-yl. Of these quinolin-3-yl and quinolin-6-yl may be preferred. The isoquinolinyl may be isoquinolin-1-yl, isoquinolin-3-yl, isoquinolin-4-yl, isoquinolin-5-yl, isoquinolin-6-yl, isoquinolin-7-yl and isoquinolin-8-yl. Of these isoquinolin-3-yl and isoquinolin-6-yl may be preferred.

[0120] When R^2 is a C_{5-10} aryl group, it may bear any number of substituent groups. It preferably bears from 1 to 3 substituent groups, with 1 and 2 being more preferred, and singly substituted groups being most preferred. The substituents may be any position.

[0121] Where R^2 is C_{5-7} aryl group, a single substituent is preferably on a ring atom that is not adjacent the bond to the remainder of the compound, i.e. it is preferably β or γ to the bond to the remainder of the compound. Therefore, where the C_{57} aryl group is phenyl, the substituent is preferably in the meta- or para-positions, and more preferably is in the para-position.

[0122] Where R^2 is a C_{8-10} aryl group, for example quinolinyl or isoquinolinyl, it may bear any number of substituents at any position of the quinoline or isoquinoline rings. In some embodiments, it bears one, two or three substituents, and these may be on either the proximal and distal rings or both (if more than one substituent).

R^2 Substituents, when R^2 is a C_{5-10} Aryl Group

[0123] If a substituent on R^2 when R^2 is a C_{5-10} aryl group is halo, it is preferably F or Cl, more preferably Cl.

[0124] If a substituent on R^2 when R^2 is a C_{5-10} aryl group is ether, it may in some embodiments be an alkoxy group, for example, a C_{1-7} alkoxy group (e.g. methoxy, ethoxy) or it may in some embodiments be a C_{5-7} aryloxy group (e.g. phenoxy, pyridyloxy, furanyloxy). The alkoxy group may itself be further substituted, for example by an amino group (e.g. dimethylamino).

[0125] If a substituent on R^2 when R^2 is a C_{5-10} aryl group is C_{1-7} alkyl, it may preferably be a C_{1-4} alkyl group (e.g. methyl, ethyl, propyl, butyl).

[0126] If a substituent on R^2 when R^2 is a C_{5-10} aryl group is C_{3-7} heterocyclyl, it may in some embodiments be C_6 nitrogen containing heterocyclyl group, e.g. morpholino, thiomorpholino, piperidinyl, piperazinyl. These groups may be bound to the rest of the PBD moiety via the nitrogen atom. These groups may be further substituted, for example, by C_{1-4} alkyl groups. If the C_6 nitrogen containing heterocyclyl group is piperazinyl, the said further substituent may be on the second nitrogen ring atom.

[0127] If a substituent on R^2 when R^2 is a C_{5-10} aryl group is bis-oxy- C_{1-3} alkylene, this is preferably bis-oxy-methylene or bis-oxy-ethylene.

[0128] If a substituent on R^2 when R^2 is a C_{5-10} aryl group is ester, this is preferably methyl ester or ethyl ester.

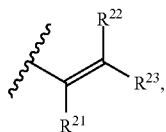
[0129] Particularly preferred substituents when R^2 is a C_{5-10} aryl group include methoxy, ethoxy, fluoro, chloro, cyano, bis-oxy-methylene, methyl-piperazinyl, morpholino and methylthiophenyl. Other particularly preferred substituent for R^2 are dimethylaminopropoxy and carboxy.

[0130] Particularly preferred substituted R^2 groups when R^2 is a C_{5-10} aryl group include, but are not limited to, 4-methoxy-phenyl, 3-methoxyphenyl, 4-ethoxy-phenyl, 3-ethoxy-phenyl, 4-fluoro-phenyl, 4-chloro-phenyl, 3,4-bisoxymethylene-phenyl, 4-methylthiophenyl, 4-cyanophenyl, 4-phenoxyphenyl, quinolin-3-yl and quinolin-6-yl, isoquinolin-3-yl and isoquinolin-6-yl, 2-thienyl, 2-furanyl, methoxynaphthyl, and naphthyl. Another possible substituted R^2 group is 4-nitrophenyl. R^2 groups of particular interest include 4-(4-methylpiperazin-1-yl)phenyl and 3,4-bisoxymethylene-phenyl.

[0131] When R^2 is C_{1-5} saturated aliphatic alkyl, it may be methyl, ethyl, propyl, butyl or pentyl. In some embodiments, it may be methyl, ethyl or propyl (n-pentyl or isopropyl). In some of these embodiments, it may be methyl. In other embodiments, it may be butyl or pentyl, which may be linear or branched.

[0132] When R^2 is C_{3-6} saturated cycloalkyl, it may be cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl. In some embodiments, it may be cyclopropyl.

[0133] When R^2 is



each of R^{21} , R^{22} and R^{23} are independently selected from H, C_{1-3} saturated alkyl, C_{2-3} alkenyl, C_{2-3} alkynyl and cyclopropyl, where the total number of carbon atoms in the R^2 group is no more than 5. In some embodiments, the total number of carbon atoms in the R^2 group is no more than 4 or no more than 3.

[0134] In some embodiments, one of R^{21} , R^{22} and R^{23} is H, with the other two groups being selected from H, C_{1-3} saturated alkyl, C_{2-3} alkenyl, C_{2-3} alkynyl and cyclopropyl.

[0135] In other embodiments, two of R^{21} , R^{22} and R^{23} are H, with the other group being selected from H, C_{1-3} saturated alkyl, C_{2-3} alkenyl, C_{2-3} alkynyl and cyclopropyl.

[0136] In some embodiments, the groups that are not H are selected from methyl and ethyl. In some of these embodiments, the groups that are not H are methyl.

[0137] In some embodiments, R^{21} is H.

[0138] In some embodiments, R^{22} is H.

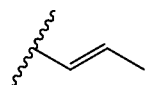
[0139] In some embodiments, R^{23} is H.

[0140] In some embodiments, R^{21} and R^{22} are H.

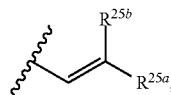
[0141] In some embodiments, R^{21} and R^{23} are H.

[0142] In some embodiments, R^{22} and R^{23} are H.

[0143] A R^2 group of particular interest is:

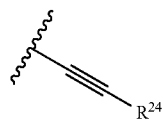


[0144] When R^2 is



one of R^{25a} and R^{25b} is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl. In some embodiments, the group which is not H is optionally substituted phenyl. If the phenyl optional substituent is halo, it is preferably fluoro. In some embodiment, the phenyl group is unsubstituted.

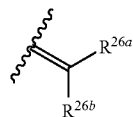
[0145] When R^2 is



R^{24} is selected from: H; C_{1-3} saturated alkyl; C_{2-3} alkenyl; C_{2-3} alkynyl; cyclopropyl; phenyl, which phenyl is optionally substituted by a group selected from halo methyl, methoxy; pyridyl; and thiophenyl. If the phenyl optional substituent is halo, it is preferably fluoro. In some embodiment, the phenyl group is unsubstituted. In some embodiments, R^{24} is selected from H, methyl, ethyl, ethenyl and ethynyl. In some of these embodiments, R^{24} is selected from H and methyl.

[0146] When there is a single bond present between C2 and C3,

[0147] R^2 is



where R^{26a} and R^{26b} are independently selected from H, F, C_{1-4} saturated alkyl, C_{2-3} alkenyl, which alkyl and alkenyl groups are optionally substituted by a group selected from

C_{1-4} alkyl amido and C_{1-4} alkyl ester; or, when one of R^{26a} and R^{26b} is H, the other is selected from nitrile and a C_{1-4} alkyl ester.

[0148] In some embodiments, it is preferred that R^{26a} and R^{26b} are both H.

[0149] In other embodiments, it is preferred that R^{26a} and R^{26b} are both methyl.

[0150] In further embodiments, it is preferred that one of R^{26a} and R^{26b} is H, and the other is selected from C_{1-4} saturated alkyl, C_{2-3} alkenyl, which alkyl and alkenyl groups are optionally substituted. In these further embodiment, it may be further preferred that the group which is not H is selected from methyl and ethyl.

R^{12}

[0151] The above preferences for R^2 apply equally to R^{12} .

[0152] In one preferred embodiment of the invention, DL is

[0158] [N] is the sidechain of the asparagine at position 302 of each SEQ ID NO.3;

[0159] [GlcNAc] is a N-acetyl glucosamine residue; and

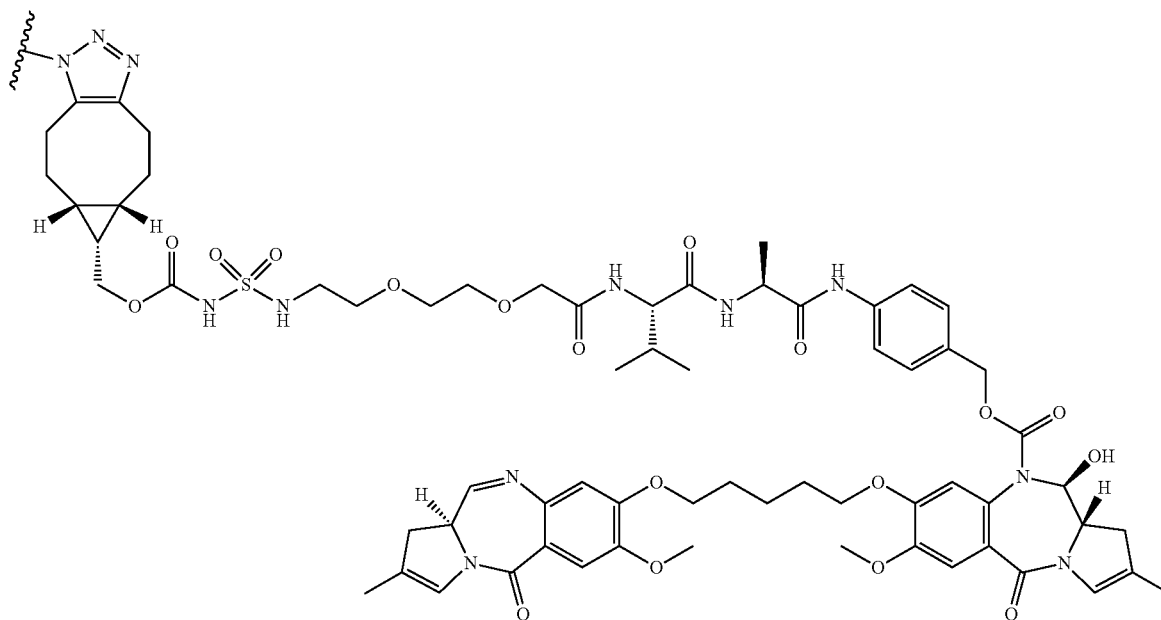
[0160] DL is the drug-linker described immediately above.

The Antibody Component of the Anti-AXL ADC

[0161] In one aspect the antibody is an antibody that binds to AXL.

1H12

[0162] In some embodiments the antibody comprises a VH domain having a VH CDR3 with the amino acid sequence of SEQ ID NO.7. In some embodiments the VH domain further comprises a VH CDR2 with the amino acid sequence of SEQ ID NO.6, and/or a VH CDR1 with the



[0153] The above DL may be preferably comprised in an ADC having the formula $Ab-(DL)_p$, wherein Ab is an antibody that binds to AXL. DL may be conjugated to the antibody through the sidechain of an antibody asparagine residue, for example Asn297 according to the numbering system of Kabat. The structure of the linkage to the antibody may be N-[sugar]-DL, wherein N is the asparagine residue, and [sugar] represents a sugar residue, such as a GlcNAc residue. p may be 1 to 4, for example 2.

[0154] For example, in one embodiment the invention provides a conjugate having the formula:



wherein:

[0155] Ab is an antibody comprising:

[0156] (a) two heavy chains, each having the sequence according to SEQ ID NO. 3; and

[0157] (b) two light chains, each having the sequence according to SEQ ID NO. 4;

amino acid sequence of SEQ ID NO.5. In some embodiments the antibody comprises a VH domain having a VH CDR1 with the amino acid sequence of SEQ ID NO.5, a VH CDR2 with the amino acid sequence of SEQ ID NO.6, and a VH CDR3 with the amino acid sequence of SEQ ID NO.7. In preferred embodiments the antibody comprises a VH domain having the sequence according to SEQ ID NO. 1.

[0163] The antibody may further comprise a VL domain. In some embodiments the antibody comprises a VL domain having a VL CDR3 with the amino acid sequence of SEQ ID NO.10. In some embodiments the VL domain further comprises a VL CDR2 with the amino acid sequence of SEQ ID NO.9, and/or a VL CDR1 with the amino acid sequence of SEQ ID NO.8. In some embodiments the antibody comprises a VL domain having a VL CDR1 with the amino acid sequence of SEQ ID NO.8, a VL CDR2 with the amino acid sequence of SEQ ID NO.9, and a VL CDR3 with the amino acid sequence of SEQ ID NO.10. In preferred embodiments the antibody comprises a VL domain having the sequence according to SEQ ID NO. 2.

[0164] In preferred embodiments the antibody comprises a VH domain and a VL domain. Preferably the VH comprises the sequence of SEQ ID NO.1 and the VL domain comprises the sequence of SEQ ID NO.2.

[0165] The VH and VL domain(s) may pair so as to form an antibody antigen binding site that binds AXL.

[0166] In some embodiments the antibody is an intact antibody comprising a VH domain paired with a VL domain, the VH and VL domains having sequences of SEQ ID NO.1 paired with SEQ ID NO.2.

[0167] In some embodiments the antibody comprises a heavy chain having the sequence of SEQ ID NO. 3 paired with a light chain having the sequence of SEQ ID NO.4. In some embodiments the antibody is an intact antibody comprising two heavy chains having the sequence of SEQ ID NO.3, each paired with a light chain having the sequence of SEQ ID NO.4.

[0168] In one aspect the antibody is an antibody as described herein which has been modified (or further modified) as described below. In some embodiments the antibody is a humanised, deimmunised or resurfaced version of an antibody disclosed herein.

[0169] In some embodiments the antibody is a fully human monoclonal IgG1 antibody, preferably IgG1, κ .

[0170] In an aspect the antibody is an antibody as described herein which has been modified (or further modified) as described below. In some embodiments the antibody is a humanised, deimmunised or resurfaced version of an antibody disclosed herein.

[0171] The most preferred anti-AXL-ADC for use with the aspects of the present disclosure is ADCxAXL, as described herein below.

5F11

[0172] In some embodiments the antibody comprises a VH domain having a VH CDR3 with the amino acid sequence of SEQ ID NO.15. In some embodiments the VH domain further comprises a VH CDR2 with the amino acid sequence of SEQ ID NO.14, and/or a VH CDR1 with the amino acid sequence of SEQ ID NO.13. In some embodiments the antibody comprises a VH domain having a VH CDR1 with the amino acid sequence of SEQ ID NO.13, a VH CDR2 with the amino acid sequence of SEQ ID NO.14, and a VH CDR3 with the amino acid sequence of SEQ ID NO.15.

[0173] In some embodiments the antibody comprises a VH domain having the sequence according to SEQ ID NO. 11. In some embodiments the antibody comprises a VH domain having the sequence according to SEQ ID NO. 19. In some embodiments the antibody comprises a VH domain having the sequence according to SEQ ID NO. 20. In some embodiments the antibody comprises a VH domain having the sequence according to SEQ ID NO. 21.

[0174] The antibody may further comprise a VL domain. In some embodiments the antibody comprises a VL domain having a VL CDR3 with the amino acid sequence of SEQ ID NO.18. In some embodiments the VL domain further comprises a VL CDR2 with the amino acid sequence of SEQ ID NO.17, and/or a VL CDR1 with the amino acid sequence of SEQ ID NO.16. In some embodiments the antibody comprises a VL domain having a VL CDR1 with the amino acid sequence of SEQ ID NO.16, a VL CDR2 with the amino acid sequence of SEQ ID NO.17, and a VL CDR3 with the amino acid sequence of SEQ ID NO.18.

[0175] In some embodiments the antibody comprises a VL domain having the sequence according to SEQ ID NO. 22.

[0176] In preferred embodiments the antibody comprises a VH domain and a VL domain. In some embodiments the VH comprises a VH CDR1 with the amino acid sequence of SEQ ID NO.13, a VH CDR2 with the amino acid sequence of SEQ ID NO.14, and a VH CDR3 with the amino acid sequence of SEQ ID NO.15; and the VL domain comprises a VL CDR1 with the amino acid sequence of SEQ ID NO.16, a VL CDR2 with the amino acid sequence of SEQ ID NO.17, and a VL CDR3 with the amino acid sequence of SEQ ID NO.18.

[0177] In some embodiments the antibody comprises a VH domain having the sequence of SEQ ID NO.19 and the VL domain having the sequence of SEQ ID NO.22. In some embodiments the antibody comprises a VH domain having the sequence of SEQ ID NO.20 and the VL domain having the sequence of SEQ ID NO.22. In some embodiments the antibody comprises a VH domain having the sequence of SEQ ID NO.21 and the VL domain having the sequence of SEQ ID NO.22.

[0178] In one aspect the antibody is an antibody as described herein which has been modified (or further modified) as described below. In some embodiments the antibody is a humanised, deimmunised or resurfaced version of an antibody disclosed herein.

[0179] In some embodiments the antibody is a fully human monoclonal IgG1 antibody, preferably IgG1, κ .

[0180] In an aspect the antibody is an antibody as described herein which has been modified (or further modified) as described below. In some embodiments the antibody is a humanised, deimmunised or resurfaced version of an antibody disclosed herein.

Modification of Antibodies

[0181] The antibodies disclosed herein may be modified. For example, to make them less immunogenic to a human subject. This may be achieved using any of a number of techniques familiar to the person skilled in the art. Some of these techniques are described in more detail below.

Humanisation

[0182] Techniques to reduce the in vivo immunogenicity of a non-human antibody or antibody fragment include those termed "humanisation".

[0183] A "humanized antibody" refers to a polypeptide comprising at least a portion of a modified variable region of a human antibody wherein a portion of the variable region, preferably a portion substantially less than the intact human variable domain, has been substituted by the corresponding sequence from a non-human species and wherein the modified variable region is linked to at least another part of another protein, preferably the constant region of a human antibody. The expression "humanized antibodies" includes human antibodies in which one or more complementarity determining region ("CDR") amino acid residues and/or one or more framework region ("FW" or "FR") amino acid residues are substituted by amino acid residues from analogous sites in rodent or other non-human antibodies. The expression "humanized antibody" also includes an immunoglobulin amino acid sequence variant or fragment thereof that comprises an FR having substantially the amino acid

sequence of a human immunoglobulin and a CDR having substantially the amino acid sequence of a non-human immunoglobulin.

[0184] “Humanized” forms of non-human (e.g., murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. Or, looked at another way, a humanized antibody is a human antibody that also contains selected sequences from non-human (e.g. murine) antibodies in place of the human sequences. A humanized antibody can include conservative amino acid substitutions or non-natural residues from the same or different species that do not significantly alter its binding and/or biologic activity. Such antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulins.

[0185] There are a range of humanisation techniques, including ‘CDR grafting’, ‘guided selection’, ‘deimmunization’, ‘resurfacing’ (also known as ‘veneering’), ‘composite antibodies’, ‘Human String Content Optimisation’ and framework shuffling.

CDR Grafting

[0186] In this technique, the humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary-determining region (CDR) of the recipient antibody are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, camel, bovine, goat, or rabbit having the desired properties (in effect, the non-human CDRs are ‘grafted’ onto the human framework). In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues (this may happen when, for example, a particular FR residue has significant effect on antigen binding).

[0187] Furthermore, humanized antibodies can comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and maximize antibody performance. Thus, in general, a humanized antibody will comprise all of at least one, and in one aspect two, variable domains, in which all or all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), or that of a human immunoglobulin.

Guided Selection

[0188] The method consists of combining the V_H or V_L domain of a given non-human antibody specific for a particular epitope with a human V_H or V_L library and specific human V domains are selected against the antigen of interest. This selected human VH is then combined with a

VL library to generate a completely human $VH \times VL$ combination. The method is described in Nature Biotechnology (N.Y.) Z2 (1994) 899-903.

Composite Antibodies

[0189] In this method, two or more segments of amino acid sequence from a human antibody are combined within the final antibody molecule. They are constructed by combining multiple human VH and VL sequence segments in combinations which limit or avoid human T cell epitopes in the final composite antibody V regions. Where required, T cell epitopes are limited or avoided by, exchanging V region segments contributing to or encoding a T cell epitope with alternative segments which avoid T cell epitopes. This method is described in US 2008/0206239 A1.

Deimmunization

[0190] This method involves the removal of human (or other second species) T-cell epitopes from the V regions of the therapeutic antibody (or other molecule). The therapeutic antibodies V-region sequence is analysed for the presence of MHC class II-binding motifs by, for example, comparison with databases of MHC-binding motifs (such as the “motifs” database hosted at www.wehi.edu.au). Alternatively, MHC class II-binding motifs may be identified using computational threading methods such as those devised by Altuvia et al. (J. Mol. Biol. 249 244-250 (1995)); in these methods, consecutive overlapping peptides from the V-region sequences are testing for their binding energies to MHC class II proteins. This data can then be combined with information on other sequence features which relate to successfully presented peptides, such as amphipathicity, Rothbard motifs, and cleavage sites for cathepsin B and other processing enzymes.

[0191] Once potential second species (e.g. human) T-cell epitopes have been identified, they are eliminated by the alteration of one or more amino acids. The modified amino acids are usually within the T-cell epitope itself, but may also be adjacent to the epitope in terms of the primary or secondary structure of the protein (and therefore, may not be adjacent in the primary structure). Most typically, the alteration is by way of substitution but, in some circumstances amino acid addition or deletion will be more appropriate.

[0192] All alterations can be accomplished by recombinant DNA technology, so that the final molecule may be prepared by expression from a recombinant host using well established methods such as Site Directed Mutagenesis. However, the use of protein chemistry or any other means of molecular alteration is also possible.

Resurfacing

[0193] This method involves:

[0194] (a) determining the conformational structure of the variable region of the non-human (e.g. rodent)

antibody (or fragment thereof) by constructing a three-dimensional model of the non-human antibody variable region;

- [0195] (b) generating sequence alignments using relative accessibility distributions from x-ray crystallographic structures of a sufficient number of non-human and human antibody variable region heavy and light chains to give a set of heavy and light chain framework positions wherein the alignment positions are identical in 98% of the sufficient number of non-human antibody heavy and light chains;
- [0196] (c) defining for the non-human antibody to be humanized, a set of heavy and light chain surface exposed amino acid residues using the set of framework positions generated in step (b);
- [0197] (d) identifying from human antibody amino acid sequences a set of heavy and light chain surface exposed amino acid residues that is most closely identical to the set of surface exposed amino acid residues defined in step (c), wherein the heavy and light chain from the human antibody are or are not naturally paired;
- [0198] (e) substituting, in the amino acid sequence of the non-human antibody to be humanized, the set of heavy and light chain surface exposed amino acid residues defined in step (c) with the set of heavy and light chain surface exposed amino acid residues identified in step (d);
- [0199] (f) constructing a three-dimensional model of the variable region of the non-human antibody resulting from the substituting specified in step (e);
- [0200] (g) identifying, by comparing the three-dimensional models constructed in steps (a) and (f), any amino acid residues from the sets identified in steps (c) or (d), that are within 5 Angstroms of any atom of any residue of the complementarity determining regions of the non-human antibody to be humanized; and
- [0201] (h) changing any residues identified in step (g) from the human to the original non-human amino acid residue to thereby define a non-human antibody humanizing set of surface exposed amino acid residues; with the proviso that step (a) need not be conducted first, but must be conducted prior to step (g).

Superhumanization

[0202] The method compares the non-human sequence with the functional human germline gene repertoire. Those human genes encoding canonical structures identical or closely related to the non-human sequences are selected. Those selected human genes with highest homology within the CDRs are chosen as FR donors. Finally, the non-human CDRs are grafted onto these human FRs. This method is described in patent WO 2005/079479 A2.

Human String Content Optimization

[0203] This method compares the non-human (e.g. mouse) sequence with the repertoire of human germline genes and the differences are scored as Human String Content (HSC) that quantifies a sequence at the level of potential MHC/T-cell epitopes. The target sequence is then humanized by maximizing its HSC rather than using a global identity measure to generate multiple diverse humanized variants (described in *Molecular Immunology*, 44, (2007) 1986-1998).

Framework Shuffling

[0204] The CDRs of the non-human antibody are fused in-frame to cDNA pools encompassing all known heavy and light chain human germline gene frameworks. Humanised antibodies are then selected by e.g. panning of the phage displayed antibody library. This is described in *Methods* 36, 43-60 (2005).

Modification of Antibody with Azide

[0205] The antibody may be prepared for conjugation with the drug linker through a three step process:

[0206] (1) Expression of antibody (Ab) bearing the core N-glycan in a suitable expression system (e.g. a CHO cell line). The core N-glycan is typically conjugated to Asn-297 of the heavy chain according to the numbering system of Kabat;

[0207] (2) trimming of all glycan isoforms (complex, hybrid, high-mannose) with an endoglycosidase to leave the core GlcNAc; and

[0208] (3) enzymatic transfer to the core GlcNAc of a N-acetylgalactose residue harboring an azide group for conjugation to the drug linker.

[0209] An overview of the above process is set out in van Geel, R., et al., *Bioconjugate Chemistry*, 2015, 26, 2233-2242; DOI: 10.1021/acs.bioconjchem.5b00224. Alternatively, a one-pot process may be used—see the examples.

ADCxAXL

[0210] ADCxAXL is an antibody drug conjugate composed of a humanized antibody against human AXL attached to a pyrrolobenzodiazepine (PBD) warhead via a cleavable linker. The mechanism of action of ADCxAXL depends on AXL binding. The AXL specific antibody targets the antibody drug conjugate (ADC) to cells expressing AXL. Upon binding, the ADC internalizes and is transported to the lysosome, where the protease sensitive linker is cleaved and free PBD dimer is released inside the target cell. The released PBD dimer inhibits transcription in a sequence-selective manner, due either to direct inhibition of RNA polymerase or inhibition of the interaction of associated transcription factors. The PBD dimer produces covalent crosslinks that do not distort the DNA double helix and which are not recognized by nucleotide excision repair factors, allowing for a longer effective period (Hartley 2011).

[0211] It has the chemical structure:

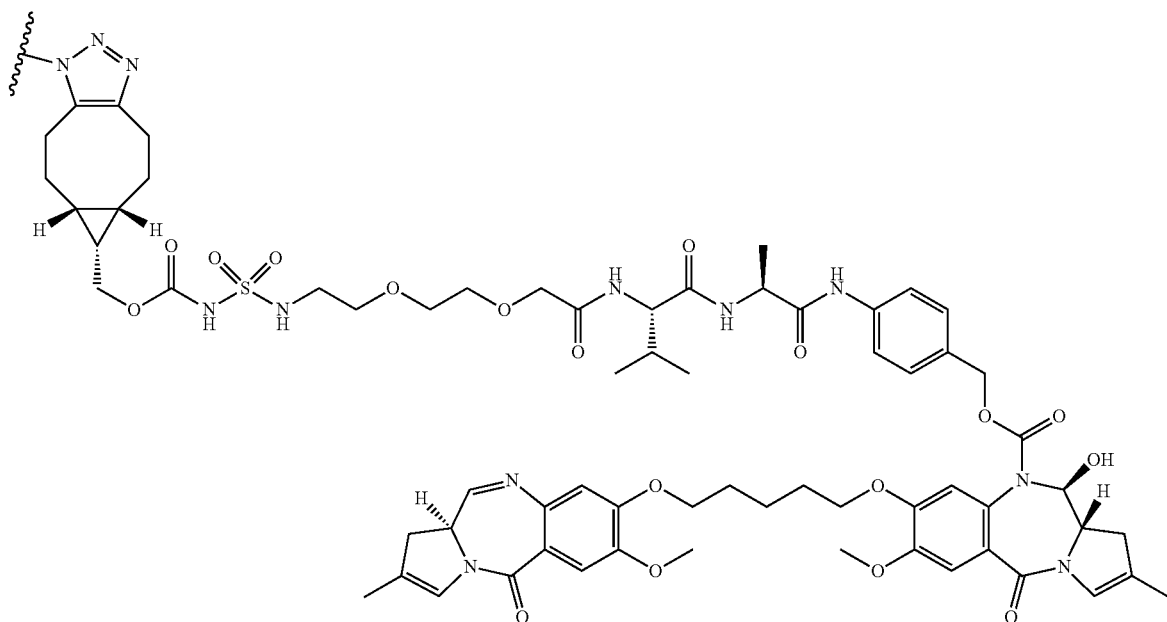


wherein:

DL is:

[0212]

50, 100, 200, 500, 1000, 2000, 5000, 10^4 , 10^5 or 10^6 -fold higher than the antibody's association constant for BSA, when measured at physiological conditions. The antibodies of the invention can bind AXL with a high affinity. For



Ab is an antibody that binds to AXL, the antibody comprising:

[0213] (a) a heavy chain having the sequence according to SEQ ID NO. 3;

[0214] (b) a light chain having the sequence according to SEQ ID NO. 4.

[0215] It is noted that “having the sequence” has the same meaning as “comprising the sequence”; in particular, in some embodiments the heavy chain of ADCxAXL is expressed with an additional terminal ‘K’ residue (so, ending . . . SPGK), with the terminal K being optionally removed post-translationally to improve the homogeneity of the final therapeutic ADC product.

[0216] DL may be conjugated to the antibody through the sidechain of the asparagine at position 302 of SEQ ID NO.3. The structure of the linkage to the antibody may be N-[GlcNAc]-DL, wherein N is the asparagine residue, and [GlcNAc] represents a GlcNAc residue. p may be up to 2, and is typically greater than 1.9.

Definitions

AXL Binding

[0217] The “first target protein” (FTP) as used herein may be AXL.

[0218] As used herein, “binds AXL” is used to mean the antibody binds AXL with a higher affinity than a non-specific partner such as Bovine Serum Albumin (BSA, Genbank accession no. CAA76847, version no. CAA76847.1 GI:3336842, record update date: Jan. 7, 2011 02:30 PM). In some embodiments the antibody binds AXL with an association constant (K_a) at least 2, 3, 4, 5, 10, 20,

example, in some embodiments the antibody can bind AXL with a K_D equal to or less than about 10^{-6} M, such as 1×10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} , 10^{-10} , 10^{-11} , 10^{-12} , 10^{-13} or 10^{-14} .

[0219] As used herein, “binds AXL” is used to mean the antibody binds AXL with a higher affinity than a non-specific partner such as Bovine Serum Albumin (BSA, Genbank accession no. CAA76847, version no. CAA76847.1 GI:3336842, record update date: Jan. 7, 2011 02:30 PM). In some embodiments the antibody binds AXL with an association constant (K_a) at least 2, 3, 4, 5, 10, 20, 50, 100, 200, 500, 1000, 2000, 5000, 104, 105 or 106-fold higher than the antibody's association constant for BSA, when measured at physiological conditions. The antibodies of the invention can bind AXL with a high affinity. For example, in some embodiments the antibody can bind AXL with a K_D equal to or less than about 10^{-6} M, such as 1×10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} , 10^{-10} , 10^{-11} , 10^{-12} , 10^{-13} or 10^{-14} .

[0220] AXL is member of the human TAM family of receptor tyrosine kinases. In some embodiments, the AXL polypeptide corresponds to Genbank accession no. AAH32229, version no. AAH32229.1 GI:21619004, record update date: Mar. 6, 2012 01:18 PM (SEQ ID NO.9). In one embodiment, the nucleic acid encoding AXL polypeptide corresponds to Genbank accession no. M76125, version no. M76125.1 GI:292869, record update date: Jun. 23, 2010 08:53 AM. In some embodiments, the AXL polypeptide has the sequence of SEQ ID NO.23.

Substituents

[0221] The phrase “optionally substituted” as used herein, pertains to a parent group which may be unsubstituted or which may be substituted.

[0222] Unless otherwise specified, the term “substituted” as used herein, pertains to a parent group which bears one or more substituents. The term “substituent” is used herein in the conventional sense and refers to a chemical moiety which is covalently attached to, or if appropriate, fused to, a parent group. A wide variety of substituents are well known, and methods for their formation and introduction into a variety of parent groups are also well known.

[0223] Examples of substituents are described in more detail below.

[0224] C_{1-12} alkyl: The term “ C_{1-12} alkyl” as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from a carbon atom of a hydrocarbon compound having from 1 to 12 carbon atoms, which may be aliphatic or alicyclic, and which may be saturated or unsaturated (e.g. partially unsaturated, fully unsaturated). The term “ C_{1-4} alkyl” as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from a carbon atom of a hydrocarbon compound having from 1 to 4 carbon atoms, which may be aliphatic or alicyclic, and which may be saturated or unsaturated (e.g. partially unsaturated, fully unsaturated). Thus, the term “alkyl” includes the sub-classes alkenyl, alkynyl, cycloalkyl, etc., discussed below.

[0225] Examples of saturated alkyl groups include, but are not limited to, methyl (C_1), ethyl (C_2), propyl (C_3), butyl (C_4), pentyl (C_5), hexyl (C_6) and heptyl (C_7).

[0226] Examples of saturated linear alkyl groups include, but are not limited to, methyl (C_1), ethyl (C_2), n-propyl (C_3), n-butyl (C_4), n-pentyl (amyl) (C_5), n-hexyl (C_6) and n-heptyl (C_7).

[0227] Examples of saturated branched alkyl groups include iso-propyl (C_3), iso-butyl (C_4), sec-butyl (C_4), tert-butyl (C_4), iso-pentyl (C_5), and neo-pentyl (C_5).

[0228] C_{2-12} Alkenyl: The term “ C_{2-12} alkenyl” as used herein, pertains to an alkyl group having one or more carbon-carbon double bonds.

[0229] Examples of unsaturated alkenyl groups include, but are not limited to, ethenyl (vinyl, $-\text{CH}=\text{CH}_2$), 1-propenyl ($-\text{CH}=\text{CH}-\text{CH}_3$), 2-propenyl (allyl, $-\text{CH}=\text{CH}_2$), isopropenyl (1-methylvinyl, $-\text{C}(\text{CH}_3)=\text{CH}_2$), butenyl (C_4), pentenyl (C_5), and hexenyl (C_6).

[0230] C_{2-12} alkynyl: The term “ C_{2-12} alkynyl” as used herein, pertains to an alkyl group having one or more carbon-carbon triple bonds.

[0231] Examples of unsaturated alkynyl groups include, but are not limited to, ethynyl ($-\text{C}\equiv\text{CH}$) and 2-propynyl (propargyl, $-\text{CH}_2-\text{C}\equiv\text{CH}$).

[0232] C_{3-12} cycloalkyl: The term “ C_{3-12} cycloalkyl” as used herein, pertains to an alkyl group which is also a cyclyl group; that is, a monovalent moiety obtained by removing a hydrogen atom from an alicyclic ring atom of a cyclic hydrocarbon (carbocyclic) compound, which moiety has from 3 to 7 carbon atoms, including from 3 to 7 ring atoms.

[0233] Examples of cycloalkyl groups include, but are not limited to, those derived from:

[0234] saturated monocyclic hydrocarbon compounds: cyclopropane (C_3), cyclobutane (C_4), cyclopentane (C_5), cyclohexane (C_6), cycloheptane (C_7), methylcyclopropane (C_4), dimethylcyclopropane (C_5), methylcyclobutane (C_5), dimethylcyclobutane (C_6), methylcyclopentane (C_6), dimethylcyclopentane (C_7) and methylcyclohexane (C_7);

[0235] unsaturated monocyclic hydrocarbon compounds: cyclopropene (C_3), cyclobutene (C_4), cyclopentene (C_5), cyclohexene (C_6), methylcyclopropene (C_4), dimethylcyclo-

propene (C_5), methylcyclobutene (C_5), dimethylcyclobutene (C_6), methylcyclopentene (C_6), dimethylcyclopentene (C_7) and methylcyclohexene (C_7); and

[0236] saturated polycyclic hydrocarbon compounds: norcarane (C_7), norpinane (C_7), norbornane (C_7).

[0237] C_{3-20} heterocyclyl: The term “ C_{3-20} heterocyclyl” as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from a ring atom of a heterocyclic compound, which moiety has from 3 to 20 ring atoms, of which from 1 to 10 are ring heteroatoms. Preferably, each ring has from 3 to 7 ring atoms, of which from 1 to 4 are ring heteroatoms.

[0238] In this context, the prefixes (e.g. C_{3-20} , C_{3-7} , C_{5-6} , etc.) denote the number of ring atoms, or range of number of ring atoms, whether carbon atoms or heteroatoms. For example, the term “ C_{5-6} heterocyclyl”, as used herein, pertains to a heterocyclyl group having 5 or 6 ring atoms.

[0239] Examples of monocyclic heterocyclyl groups include, but are not limited to, those derived from:

N_1 : aziridine (C_3), azetidine (C_4), pyrrolidine (tetrahydropyrrole) (C_5), pyrroline (e.g., 3-pyrroline, 2,5-dihydropyrrole) (C_5), 2H-pyrrole or 3H-pyrrole (isopyrrole, isoazole) (C_5), piperidine (C_6), dihydropyridine (C_6), tetrahydropyridine (C_6), azepine (C_7);

O_1 : oxirane (C_3), oxetane (C_4), oxolane (tetrahydrofuran) (C_5), oxole (dihydrofuran) (C_5), oxane (tetrahydropyran) (C_6), dihydropyran (C_6), pyran (C_6), oxepin (C_7);

S_1 : thiirane (C_3), thietane (C_4), thiolane (tetrahydrothiophene) (C_5), thiane (tetrahydrothiopyran) (C_6), thiopane (C_7);

O_2 : dioxolane (C_5), dioxane (C_6), and dioxepane (C_7);

O_3 : trioxane (C_6);

N_2 : imidazolidine (C_5), pyrazolidine (diazolidine) (C_5), imidazoline (C_5), pyrazoline (dihydropyrazole) (C_5), piperazine (C_6);

N_1O_1 : tetrahydrooxazole (C_5), dihydrooxazole (C_5), tetrahydroisoxazole (C_5), dihydroisoxazole (C_5), morpholine (C_6), tetrahydrooxazine (C_6), dihydrooxazine (C_6), oxazine (C_6);

N_1S_1 : thiazoline (C_5), thiazolidine (C_5), thiomorpholine (C_6);

N_2O_1 : oxadiazine (C_6);

O_1S_1 : oxathiole (C_5) and oxathiane (thioxane) (C_6); and,

$N_1O_1S_1$: oxathiazine (C_6).

[0240] Examples of substituted monocyclic heterocyclyl groups include those derived from saccharides, in cyclic form, for example, furanoses (C_5), such as arabinofuranose, lyxofuranose, ribofuranose, and xylofuranose, and pyranoses (C_6), such as allopentanose, altropentanose, glucopyranose, mannopyranose, gulopyranose, idopyranose, galactopyranose, and talopyranose.

[0241] C_{5-20} aryl: The term “ C_{5-20} aryl”, as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from an aromatic ring atom of an aromatic compound, which moiety has from 3 to 20 ring atoms. The term “ C_{5-7} aryl”, as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from an aromatic ring atom of an aromatic compound, which moiety has from 5 to 7 ring atoms and the term “ C_{5-10} aryl”, as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from an aromatic ring atom of an

aromatic compound, which moiety has from 5 to 10 ring atoms. Preferably, each ring has from 5 to 7 ring atoms.

[0242] In this context, the prefixes (e.g., C₃₋₂₀, C₅₋₇, C₅₋₆, C₅₋₁₀, etc.) denote the number of ring atoms, or range of number of ring atoms, whether carbon atoms or heteroatoms. For example, the term “C₅₋₆ aryl” as used herein, pertains to an aryl group having 5 or 6 ring atoms.

[0243] The ring atoms may be all carbon atoms, as in “carboaryl groups”.

[0244] Examples of carboaryl groups include, but are not limited to, those derived from benzene (i.e. phenyl) (C₆), naphthalene (C₁₀), azulene (C₁₀), anthracene (C₁₄), phenanthrene (C₁₄), naphthacene (C₁₈), and pyrene (C₁₆).

[0245] Examples of aryl groups which comprise fused rings, at least one of which is an aromatic ring, include, but are not limited to, groups derived from indane (e.g. 2,3-dihydro-1H-indene) (C₉), indene (C₉), isoindene (C₉), tetraline (1,2,3,4-tetrahydronaphthalene (C₁₀), acenaphthene (C₁₂), fluorene (C₁₃), phenalene (C₁₃), acephenanthrene (C₁₅), and aceanthrene (C₁₆).

[0246] Alternatively, the ring atoms may include one or more heteroatoms, as in “heteroaryl groups”. Examples of monocyclic heteroaryl groups include, but are not limited to, those derived from:

N₁: pyrrole (azole) (C₅), pyridine (azine) (C₆);

O₁: furan (oxole) (C₅);

S₁: thiophene (thiole) (C₅);

N₁O₁: oxazole (C₅), isoxazole (C₅), isoxazine (C₆);

N₂O₁: oxadiazole (furazan) (C₅);

N₃O₁: oxatriazole (C₅);

N₁S₁: thiazole (C₅), isothiazole (C₅);

N₂: imidazole (1,3-diazole) (C₅), pyrazole (1,2-diazole) (C₅), pyridazine (1,2-diazine) (C₆), pyrimidine (1,3-diazine) (C₆) (e.g., cytosine, thymine, uracil), pyrazine (1,4-diazine) (C₆);

N₃: triazole (C₅), triazine (C₆); and,

N₄: tetrazole (C₅).

[0247] Examples of heteroaryl which comprise fused rings, include, but are not limited to:

[0248] C₉ (with 2 fused rings) derived from benzofuran (O₁), isobenzofuran (O₁), indole (N₁), isoindole (N₁), indolizine (N₁), indoline (N₁), isoindoline (N₁), purine (N₄) (e.g., adenine, guanine), benzimidazole (N₂), indazole (N₂), benzoxazole (N₁O₁), benzisoxazole (N₁O₁), benzodioxole (O₂), benzofurazan (N₂O₁), benzotriazole (N₃), benzothiofuran (S₁), benzothiazole (N₁S₁), benzothiadiazole (N₂S);

[0249] C₁₀ (with 2 fused rings) derived from chromene (O₁), isochromene (O₁), chroman (O₁), isochroman (O₁), benzodioxan (O₂), quinoline (N₁), isoquinoline (N₁), quinolizine (N₁), benzoxazine (N₁O₁), benzodiazine (N₂), pyridopyridine (N₂), quinoxaline (N₂), quinazoline (N₂), cinnoline (N₂), phthalazine (N₂), naphthyridine (N₂), pteridine (N₄);

[0250] C₁₁ (with 2 fused rings) derived from benzodiazepine (N₂);

[0251] C₁₃ (with 3 fused rings) derived from carbazole (N₁), dibenzofuran (O₁), dibenzothiophene (S₁), carboline (N₂), perimidine (N₂), pyridoindole (N₂); and,

[0252] C₁₄ (with 3 fused rings) derived from acridine (N₁), xanthene (O₁), thioxanthene (S₁), oxanthrene (O₂), phenoxathiin (O₁S₁), phenazine (N₂), phenox-

azine (N₁O₁), phenothiazine (N₁S₁), thianthrene (S₂), phenanthridine (N₁), phenanthroline (N₂), phenazine (N₂).

[0253] The above groups, whether alone or part of another substituent, may themselves optionally be substituted with one or more groups selected from themselves and the additional substituents listed below.

[0254] Halo: —F, —Cl, —Br, and —I.

[0255] Hydroxy: —OH.

[0256] Ether: —OR, wherein R is an ether substituent, for example, a C₁₋₇ alkyl group (also referred to as a C₁₋₇ alkoxy group, discussed below), a C₃₋₂₀ heterocyclyl group (also referred to as a C₃₋₂₀ heterocycloxy group), or a C₅₋₂₀ aryl group (also referred to as a C₅₋₂₀ aryloxy group), preferably a C₁₋₇alkyl group.

[0257] Alkoxy: —OR, wherein R is an alkyl group, for example, a C₁₋₇ alkyl group. Examples of C₁₋₇ alkoxy groups include, but are not limited to, —OMe (methoxy), —OEt (ethoxy), —O(nPr) (n-propoxy), —O(iPr) (isopropoxy), —O(nBu) (n-butoxy), —O(sBu) (sec-butoxy), —O(iBu) (isobutoxy), and —O(tBu) (tert-butoxy).

[0258] Carboxy (carboxylic acid): —C(=O)OH.

[0259] Ester (carboxylate, carboxylic acid ester, oxycarbonyl): —C(=O)OR, wherein R is an ester substituent, for example, a C₁₋₇ alkyl group, a C₃₋₂₀ heterocyclyl group, or a C₅₋₂₀ aryl group, preferably a C₁₋₇ alkyl group. Examples of ester groups include, but are not limited to, —C(=O)OCH₃, —C(=O)OCH₂CH₃, —C(=O)OC(CH₃)₃, and —C(=O)OPh.

[0260] Amino: —NR¹R², wherein R¹ and R² are independently amino substituents, for example, hydrogen, a C₁₋₇ alkyl group (also referred to as C₁₋₇ alkylamino or di-C₁₋₇ alkylamino), a C₃₋₂₀ heterocyclyl group, or a C₅₋₂₀ aryl group, preferably H or a C₁₋₇ alkyl group, or, in the case of a “cyclic” amino group, R¹ and R², taken together with the nitrogen atom to which they are attached, form a heterocyclic ring having from 4 to 8 ring atoms. Amino groups may be primary (—NH₂), secondary (—NHR¹), or tertiary (—NHR¹R²), and in cationic form, may be quaternary (—NR¹R²R³). Examples of amino groups include, but are not limited to, —NH₂, —NHCH₃, —NHC(CH₃)₂, —N(CH₃)₂, —N(CH₂CH₃)₂, and —NHPh. Examples of cyclic amino groups include, but are not limited to, aziridino, azetidino, pyrrolidino, piperidino, piperazino, morpholino, and thiomorpholino.

[0261] Amido (carbamoyl, carbamyl, aminocarbonyl, carboxamide): —C(=O)NR¹R², wherein R¹ and R² are independently amino substituents, as defined for amino groups. Examples of amido groups include, but are not limited to, —C(=O)NH₂, —C(=O)NHCH₃, —C(=O)N(CH₃)₂, —C(=O)NHCH₂CH₃, and —C(=O)N(CH₂CH₃)₂, as well as amido groups in which R¹ and R², together with the nitrogen atom to which they are attached, form a heterocyclic structure as in, for example, piperidinocarbonyl, morpholinocarbonyl, thiomorpholinocarbonyl, and piperazinocarbonyl.

[0262] Nitro: —NO₂.

[0263] Azido: —N₃.

[0264] Cyano (nitrile, carbonitrile): —CN.

Drug Loading

[0265] The drug loading is the average number of PBD drugs per antibody, e.g. antibody.

[0266] The average number of drugs per antibody in preparations of ADC from conjugation reactions may be characterized by conventional means such as UV, reverse phase HPLC, HIC, mass spectroscopy, ELISA assay, and electrophoresis. The quantitative distribution of ADC in terms of p may also be determined. By ELISA, the averaged value of p in a particular preparation of ADC may be determined (Hamblett et al (2004) Clin. Cancer Res. 10:7063-7070; Sanderson et al (2005) Clin. Cancer Res. 11:843-852). However, the distribution of p (drug) values is not discernible by the antibody-antigen binding and detection limitation of ELISA. Also, ELISA assay for detection of antibody-drug conjugates does not determine where the drug moieties are attached to the antibody, such as the heavy chain or light chain fragments, or the particular amino acid residues. In some instances, separation, purification, and characterization of homogeneous ADC where p is a certain value from ADC with other drug loadings may be achieved by means such as reverse phase HPLC or electrophoresis. Such techniques are also applicable to other types of conjugates.

[0267] For the present antibody-drug conjugates, p is limited by the number of attachment sites on the antibody, i.e. the number of azide groups. For example, the antibody may have only one or two azide groups to which the drug linker may be attached.

[0268] Typically, fewer than the theoretical maximum of drug moieties are conjugated to an antibody during a conjugation reaction. The loading (drug/antibody ratio) of an ADC may be controlled in several different manners, including: (i) limiting the molar excess of drug-linker intermediate (D-L) or linker reagent relative to antibody, and (ii) limiting the conjugation reaction time or temperature.

[0269] Where more than one nucleophilic or electrophilic group of the antibody reacts with a drug-linker intermediate, or linker reagent followed by drug moiety reagent, then the resulting product is a mixture of ADC compounds with a distribution of drug moieties attached to an antibody, e.g. 1, 2, 3, etc. Liquid chromatography methods such as polymeric reverse phase (PLRP) and hydrophobic interaction (HIC) may separate compounds in the mixture by drug loading value. Preparations of ADC with a single drug loading value (p) may be isolated, however, these single loading value ADCs may still be heterogeneous mixtures because the drug moieties may be attached, via the linker, at different sites on the antibody.

[0270] Thus the antibody-drug conjugate compositions of the invention include mixtures of antibody-drug conjugate compounds where the antibody has one or more PBD drug moieties and where the drug moieties may be attached to the antibody at various amino acid residues.

[0271] In one embodiment, the average number of dimer pyrrolobenzodiazepine groups per antibody is in the range 1 to 8. In some embodiments the range is selected from 1 to 4, 1 to 4, 2 to 4, and 1 to 3.

[0272] In some embodiments, there are one or two dimer pyrrolobenzodiazepine groups per antibody.

Includes Other Forms

[0273] Unless otherwise specified, included in the above are the well known ionic, salt, solvate, and protected forms of these substituents. For example, a reference to carboxylic acid ($-\text{COOH}$) also includes the anionic (carboxylate) form ($-\text{COO}^-$), a salt or solvate thereof, as well as conventional

protected forms. Similarly, a reference to an amino group includes the protonated form ($-\text{N}^+\text{HR}^1\text{R}^2$), a salt or solvate of the amino group, for example, a hydrochloride salt, as well as conventional protected forms of an amino group. Similarly, a reference to a hydroxyl group also includes the anionic form ($-\text{O}^-$), a salt or solvate thereof, as well as conventional protected forms.

Salts

[0274] It may be convenient or desirable to prepare, purify, and/or handle a corresponding salt of the active compound, for example, a pharmaceutically-acceptable salt. Examples of pharmaceutically acceptable salts are discussed in Berge, et al., *J. Pharm. Sci.*, 66, 1-19 (1977).

[0275] For example, if the compound is anionic, or has a functional group which may be anionic (e.g. $-\text{COOH}$ may be $-\text{COO}^-$), then a salt may be formed with a suitable cation. Examples of suitable inorganic cations include, but are not limited to, alkali metal ions such as Na^+ and K^+ , alkaline earth cations such as Ca^{2+} and Mg^{2+} , and other cations such as Al^{+3} . Examples of suitable organic cations include, but are not limited to, ammonium ion (i.e. NH_4^+) and substituted ammonium ions (e.g. NH_3R^+ , NH_2R_2^+ , NHR_3^+ , NR_4^+). Examples of some suitable substituted ammonium ions are those derived from: ethylamine, diethylamine, dicyclohexylamine, triethylamine, butylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine, benzylamine, phenylbenzylamine, choline, meglumine, and tromethamine, as well as amino acids, such as lysine and arginine. An example of a common quaternary ammonium ion is $\text{N}(\text{CH}_3)_4^+$.

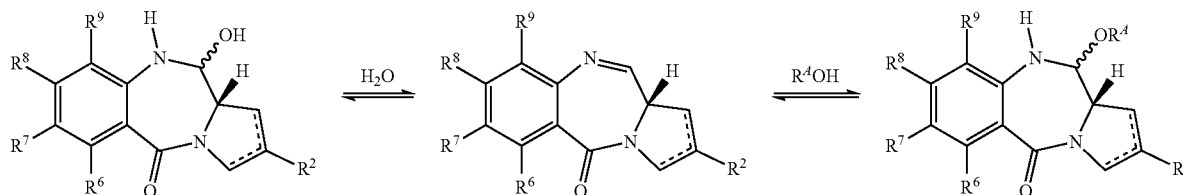
[0276] If the compound is cationic, or has a functional group which may be cationic (e.g. $-\text{NH}_2$ may be $-\text{NH}_3^+$), then a salt may be formed with a suitable anion. Examples of suitable inorganic anions include, but are not limited to, those derived from the following inorganic acids: hydrochloric, hydrobromic, hydroiodic, sulfuric, sulfurous, nitric, nitrous, phosphoric, and phosphorous.

[0277] Examples of suitable organic anions include, but are not limited to, those derived from the following organic acids: 2-acetoxybenzoic, acetic, ascorbic, aspartic, benzoic, camphorsulfonic, cinnamic, citric, edetic, ethanedithiolonic, ethanesulfonic, fumaric, glucoheptonic, gluconic, glutamic, glycolic, hydroxymaleic, hydroxynaphthalene carboxylic, isethionic, lactic, lactobionic, lauric, maleic, malic, methanesulfonic, mucic, oleic, oxalic, palmitic, pantoic, pantothenic, phenylacetic, phenylsulfonic, propionic, pyruvic, salicylic, stearic, succinic, sulfanilic, tartaric, toluenesulfonic, trifluoroacetic acid and valeric. Examples of suitable polymeric organic anions include, but are not limited to, those derived from the following polymeric acids: tannic acid, carboxymethyl cellulose.

Solvates

[0278] It may be convenient or desirable to prepare, purify, and/or handle a corresponding solvate of the active compound. The term "solvate" is used herein in the conventional sense to refer to a complex of solute (e.g. active compound, salt of active compound) and solvent. If the solvent is water, the solvate may be conveniently referred to as a hydrate, for example, a mono-hydrate, a di-hydrate, a tri-hydrate, etc.

[0279] The invention includes compounds where a solvent adds across the imine bond of the PBD moiety, which is illustrated below where the solvent is water or an alcohol (R^4OH , where R^4 is C_{1-4} alkyl):



[0280] These forms can be called the carbinolamine and carbinolamine ether forms of the PBD (as described in the section relating to R^{10} above). The balance of these equilibria depend on the conditions in which the compounds are found, as well as the nature of the moiety itself.

[0281] These particular compounds may be isolated in solid form, for example, by lyophilisation.

Isomers

[0282] Certain compounds of the invention may exist in one or more particular geometric, optical, enantiomeric, diastereomeric, epimeric, atropic, stereoisomeric, tautomeric, conformational, or anomeric forms, including but not limited to, cis- and trans-forms; E- and Z-forms; c-, t-, and r-forms; endo- and exo-forms; R-, S-, and meso-forms; D- and L-forms; d- and l-forms; (+) and (−) forms; keto-, enol-, and enolate-forms; syn- and anti-forms; synclinal- and anticlinal-forms; α - and β -forms; axial and equatorial forms; boat-, chair-, twist-, envelope-, and halfchair-forms; and combinations thereof, hereinafter collectively referred to as “isomers” (or “isomeric forms”).

[0283] The term “chiral” refers to molecules which have the property of non-superimposability of the mirror image partner, while the term “achiral” refers to molecules which are superimposable on their mirror image partner.

[0284] The term “stereoisomers” refers to compounds which have identical chemical constitution, but differ with regard to the arrangement of the atoms or groups in space.

[0285] “Diastereomer” refers to a stereoisomer with two or more centers of chirality and whose molecules are not mirror images of one another. Diastereomers have different physical properties, e.g. melting points, boiling points, spectral properties, and reactivities. Mixtures of diastereomers may separate under high resolution analytical procedures such as electrophoresis and chromatography.

[0286] “Enantiomers” refer to two stereoisomers of a compound which are non-superimposable mirror images of one another.

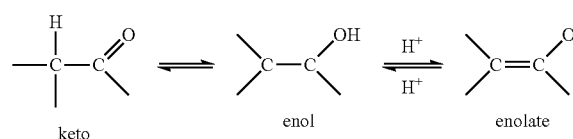
[0287] Stereochemical definitions and conventions used herein generally follow S. P. Parker, Ed., *McGraw-Hill Dictionary of Chemical Terms* (1984) McGraw-Hill Book Company, New York; and Eliel, E. and Wilen, S., “Stereochemistry of Organic Compounds”, John Wiley & Sons, Inc., New York, 1994. The compounds of the invention may contain asymmetric or chiral centers, and therefore exist in different stereoisomeric forms. It is intended that all stereoisomeric forms of the compounds of the invention, including but not limited to, diastereomers, enantiomers and atropisomers, as well as mixtures thereof such as racemic mixtures,

form part of the present invention. Many organic compounds exist in optically active forms, i.e., they have the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L, or R and

S, are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and l or (+) and (−) are employed to designate the sign of rotation of plane-polarized light by the compound, with (−) or l meaning that the compound is levorotatory. A compound prefixed with (+) or d is dextrorotatory. For a given chemical structure, these stereoisomers are identical except that they are mirror images of one another. A specific stereoisomer may also be referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture or a racemate, which may occur where there has been no stereoselection or stereospecificity in a chemical reaction or process. The terms “racemic mixture” and “racemate” refer to an equimolar mixture of two enantiomeric species, devoid of optical activity.

[0288] Note that, except as discussed below for tautomeric forms, specifically excluded from the term “isomers”, as used herein, are structural (or constitutional) isomers (i.e. isomers which differ in the connections between atoms rather than merely by the position of atoms in space). For example, a reference to a methoxy group, $—OCH_3$, is not to be construed as a reference to its structural isomer, a hydroxymethyl group, $—CH_2OH$. Similarly, a reference to ortho-chlorophenyl is not to be construed as a reference to its structural isomer, meta-chlorophenyl. However, a reference to a class of structures may well include structurally isomeric forms falling within that class (e.g. C_{1-7} alkyl includes n-propyl and iso-propyl; butyl includes n-, iso-, sec-, and tert-butyl; methoxyphenyl includes ortho-, meta-, and para-methoxyphenyl).

[0289] The above exclusion does not pertain to tautomeric forms, for example, keto-, enol-, and enolate-forms, as in, for example, the following tautomeric pairs: keto/enol (illustrated below), imine/enamine, amide/imino alcohol, amidine/amidine, nitroso/oxime, thioketone/enethiol, N-nitroso/hydroxyazo, and nitro/aci-nitro.



[0290] The term “tautomer” or “tautomeric form” refers to structural isomers of different energies which are interconvertible via a low energy barrier. For example, proton

tautomers (also known as prototropic tautomers) include interconversions via migration of a proton, such as keto-enol and imine-enamine isomerizations. Valence tautomers include interconversions by reorganization of some of the bonding electrons.

[0291] Note that specifically included in the term “isomer” are compounds with one or more isotopic substitutions. For example, H may be in any isotopic form, including ^1H , ^2H (D), and ^3H (T); C may be in any isotopic form, including ^{12}C , ^{13}C , and ^{14}C ; O may be in any isotopic form, including ^{16}O and ^{18}O ; and the like.

[0292] Examples of isotopes that can be incorporated into compounds of the invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorus, fluorine, and chlorine, such as, but not limited to ^2H (deuterium, D), ^3H (tritium), ^{11}C , ^{13}C , ^{14}C , ^{15}N , ^{18}F , ^{31}P , ^{32}P , ^{35}S , ^{36}Cl , and ^{125}I . Various isotopically labeled compounds of the present invention, for example those into which radioactive isotopes such as ^3H , ^{13}C , and ^{14}C are incorporated. Such isotopically labeled compounds may be useful in metabolic studies, reaction kinetic studies, detection or imaging techniques, such as positron emission tomography (PET) or single-photon emission computed tomography (SPECT) including drug or substrate tissue distribution assays, or in radioactive treatment of patients. Deuterium labeled or substituted therapeutic compounds of the invention may have improved DMPK (drug metabolism and pharmacokinetics) properties, relating to distribution, metabolism, and excretion (ADME). Substitution with heavier isotopes such as deuterium may afford certain therapeutic advantages resulting from greater metabolic stability, for example increased in vivo half-life or reduced dosage requirements. An ^{18}F labeled compound may be useful for PET or SPECT studies. Isotopically labeled compounds of this invention and prodrugs thereof can generally be prepared by carrying out the procedures disclosed in the schemes or in the examples and preparations described below by substituting a readily available isotopically labeled reagent for a non-isotopically labeled reagent. Further, substitution with heavier isotopes, particularly deuterium (i.e., ^2H or D) may afford certain therapeutic advantages resulting from greater metabolic stability, for example increased in vivo half-life or reduced dosage requirements or an improvement in therapeutic index. It is understood that deuterium in this context is regarded as a substituent. The concentration of such a heavier isotope, specifically deuterium, may be defined by an isotopic enrichment factor. In the compounds of this invention any atom not specifically designated as a particular isotope is meant to represent any stable isotope of that atom.

[0293] Unless otherwise specified, a reference to a particular compound includes all such isomeric forms, including (wholly or partially) racemic and other mixtures thereof. Methods for the preparation (e.g. asymmetric synthesis) and separation (e.g. fractional crystallisation and chromatographic means) of such isomeric forms are either known in the art or are readily obtained by adapting the methods taught herein, or known methods, in a known manner.

Secondary Agents

[0294] The recent development of agents that enhance anti-tumor immunity is rapidly changing the treatment of a broad range of cancers. However, these treatments are not effective in all cancer types, responses are often not durable,

and many patients receive little or no benefit from treatment. The prevailing assumption in the oncology field is that only combinations of immune-therapies with other treatment options will ultimately be able to cure cancer patients.

[0295] The ADC is well tolerated and active across a range of cancer types, and will likely be one component of combination therapies that increase the response rate and durability of treatment. The purpose of this disclosure is to combine the ADC with the secondary agent.

[0296] A secondary agent as described herein may be an Immune-oncology (IO) drug.

[0297] Immune-oncology (IO) drugs, a type of cancer therapy relying on the body's immune system to help fight cancer, have shown enhanced durability of anti-tumor response. There are different types of IO, including but not limited to PD1 inhibitors, PD-L1 inhibitors, CTLA4 inhibitors, GITR agonists and OX40 agonists. Due to the considerable fraction of patients who are not cured by single agent immunotherapies and ultimately relapse, combination treatments with alternative IO drugs or different therapeutic modalities are needed (see K S Peggs et al. 2009, *Clinical and Experimental Immunology*, 157: 9-19 [doi:10.1111/j.1365-2249.2009.03912.x]; D M Pardoll 2012 [doi:10.1038/nrc3239]).

[0298] Immunogenic cell death (ICD) is a particular form of cell death that stimulates an immune response against dead-cell antigens (released by dying cells) and it is considered as one of the best way to induce an adaptive immune response and improve the efficacy of anti-cancer treatment. This process is frequently suboptimal, calling for combinatorial strategies that attempt to restore the full immunogenicity of cell death for therapeutic purposes. There are several anti-neoplastic agents that can induce ICD such as various anthracyclines (including doxorubicin, epirubicin and idarubicin), alkylating agents (including oxaliplatin and cyclophosphamide), the topoisomerase II inhibitor mitoxantrone, and the proteasomal inhibitor Bortezomib.

[0299] Antibody-drug conjugates, including those with a PBD warhead, may be particularly suited as combination partners because they are more targeted compared to conventional chemotherapy and expected to offer an increased antigen presentation to infiltrating cells as has been shown for auristatin-based ADCs.

[0300] Combining ADCs with IO therefore allows for dual benefits: on the one hand, the ADC will directly kill the tumor expressing the target, providing immediate anti-tumor activity, and on the other the immunogenic cell death induced by ADC mediated cell kill may boost a stronger and more durable adaptive immune response, as compared to when the IO is given as a single agent.

[0301] The secondary agent may be:

[0302] (a) a PD1 antagonist, such as pembrolizumab, nivolumab, MEDI0680, PDR001 (spartalizumab), Camrelizumab, AUNP12, Pidilizumab, Cemiplimab (REGN-2810), AMP-224, BGB-A317 (Tislelizumab), or BGB-108;

[0303] (b) a PD-L1 antagonist, such as atezolizumab (Tecentriq), BMS-936559/MDX-1105, durvalumab/MEDI4736, or MSB0010718C (Avelumab);

[0304] (c) a GITR (Glucocorticoid-Induced TNFR-Related protein) agonist, such as MEDI1873, TRX518, GWN323, MK-1248, MK-4166, BMS-986156 or INCAGN1876;

- [0305] (d) an OX40 agonist, such as MEDI0562, MEDI6383, MOXR0916, RG7888, OX40mAb24, INCAGN1949, GSK3174998, or PF-04518600;
 - [0306] (e) a CTLA-4 antagonist, such as ipilimumab (brand name Yervoy) or Tremelimumab (Originally developed by Pfizer, now Medimmune);
 - [0307] (f) Fludarabine or Cytarabine;
 - [0308] (g) a hypomethylating agent, such as cytidine analogs—for example, 5-azacytidine (azacitidine) and 5-aza-2'-deoxycytidine (decitabine); or
 - [0309] (h) a PARP inhibitor (PARPi), such as Olaparib, CEP-9722, BMN-673/talazoparib, Rucaparib, Iniparib/SAR24-550/BSI-201, Veliparib (ABT-888), Niraparib/MK-4827, BGB-290, 3-aminobenzamide, and E7016;
 - [0310] (i) an agent that upregulates HER2 expression, such as gemcitabine and tamoxifen;
 - [0311] (j) an AXL-kinase inhibitor (AXLi) such as BGB324 (bemcentinib), TP0903, Gilteritinib (ASP2215), Cabozantinib (XL184), SG17079, Meresitinib, amuvatinib (MP-470), bosutinib (SKI-606), MGCD265, and foretinib (GSK1363089/XL880);
 - [0312] (k) a BRAF inhibitor (BRAFi), such as vemurafenib, PLX4720, dabrafenib, Sorafenib, Encorafenib, and GDC0879; or
 - [0313] (l) a MEK inhibitor (MEKi), such as Trametinib, Cobimetinib, Binimetinib, Selumetinib, PD-325901, CI-1040, PD035901, U0126 and TAK-733.
- [0314] Each of these classes of secondary agent is described in more detail below.

PD1 Antagonists

[0315] Programmed death receptor I (PD1) is an immune-inhibitory receptor that is primarily expressed on activated T and B cells. Interaction with its ligands has been shown to attenuate T-cell responses both in vitro and in vivo. Blockade of the interaction between PD1 and one of its ligands, PD-L1, has been shown to enhance tumor-specific CD8+ T-cell immunity and may therefore be helpful in clearance of tumor cells by the immune system.

[0316] PD1 (encoded by the gene *Pdcd1*) is an Immunoglobulin superfamily member related to CD28, and CTLA-4. PD1 has been shown to negatively regulate antigen receptor signalling upon engagement of its ligands (PD-L1 and/or PD-L2). The structure of murine PD1 has been solved as well as the co-crystal structure of mouse PD1 with human PD-L1 (Zhang, X., et al., (2004) *Immunity* 20: 337-347; Lin, et al., (2008) *Proc. Natl. Acad. Sci. USA* 105: 301 1-6). PD1 and like family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail that is responsible for the binding of signaling molecules. The cytoplasmic tail of PD1 contains two tyrosine-based signaling motifs, an ITIM (immunoreceptor tyrosine-based inhibition motif) and an ITSM (immunoreceptor tyrosine-based switch motif).

[0317] In humans, expression of PD1 (on tumor infiltrating lymphocytes) and/or PD-L1 (on tumor cells) has been found in a number of primary tumor biopsies assessed by immunohistochemistry. Such tissues include cancers of the lung, liver, ovary, cervix, skin, colon, glioma, bladder, breast, kidney, esophagus, stomach, oral squamous cell, urothelial cell, and pancreas as well as tumors of the head and neck (Brown, J. A., et al., (2003) *J Immunol.* 170: 1257-1266; Dong H., et al., (2002) *Nat. Med.* 8: 793-800;

Winterle, et al., (2003) *Cancer Res.* 63: 7462-7467; Strome, S. E., et al., (2003) *Cancer Res.* 63: 6501-6505; Thompson, R. H., et al., (2006) *Cancer Res.* 66: 3381-5; Thompson, et al., (2007) *Clin. Cancer Res.* 13: 1757-61; Nomi, T., et al., (2007) *Clin. Cancer Res.* 13: 2151-7). More strikingly, PD-ligand expression on tumor cells has been correlated to poor prognosis of cancer patients across multiple tumor types (reviewed in Okazaki and Honjo, (2007) *Int. Immunol.* 19: 813-824).

[0318] To date, numerous studies have shown that interaction of PD1 with its ligands (PD-L1 and PD-L2) leads to the inhibition of lymphocyte proliferation in vitro and in vivo. Blockade of the PD1/PD-L1 interaction could lead to enhanced tumor-specific T-cell immunity and therefore be helpful in clearance of tumor cells by the immune system. To address this issue, a number of studies were performed. In a murine model of aggressive pancreatic cancer (Nomi, T., et al. (2007) *Clin. Cancer Res.* 13: 2151-2157), the therapeutic efficacy of PD1/PD-L1 blockade was demonstrated. Administration of either PD1 or PD-L1 directed antibody significantly inhibited tumor growth. Antibody blockade effectively promoted tumor reactive CD8+ T cell infiltration into the tumor resulting in the up-regulation of anti-tumor effectors including IFN gamma, granzyme B and perforin. Additionally, the authors showed that PD1 blockade can be effectively combined with chemotherapy to yield a synergistic effect. In another study, using a model of squamous cell carcinoma in mice, antibody blockade of PD1 or PD-L1 significantly inhibited tumor growth (Tsushima, F., et al., (2006) *Oral Oncol.* 42: 268-274).

[0319] "PD1 antagonist" means any chemical compound or biological molecule that stimulates an immune reaction through inhibition of PD1 signalling.

[0320] To examine the extent of enhancement of, e.g., PD1 activity, samples or assays comprising a given, e.g., protein, gene, cell, or organism, are treated with a potential activating or inhibiting agent and are compared to control samples treated with an inactive control molecule. Control samples are assigned a relative activity value of 100%. Inhibition is achieved when the activity value relative to the control is about 90% or less, typically 85% or less, more typically 80% or less, most typically 75% or less, generally 70% or less, more generally 65% or less, most generally 60% or less, typically 55% or less, usually 50% or less, more usually 45% or less, most usually 40% or less, preferably 35% or less, more preferably 30% or less, still more preferably 25% or less, and most preferably less than 20%. Activation is achieved when the activity value relative to the control is about 110%, generally at least 120%, more generally at least 140%, more generally at least 160%, often at least 180%, more often at least 2-fold, most often at least 2.5-fold, usually at least 5-fold, more usually at least 10-fold, preferably at least 20-fold, more preferably at least 40-fold, and most preferably over 40-fold higher.

[0321] Combining an ADC, which targets a first target protein (FTP) with PD1 inhibitors is advantageous, because on the one hand, the ADC will directly kill the FTP positive tumor cells, while on the other hand the PD1 inhibitor will engage the patient's own immune system to eliminate the cancer cells. Next to FTP(+) tumor cells, FTP negative tumor cells in close proximity to FTP(+) tumor cells will potentially be killed by the bystander mechanism of the PBD-dimer released after cell kill of CD19(+) or CD22(+) cells. Hence, the ADC will directly kill the tumor cells.

[0322] The resulting release of tumor associated antigens from cells that are killed with the PBD dimer will trigger the immune system, which will be further enhanced by the use of programmed cell death protein 1 (PD1) inhibitors, expressed on a large proportion of tumour infiltrating lymphocytes (TILs) from many different tumour types. Blockade of the PD1 pathway may enhance antitumour immune responses against the antigens released from the tumors killed by the ADC by diminishing the number and/or suppressive activity of intratumoral TReg cells.

[0323] The major function of PD1 is to limit the activity of T-cells at the time of an anti-inflammatory response to infection and to limit autoimmunity. PD1 expression is induced when T-cells become activated, and binding of one of its own ligands inhibits kinases involved in T-cell activation. Hence, in the tumor environment this may translate into a major immune resistance, because many tumours are highly infiltrated with TReg cells that probably further suppress effector immune responses. This resistance mechanism is alleviated by the use of PD1 inhibitors in combination with the ADC.

[0324] PD1 antagonists suitable for use as secondary agents in the present disclosure include:

[0325] a) a PD1 antagonist which inhibits the binding of PD1 to its ligand binding partners.

[0326] b) a PD1 antagonist which inhibits the binding of PD1 to PD-L1.

[0327] c) a PD1 antagonist which inhibits the binding of PD-1 to PDL2.

[0328] d) a PD1 antagonist which inhibits the binding of PD-1 to both PDL1 and PDL2.

[0329] e) a PD1 antagonist of parts (a) to (d) which is an antibody.

[0330] Specific PD1 antagonists suitable for use as secondary agents in the present disclosure include:

[0331] a) pembrolizumab (brand name Keytruda)

[0332] i. CAS Number→1374853-91-4

[0333] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0334] ii. NCBI Pubchem reference→254741536

[0335] (see <https://pubchem.ncbi.nlm.nih.gov/>)

[0336] iii. DrugBank reference→DB09037

[0337] (see <https://www.drugbank.ca/>)

[0338] iv. Unique Ingredient Identifier (UNII)→DPT003T46P

[0339] (see <http://www.fda.gov/ForIndustry/Data-Standards/SubstanceRegistrationSystem-Unique-IngredientIdentifierUNII/default.htm>)

[0340] b) nivolumab (brand name Opdivo)

[0341] i. CAS Number→946414-94-4

[0342] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0343] ii. DrugBank reference→DB09035

[0344] (see <https://www.drugbank.ca/>)

[0345] c) MEDI0680 (formerly AMP-514)

[0346] As described in WO2014/055648, WO2015/042246, WO2016/127052, WO2017/004016, WO2012/145493, U.S. Pat. No. 8,609,089, WO2016/007235, WO2016/011160; Int. J. Mol. Sci. 2016 July; 17(7): 1151, doi: 10.3390/ijms17071151; and Drug Discov Today, 2015 September; 20(9): 1127-34. doi: 10.1016/j.drudis.2015.07.003.

[0347] See also clinical trials NCT02271945 and NCT02013804 at <https://clinicaltrials.gov/ct2/home>

[0348] d) PDR001 (spartalizumab)

[0349] i. CAS Number→1935694-88-4

[0350] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0351] ii. Unique Ingredient Identifier (UNII)→QOG25L6Z8Z

[0352] (see <http://www.fda.gov/ForIndustry/Data-Standards/SubstanceRegistrationSystem-Unique-IngredientIdentifierUNII/default.htm>)

[0353] As described in WO2016/007235 and WO2016/011160

[0354] NCI thesaurus code→C121625

[0355] (see <https://ncit.nci.nih.gov/ncitbrowser/>)

[0356] e) Camrelizumab [INCSHR-1210] (Incyte)

[0357] i. CAS Number→1798286-48-2

[0358] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0359] ii. Unique Ingredient Identifier (UNII)→73096E137E

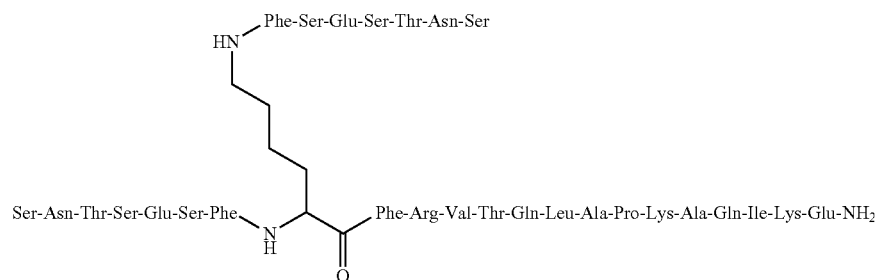
[0360] (see <http://www.fda.gov/ForIndustry/Data-Standards/SubstanceRegistrationSystem-Unique-IngredientIdentifierUNII/default.htm>)

[0361] f) AUNP12 (peptide) (Aurigene/PierreFabre)

[0362] i. Disclosed in WO2011/161699 as SEQ ID NO:49 a.k.a. "compound 8", see Example 2 on page 77 of the A2 publication of WO2011/161699.

[0363] ii. CAS Number→1353563-85-5

[0364] (see <http://www.cas.org/content/chemical-substances/faqs>)



-continued

or:



- [0365] g) Pidilizumab (CT-01 1)
- [0366] i. CAS Number→1036730-42-3
- [0367] (see <http://www.cas.org/content/chemical-substances/faqs>)
- [0368] ii. Unique Ingredient Identifier (UNII)→B932PAQ1BQ
- [0369] (see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)
- [0370] h) Cemiplimab (formerly REGN-2810, SAR-439684)
- [0371] i. CAS Number→1801342-60-8
- [0372] (see <http://www.cas.org/content/chemical-substances/faqs>)
- [0373] ii. Unique Ingredient Identifier (UNII)→6QVL0571NT
- [0374] (see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)
- [0375] As described in WO2016/007235
- [0376] NCI thesaurus code→C121540
- [0377] (see <https://ncit.nci.nih.gov/ncit-browser/>)
- [0378] i) BGB-A317 (Tislelizumab)
- [0379] i. As described in U.S. Pat. No. 9,834,606 B2
- [0380] ii. See clinical trial NCT03209973 (<https://clinicaltrials.gov/>)
- [0381] iii. NCI thesaurus code C121775
- [0382] (see <https://ncit.nci.nih.gov/ncitbrowser/>)
- [0383] j) BGB-108
- [0384] See WO2016/000619 and U.S. Pat. No. 8,735,553
- [0385] k) AMP-224
- [0386] see clinical trial NCT02298946, <https://clinicaltrials.gov/ct2/home>
- [0387] In some embodiments, PD1 polypeptide corresponds to Genbank accession no. AAC51773, version no. AAC51773.1, record update date: Jun. 23, 2010 09:24 AM. In one embodiment, the nucleic acid encoding PD1 polypeptide corresponds to Genbank accession no. U64863, version no. U64863.1, record update date: Jun. 23, 2010 09:24 AM. In some embodiments, PD1 polypeptide corresponds to Uniprot/Swiss-Prot accession No. Q15116.

PD-L1 Antagonists

[0388] “PD-L1 antagonist” means any chemical compound or biological molecule that stimulates an immune reaction through inhibition of PD-L1 signalling.

[0389] To examine the extent of enhancement of, e.g., PD-L1 activity, samples or assays comprising a given, e.g., protein, gene, cell, or organism, are treated with a potential activating or inhibiting agent and are compared to control samples treated with an inactive control molecule. Control samples are assigned a relative activity value of 100%. Inhibition is achieved when the activity value relative to the control is about 90% or less, typically 85% or less, more

typically 80% or less, most typically 75% or less, generally 70% or less, more generally 65% or less, most generally 60% or less, typically 55% or less, usually 50% or less, more usually 45% or less, most usually 40% or less, preferably 35% or less, more preferably 30% or less, still more preferably 25% or less, and most preferably less than 20%. Activation is achieved when the activity value relative to the control is about 110%, generally at least 120%, more generally at least 140%, more generally at least 160%, often at least 180%, more often at least 2-fold, most often at least 2.5-fold, usually at least 5-fold, more usually at least 10-fold, preferably at least 20-fold, more preferably at least 40-fold, and most preferably over 40-fold higher.

[0390] Combining an ADC, which targets a first target protein (FTP) positive lymphomas and leukemias with PD-L1 inhibitors is advantageous because, on the one hand, the ADC will directly kill the FTP positive tumor cells while, on the other hand, the PD-L1 inhibitor will engage the patient's own immune system to eliminate the cancer cells.

[0391] Next to FTP(+) tumor cells, target negative tumor cells in close proximity to FTP(+) tumor cells will potentially be killed by the bystander mechanism of the PBD-dimer released after cell kill of FTP(+) cells. Hence, the ADC will directly kill the tumor cells. The resulting release of tumor associated antigens from cells that are killed with the PBD dimer will trigger the immune system, which will be further enhanced by the use of programmed cell death protein 1 ligand inhibitors (PD-L1, aka B7-H1 or CD274).

[0392] PD-L1 is commonly upregulated on the tumour cell surface from many different human tumours. Interfering with the PD1 ligand expressed on the tumor will avoid the immune inhibition in the tumor microenvironment and therefore blockade of the PD1 pathway using PDL1 inhibitors may enhance antitumour immune responses against the antigens released from the tumors killed by the ADC.

[0393] Combining an ADC, which targets a first target protein (FTP) with PD1 inhibitors is advantageous, because on the one hand, the ADC will directly kill the FTP positive tumor cells, while on the other hand the PD1 inhibitor will engage the patient's own immune system to eliminate the cancer cells. Next to FTP(+) tumor cells, FTP negative tumor cells in close proximity to FTP(+) tumor cells will potentially be killed by the bystander mechanism of the PBD-dimer released after cell kill of CD19(+) or CD22 (+) cells. Hence, the ADC will directly kill the tumor cells.

[0394] PD-L1 antagonists suitable for use as secondary agents in the present disclosure include PD-L1 antagonists that:

- [0395] (a) are PD-L1 binding antagonists;
- [0396] (b) inhibit the binding of PD-L1 to PD1;
- [0397] (c) inhibit the binding of PD-L1 to B7-1;
- [0398] (d) inhibit the binding of PD-L1 to both PD1 and B7-1;
- [0399] (e) are anti-PD-L1 antibodies.

[0400] Specific PD-L1 antagonists suitable for use as secondary agents in the present disclosure include:

[0401] a) atezolizumab (MPDL3280A, brand name Tecentriq)

[0402] i. CAS Number→1380723-44-3

[0403] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0404] ii. DrugBank reference→DB11595

[0405] (see <https://www.drugbank.ca/>)

[0406] iii. Unique Ingredient Identifier (UNII)→52CMI0WC3Y

[0407] (see <http://www.fda.gov/ForIndustry/Data-Standards/SubstanceRegistrationSystem-Unique-IngredientIdentifierUNII/default.htm>)

[0408] b) BMS-936559/MDX-1105

[0409] I. CAS Number→1422185-22-5

[0410] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0411] II. see clinical trial NCT02028403, <https://clinicaltrials.gov/ct2/home>

[0412] III. See WO2007/005874 for antibody sequences, in particular the:

i. Antibody having:

a. VH CDR1 = DYGFS
b. VH CDR2 = WITAYNGTNTNYAQLQG
c. VH CDR3 = DYFYGM DV
d. VL CDR1 = RASQSVSSYL V
e. VL CDR2 = DASNRAT
f. VL CDR3 = QQRSNWPRT

ii. Antibody having:

a. VH CDR1 = TYAIS
b. VH CDR2 = GIIPFGKAHYAQKFQ G
c. VH CDR3 = KFHFVSGSPFGMDV
d. VL CDR1 = RASQSVSSYL A
e. VL CDR2 = DASNRAT
f. VL CDR3 = QQRSNWPRT

iii. Antibody having:

a. VH CDR1 = SYDVH
b. VH CDR2 = WLHADTGITKFSQKFQ G
c. VH CDR3 = ERIQLWFDY
d. VL CDR1 = RASQGISSWLA
e. VL CDR2 = AASSLQ S
f. VL CDR3 = QQYNSYPYT

[0413] c) durvalumab/MEDI4736

[0414] i. CAS Number→1428935-60-7

[0415] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0416] ii. Unique Ingredient Identifier (UNII)→28X28X9OKV

[0417] (see <http://www.fda.gov/ForIndustry/Data-Standards/SubstanceRegistrationSystem-Unique-IngredientIdentifierUNII/default.htm>)

VH sequence

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWMSWVRQAPGKGLEWV

ANIKQDGSEKYYVDSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYC

AREGGWFGEALFDYWGQGLTVTVSS

-continued

VL sequence

EIVLTQSPGTLSSLSPGERATLSCRASQVRVSSSYLAWYQQKPGQAPRL

IYDASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGS

WTFGGQGTKVEIK

[0418] d) Avelumab/MSB0010718C

[0419] i. CAS Number→1537032-82-8

[0420] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0421] ii. Unique Ingredient Identifier (UNII)→KXG2PJ5511

[0422] (see <http://www.fda.gov/ForIndustry/Data-Standards/SubstanceRegistrationSystem-Unique-IngredientIdentifierUNII/default.htm>)

[0423] In some embodiments, PD-L1 polypeptide corresponds to Genbank accession no. AAF25807, version no. AAF25807.1, record update date: Mar. 10, 2010 10:14 PM. In one embodiment, the nucleic acid encoding PD1 polypeptide corresponds to Genbank accession no. AF177937, version no. AF177937.1, record update date: Mar. 10, 2010 10:14 PM. In some embodiments, PD1 polypeptide corresponds to Uniprot/Swiss-Prot accession No. Q9NZQ7.

GITR Agonists

[0424] The term “glucocorticoid-induced TNF receptor” (abbreviated herein as “GITR”), also known as TNF receptor superfamily 18 (TNFRSF18, CD357), TEASR, and 312C2, as used herein, refers to a member of the tumor necrosis factor/nerve growth factor receptor family. GITR is a 241 amino acid type I transmembrane protein characterized by three cysteine pseudo-repeats in the extracellular domain and specifically protects T-cell receptor induced apoptosis, although it does not protect cells from other apoptotic signals, including Fas triggering, dexamethasone treatment, or UV irradiation (Nocentini, G., et al. (1997) Proc. Natl. Acad. Sci. USA 94:6216-622).

[0425] GITR activation increases resistance to tumors and viral infections, is involved in autoimmune/inflammatory processes and regulates leukocyte extravasation (Nocentini supra; Cuzzocrea, et al. (2004) J Leukoc. Biol. 76:933-940; Shevach, et al. (2006) Nat. Rev. Immunol. 6:613-618; Cuzzocrea, et al. (2006) J Immunol. 177:631-641; and Cuzzocrea, et al. (2007) FASEB J 21:117-129). In tumor mouse models, agonist GITR antibody, DTA-I, was combined with an antagonist CTLA-4 antibody, and showed synergistic results in complete tumor regression of advanced stage tumors in some test group mice (Ko, et al. (2005) J Exp. Med. 7:885-891).

[0426] The nucleic acid and amino acid sequences of human GITR (hGITR), of which there are three splice variants, are known and can be found in, for example GenBank Accession Nos. gi:40354198, gi:23238190, gi:23238193, and gi:23238196.

[0427] “GITR agonist” means any chemical compound or biological molecule that stimulates an immune reaction through activation of GITR signalling. Also contemplated are soluble GITR-L proteins, a GITR binding partner.

[0428] To examine the extent of enhancement of, e.g., GITR activity, samples or assays comprising a given, e.g., protein, gene, cell, or organism, are treated with a potential activating or inhibiting agent and are compared to control

samples treated with an inactive control molecule. Control samples are assigned a relative activity value of 100%. Inhibition is achieved when the activity value relative to the control is about 90% or less, typically 85% or less, more typically 80% or less, most typically 75% or less, generally 70% or less, more generally 65% or less, most generally 60% or less, typically 55% or less, usually 50% or less, more usually 45% or less, most usually 40% or less, preferably 35% or less, more preferably 30% or less, still more preferably 25% or less, and most preferably less than 20%. Activation is achieved when the activity value relative to the control is about 110%, generally at least 120%, more generally at least 140%, more generally at least 160%, often at least 180%, more often at least 2-fold, most often at least 2.5-fold, usually at least 5-fold, more usually at least 10-fold, preferably at least 20-fold, more preferably at least 40-fold, and most preferably over 40-fold higher.

[0429] Combining an ADC, which targets a first target protein (FTP) positive lymphomas and leukemias with G1TR agonists is advantageous, because on the one hand the ADC will directly kill the FTP positive tumor cells, while on the other hand the G1TR agonist will engage the patient's own immune system to eliminate the cancer cells. Next to FTP(+) tumor cells, target negative tumor cells in close proximity to FTP(+) tumor cells will potentially be killed by the bystander mechanism of the PBD-dimer released after cell kill of FTP(+) cells. Hence, the ADC will directly kill the tumor. The resulting release of tumor associated antigens from cells killed with the PBD dimer will trigger the immune system, which will be further enhanced by the use of a G1TR agonist.

[0430] G1TR (Glucocorticoid-Induced TNFR-Related protein) is expressed transiently on activated T-cells and expressed constitutively at high levels on T-regs with further induction following activation. G1TR ligation via its ligand G1TRL stimulates both proliferation and function of both effector and regulatory CD4+ T cells. This promotes T-cell survival, and differentiation into effector cells, while abrogating suppression. Therefore it will be beneficial to target a FTP(+) tumor with the ADC, causing the antigenic cell death, while the G1TR agonist induces a stronger, durable immune response.

[0431] Specific G1TR agonists suitable for use as secondary agents in the present disclosure include:

[0432] a) MEDI1873, a G1TR ligand fusion protein developed by MedImmune

[0433] See WO2016/196792, US20160304607

[0434] NCI thesaurus code→C124651

[0435] (see <https://ncit.nci.nih.gov/ncitbrowser/>)

[0436] See also clinical trial NCT023126110 at <https://clinicaltrials.gov/ct2/home>

[0437] See Tighe N J, Bamber L, Andrews J, et al. MEDI1873, a potent, stabilized hexameric agonist of human G1TR with regulatory T-cell targeting potential. *Oncoimmunology*. 2017; 6(3):e1280645. doi: 10.1080/2162402X.2017.1280645.

[0438] b) INCAGN1876, is an agonist antibody targeting the glucocorticoid-induced TNFR-related protein, or G1TR. Discovered during a collaboration with Ludwig Cancer Research. INCAGN1876 is being co-developed with Incyte

[0439] See clinical trials NCT02583165 and NCT03277352 at <https://clinicaltrials.gov/ct2/home>

[0440] c) TRX518, a humanized aglycosylated (Fc disabled) IgG1 anti-G1TR mAb with immune-modulating activity developed by Leap Therapeutics

[0441] See WO2006/105021 for sequences 58, 60-63; and EP2175884 sequences 1-7:

VL comprising the sequence (CDR underline):
EIVMTQSPATLSVSPGERATLSCKASQNVGTNVAWYQQKPGQAPRLLI

YSASYRYSGIPARFSGSGSGTEFTLTISLSQSEDAFVYYCQQYNTDPL

TFGGGTKVEIK

VH comprising the sequence (CDR underline):
QVTLRESGPALVKPTQTLTLCTFSGFSLSTSGMGVGVIRQPPGKALE

WLAHIWDDDKYYNPSSLKSLRTISKDTSKNQVLTMTNMDPVDATYY

CARTRRYFPFAYWGQGLVTVS

QVTLRESGPALVKPTQTLTLCTFSGFSLSTSGMGVGVIRQPPGKALE

WLAHIWDDDKYYQPSLKSRLTISKDTSKNQVLTMTNMDPVDATYY

CARTRRYFPFAYWGQGLVTVS

[0442] See clinical trials NCT01239134 and NCT02628574 at <https://clinicaltrials.gov/ct2/home>

[0443] NCI thesaurus code→C95023

[0444] (see <https://ncit.nci.nih.gov/ncitbrowser/>)

[0445] d) GWN323, an anti-G1TR agonistic monoclonal antibody, which activates G1TRs found on multiple types of T-cells. GWN323 is developed by Novartis

[0446] See WO2016/196792

[0447] NCI thesaurus code→C128028

[0448] (see <https://ncit.nci.nih.gov/ncitbrowser/>)

[0449] See clinical trial NCT02740270 at <https://clinicaltrials.gov/ct2/home>

[0450] e) MK-1248, a humanized IgG4 anti-human glucocorticoid-induced tumor necrosis factor receptor (G1TR) agonistic monoclonal antibody (MoAb) with significantly reduced effector function

[0451] See clinical trial NCT02553499 at <https://clinicaltrials.gov/ct2/home>

[0452] MK-1248 has the same CDR as MK4166 (see Sukumar et al., *Cancer Res.* 2017)

[0453] f) MK-4166, a humanized IgG1 anti-human glucocorticoid-induced tumor necrosis factor receptor (G1TR) agonistic monoclonal antibody (MoAb) with potential immunomodulating activity (see Sukumar et al., *Cancer Res.* 2017).

[0454] See clinical trial NCT02132754 at <https://clinicaltrials.gov/ct2/home>

[0455] See Sukumar, et al., (2017), *Cancer Research*. 77. canres.1439.2016. 10.1158/0008-5472.CAN-16-1439.

[0456] NCI thesaurus code C116065

[0457] (see <https://ncit.nci.nih.gov/ncitbrowser/>)

[0458] g) BMS-986156, An anti-human glucocorticoid-induced tumor necrosis factor receptor (G1TR; tumor necrosis factor superfamily member 18; TNFRSF18; CD357) agonistic monoclonal antibody

[0459] See clinical trial NCT02598960 at <https://clinicaltrials.gov/ct2/home>

[0460] NCI thesaurus code C132267

[0461] (see <https://ncit.nci.nih.gov/ncitbrowser/>)

[0462] Sequences of agonist anti-GITR antibodies are provided in WO2011/028683 and WO2006/105021.

[0463] In some embodiments, GITR polypeptide corresponds to Genbank accession no. AAD22635, version no. AAD22635.1, record update date: Mar. 10, 2010 09:42 PM. In one embodiment, the nucleic acid encoding GITR polypeptide corresponds to Genbank accession no. AF125304, version no. AF125304.1, record update date: Mar. 10, 2010 09:42 PM. In some embodiments, GITR polypeptide corresponds to Uniprot/Swiss-Prot accession No. Q9Y5U5.

OX40 Agonists

[0464] OX40 (CD134; TNFRSF4) is a member of the TNFR super-family and is expressed by CD4 and CD8 T cells during antigen-specific priming. OX40 expression is largely transient following TCR/CD3 cross-linking, and by the presence of inflammatory cytokines. In the absence of activating signals, relatively few mature T cell subsets express OX40 at biologically relevant levels. Generating optimal “killer” CD8 T cell responses requires T cell receptor activation plus co-stimulation, which can be provided through ligation of OX40 using a OX40 agonist. This activating mechanism augments T cell differentiation and cytolytic function leading to enhanced anti-tumor immunity. Therefore it will be beneficial to target a FTP(+) tumor with the ADC, causing the antigenic cell death, while the OX40 agonist induces a stronger, durable immune response.

[0465] The OX40 agonist may be selected from the group consisting of an OX40 agonist antibody, an OX40L agonist fragment, an OX40 oligomeric receptor, and an OX40 immunoadhesin. In some embodiments, the OX40 binding agonist is a trimeric OX40L-Fc protein.

[0466] In some embodiments, the OX40 binding agonist is an OX40L agonist fragment comprising one or more extracellular domains of OX40L. In some embodiments, the OX40 binding agonist is an OX40 agonist antibody that binds human OX40. In some embodiments, the OX40 agonist antibody depletes cells that express human OX40. In some embodiments, the OX40 agonist antibody depletes cells that express human OX40 in vitro. In some embodiments, the cells are CD4+ effector T cells. In some embodiments, the cells are Treg cells. In some embodiments, the depleting is by ADCC and/or phagocytosis. In some embodiments, the depleting is by ADCC. In some embodiments, the OX40 agonist antibody binds human OX40 with an affinity of less than or equal to about 1 nM. In some embodiments, the OX40 agonist antibody increases CD4+ effector T cell proliferation

and/or increasing cytokine production by the CD4+ effector T cell as compared to proliferation and/or cytokine production prior to treatment with anti-human OX40 agonist antibody. In some embodiments, the cytokine is gamma interferon. In some embodiments, the OX40 agonist antibody increases memory T cell proliferation and/or increasing cytokine production by the memory cell. In some embodiments, the cytokine is gamma interferon. In some embodiments, the OX40 agonist antibody inhibits Treg function. In some embodiments, the OX40 agonist antibody inhibits Treg suppression of effector T cell function. In some embodiments, effector T cell function is effector T cell proliferation and/or cytokine production. In some embodiments, the effector T cell is a CD4+ effector T cell. In some embodiments, the OX40 agonist antibody increases OX40 signal transduction in a target cell that expresses OX40. In

some embodiments, OX40 signal transduction is detected by monitoring NFkB downstream signalling.

[0467] “OX40 agonist” means any chemical compound or biological molecule that stimulates an immune reaction through activation of OX40 signalling.

[0468] To examine the extent of enhancement of, e.g., OX40 activity, samples or assays comprising a given, e.g., protein, gene, cell, or organism, are treated with a potential activating or inhibiting agent and are compared to control samples treated with an inactive control molecule. Control samples are assigned a relative activity value of 100%. Inhibition is achieved when the activity value relative to the control is about 90% or less, typically 85% or less, more typically 80% or less, most typically 75% or less, generally 70% or less, more generally 65% or less, most generally 60% or less, typically 55% or less, usually 50% or less, more usually 45% or less, most usually 40% or less, preferably 35% or less, more preferably 30% or less, still more preferably 25% or less, and most preferably less than 20%. Activation is achieved when the activity value relative to the control is about 110%, generally at least 120%, more generally at least 140%, more generally at least 160%, often at least 180%, more often at least 2-fold, most often at least 2.5-fold, usually at least 5-fold, more usually at least 10-fold, preferably at least 20-fold, more preferably at least 40-fold, and most preferably over 40-fold higher.

[0469] Combining an ADC, which targets a first target protein (FTP) positive lymphomas and leukemias with OX40 agonists is advantageous, because on the one hand the ADC will directly kill the FTP positive tumor cells, while on the other hand the OX40 agonist will engage the patient's own immune system to eliminate the cancer cells. Next to FTP(+) tumor cells, target negative tumor cells in close proximity to FTP(+) tumor cells will potentially be killed by the bystander mechanism of the PBD-dimer released after cell kill of FTP(+) cells. Hence, the ADC will directly kill the tumor. The resulting release of tumor associated antigens from cells killed with the PBD dimer will trigger the immune system, which will be further enhanced by the use of a OX40 agonist.

[0470] Specific OX40 agonists suitable for use as secondary agents in the present disclosure include:

[0471] a) MEDI0562 (aka Tavolixizumab, Tavolimab)

[0472] a) CAS Number→1635395-25-3

[0473] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0474] b) Unique Ingredient Identifier (UNII) →4LU9B48U4D

[0475] (see <http://www.fda.gov/ForIndustry/Standards/SubstanceRegistrationSystem-Unique-IngredientIdentifierUNII/default.htm>)

[0476] See clinical trial NCT02318394 at <https://clinicaltrials.gov/ct2/home>

[0477] As described in WO2015/095423, WO2015/153514, WO2016/073380 & WO2016/081384

[0478] NCI thesaurus code→C120041

[0479] (see <https://ncit.nci.nih.gov/ncitbrowser/>)

Heavy Chain sequence:

QVQLQESGPGLVKPSQTLSTCAVYGGSFSSGYWNWIRKHPGKGLYI

GYISYNGITYHNPSLKSRTINRDTSKNQYSLQLNSVTPEDTAVYYCA

-continued

RYKYDYDGGHAMDYWGQGLTVTVSSASTKGPSVFPPLAPSSKSTSGGTA
 ALGCLVKDYFPEPVTWNSGALSTSGVHTFPAVLQSSGLYSLSSVVTV
 PSSSLGTQTYICNVNHKPSNTKVDKRVKPKCDKTHCTCPPELLE
 GPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEV
 HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
 EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
 WESNGQPENNYKTTTPVLDSDGSFFLYSLKTVDKSRWQQGNVFCSCVM
 HEALHNHYTQKSLSLSPG
 Light chain sequence:
 DIQMTQSPSSLSASVGRVTITCRASQDISNYLNWYQQKPKAPKLLI
 YYTSKLHSGVPSRFGSGSGTDYTLTISSLQPEDFATYYCQQGSALPW
 TFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
 KVQWKVDNALQSGNSQESVTEQDSKDSSTYSSTLTLSKADYEKHKVY
 ACEVTHQGLSSPVTKSFN RGE

[0480] b) MEDI6383 (Efizonerimod alfa)

[0481] a) CAS Number→1635395-27-5

[0482] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0483] b) Unique Ingredient Identifier (UNII)
 →1MH7C2X8KE

[0484] (see <http://www.fda.gov/ForIndustry/Data-Standards/SubstanceRegistrationSystem-Unique-IngredientIdentifierUNII/default.htm>)

[0485] See clinical trial NCT02221960 at <https://clinicaltrials.gov/ct2/home>

[0486] As described in WO2015/095423, WO2016/081384, and WO2016/189124

[0487] NCI thesaurus code→C118282

[0488] (see <https://ncit.nci.nih.gov/ncitbrowser/>)

Amino acid sequence (Seq ID no. 17 from
 WO2016/189124):
 ESKYGPPCPPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDV
 SQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWL
 NGKEYKCKVSNKGLPSSIEKTKISKAKGQPREPQVYTLPPSQEEMTKNQ
 VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRL
 TVDKSRWQEGNVFSCVMHEALHNHYTQKSLSLGLKDKQDKIEALSSK
 VQQLERSIGLKDAMADLEQKVLMEASTQVSHRYPIQSIVKQFTEY
 KKEKGFIILTSQKEDEIMKVQNNSVIINCDFYLISLKGYSQEVNISL
 HYQKDEEPLFQLKKVRSVNSLMVASLTYKDKVYLVNVTDTNTSLDDFHV
 NGGELILIHQNPGEFCVL

[0489] c) MOXR0916 (also known as RG7888, Pogalizumab), a humanized anti-OX40 monoclonal antibody

[0490] a) CAS Number→1638935-72-4

[0491] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0492] b) Unique Ingredient Identifier (UNII)
 →C78148TF1D

[0493] (see <http://www.fda.gov/ForIndustry/Data-Standards/SubstanceRegistrationSystem-Unique-IngredientIdentifierUNII/default.htm>)

[0494] c) NCI thesaurus code→C121376

[0495] (see <https://ncit.nci.nih.gov/ncitbrowser/>)

[0496] d) OX40mAb24 (9B12)

[0497] a) OX40mAb24 is a humanised version of 9B12. 9B12 is a murine IgG1, anti-OX40 mAb directed against the extracellular domain of human OX40 (CD134) (Weinberg, A. D., et al. J Immunother 29, 575-585 (2006)).

[0498] b) See WO2016/057667 Seq ID no.59 for OX40mAb24 VH sequence, no.29 for VL sequence (no.32 is an alternative VL):

VH sequence
 QVQLQESGPGLVKPSQTLSTLCAVYGGSFSSGYWNWIRKHPGKLEYI
 GYISYNGITYHNPSLKSRTITNRDTSKNQYSLQLNSVTPEDTAVYYCA
 RYKYDYDGGHAMDYWGQGLTVTVSS
 VL sequence
 DIQMTQSPSSLSASVGRVTITCRASQDISNYLNWYQQKPKAPKLLI
 YYTSKLHSGVPSRFGSGSGTDYTLTISSLQPEDFATYYCQQGSALPW
 TFGQGTKVEIK

[0499] e) INCAGN1949

[0500] a) See Gonzalez et al. 2016, DOI: 10.1158/1538-7445.AM2016-3204

[0501] b) See clinical trial NCT02923349 at <https://clinicaltrials.gov/ct2/home>

[0502] c) Antibody sequences are disclosed in WO2016/179517 A1:

[0503] i. In particular, an antibody comprising the sequences:

VH CDR1 -> GSAMH
 VH CDR2 -> RIRSKANSYATAYAASVKG
 VH CDR3 -> GIYDSSGYDY
 VL CDR1 -> RSSQSLLHSNGYNYLD
 VL CDR2 -> LGSNRAS
 VL CDR3 -> MQALQTPLT

[0504] ii. Such as, an antibody comprising the sequences:

VH ->
 EVQLVESGGGLVQPGGSLKLSCAASGFTFSGSAMHWVRQASGKGLEWV
 GRIRSKANSYATAYAASVKGRFTISRDDSKNTAYLQMNSLKTEDTAVY
 YCTSGIYDSSGYDYWGQGLTVTVSS

-continued

VL ->

DIVMTQSPSLPVPVTPGEPASISCRSSQSLHNSGNYLDWYLQKPGQS

PQLLIYLGNSNRASGVPRDFSGSGSGDTFLKISRVEAEDVGVIYCMQA

LQTPLTFGGGKVEIK

[0505] g) GSK3174998, a humanized IgG1 agonistic anti-OX40 monoclonal antibody (mAb)

[0506] See clinical trial NCT02528357 at <https://clinicaltrials.gov/ct2/home>

[0507] h) PF-04518600 (PF-8600) is an investigational, fully human, monoclonal antibody (mAb) that targets OX40 protein

[0508] See patent WO 2017/130076 A1

[0509] See clinical trial NCT02315066 at <https://clinicaltrials.gov/ct2/home-NCI> thesaurus code→C121927

[0510] (see <https://ncit.nci.nih.gov/ncit-browser/>)

[0511] In some embodiments, OX40 polypeptide corresponds to Genbank accession no. CAA53576, version no. CAA53576.1, record update date: Feb. 2, 2011 10:10 AM. In one embodiment, the nucleic acid encoding OX40 polypeptide corresponds to Genbank accession no. X75962, version no. X75962.1, record update date: Feb. 2, 2011 10:10 AM. In some embodiments, OX40 polypeptide corresponds to Uniprot/Swiss-Prot accession No. P43489.

CTLA Antagonist

[0512] CTLA4 (CD152) is expressed on activated T cells and serves as a co-inhibitor to keep T cell responses in check following CD28-mediated T cell activation. CTLA4 is believed to regulate the amplitude of the early activation of naive and memory T cells following TCR engagement and to be part of a central inhibitory pathway that affects both antitumor immunity and autoimmunity. CTLA4 is expressed exclusively on T cells, and the expression of its ligands CD80 (B7.1) and CD86 (B7.2), is largely restricted to antigen-presenting cells, T cells, and other immune mediating cells. Antagonistic anti-CTLA4 antibodies that block the CTLA4 signalling pathway have been reported to enhance T cell activation. One such antibody, ipilimumab, was approved by the FDA in 2011 for the treatment of metastatic melanoma. Another anti-CTLA4 antibody, tremelimumab, was tested in phase III trials for the treatment of advanced melanoma, but did not significantly increase the overall survival of patients compared to the standard of care (temozolomide or dacarbazine) at that time.

[0513] "CTLA4 agonist" means any chemical compound or biological molecule that stimulates an immune reaction through inhibition of CTLA4 signalling.

[0514] To examine the extent of enhancement of, e.g., CTLA4 activity, samples or assays comprising a given, e.g., protein, gene, cell, or organism, are treated with a potential activating or inhibiting agent and are compared to control samples treated with an inactive control molecule. Control samples are assigned a relative activity value of 100%. Inhibition is achieved when the activity value relative to the control is about 90% or less, typically 85% or less, more typically 80% or less, most typically 75% or less, generally 70% or less, more generally 65% or less, most generally 60% or less, typically 55% or less, usually 50% or less, more

usually 45% or less, most usually 40% or less, preferably 35% or less, more preferably 30% or less, still more preferably 25% or less, and most preferably less than 20%. Activation is achieved when the activity value relative to the control is about 110%, generally at least 120%, more generally at least 140%, more generally at least 160%, often at least 180%, more often at least 2-fold, most often at least 2.5-fold, usually at least 5-fold, more usually at least 10-fold, preferably at least 20-fold, more preferably at least 40-fold, and most preferably over 40-fold higher.

[0515] Combining an ADC, which targets a first target protein (FTP) positive lymphomas and leukemias with CTLA4 inhibitors is advantageous, because on the one hand, the ADC will directly kill the FTP positive tumor cells, while on the other hand the CTLA4 inhibitor will engage the patient's own immune system to eliminate the cancer cells. Next to FTP(+) tumor cells, target negative tumor cells in close proximity to FTP(+) tumor cells will potentially be killed by the bystander mechanism of the PBD-dimer released after cell kill of FTP(+) cells. Hence, the ADC will directly kill the tumor. The resulting release of tumor associated antigens from cells killed with the PBD dimer will trigger the immune system, which will be further enhanced by the use of CTLA4 inhibitors expressed on a large proportion of tumour infiltrating lymphocytes (TILs) from many different tumour types.

[0516] The major function of CTLA4 (CD152) is to regulate the amplitude of the early stages of T cell activation, and as such it counteracts the activity of the T cell costimulatory receptor, CD28, in the tumor microenvironment. Blockade of the CTLA4 pathway may therefore enhance enhancement of effector CD4+ T cell activity, while it inhibits TReg cell-dependent immunosuppression. Therefore it will be beneficial to target a FTP(+) tumor with the ADC, causing the antigenic cell death, while the CTLA4 blockade induces a stronger immune, durable response.

[0517] Specific CTLA4 antagonists suitable for use as secondary agents in the present disclosure include:

[0518] a) ipilimumab

[0519] i. CAS Number→477202-00-9

[0520] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0521] ii. Unique Ingredient Identifier (UNII) →6T8C155666

[0522] (see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

[0523] b) Tremelimumab

[0524] i. CAS Number→745013-59-6

[0525] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0526] ii. Unique Ingredient Identifier (UNII) →QEN1X95CIX

[0527] (see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

iii. VH sequence

[SEQ ID NO. 1]

GVVQPGRLRLSCAASGFTFSSYGMHWRQAPGKGLEWVAVIWDGNSN

KYYADSVKGRFTISRDNKNTLYLQMNSLRADTVAVYVCARDPRGATL

-continued

YYYYYGMVDVWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCL

VKDYFPEPVTVSWNSGALTSGVH

iv. VL sequence

[SEQ ID NO. 2]

PSLSASVGDVRTITCRASQSINSYLDWYQKPGKAPKLLIYAASSLQ

SGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQYYSTPFTFGPGTK

VEIKRTVAAPSVPFIPPSDEQLKSGTASVVCLLNFPYPREAKV

[0528] In some embodiments, CTLA polypeptide corresponds to Genbank accession no. AAL07473, version no. AAL07473.1, record update date: Mar. 11, 2010 01:28 AM. In one embodiment, the nucleic acid encoding CTLA4 polypeptide corresponds to Genbank accession no. AF414120, version no. AF414120.1, record update date: Mar. 11, 2010 01:28 AM. In some embodiments, OX40 polypeptide corresponds to Uniprot/Swiss-Prot accession No. P16410.

Fludarabine and Cytarabine

[0529] Combination of agents with different action mechanisms is an established therapeutic principle for combating cancer. It can be a way of increasing anti-tumour activity when a synergic effect is shown and/or when reduced toxicity is observed. Antibody-drug conjugates, including those with a PBD warhead, may be particularly suited as combination partners because they are more targeted compared to conventional chemotherapy. As PBD dimers cross-link DNA in a covalent fashion, combining them with other agents that interfere with DNA synthesis via a different mechanism is likely to provide a benefit. Examples of such potential combinations are Fludarabine and Cytarabine.

Fludarabine

[0530] Fludarabine or fludarabine phosphate (Fludara) is a chemotherapy drug used in the treatment of hematological malignancies such as leukemias and lymphomas. It is a purine analog, which interferes with DNA by interfering with ribonucleotide reductase (RNAR) and DNA polymerase. It is active against both dividing and resting cells. Fludarabine has also been shown to suppress ERCC1 transcription and this may explain the observed synergy between Fludarabine and the PBD Dimer SJG136 (SG2000) against chronic lymphocytic leukaemia cells. CLAG/CLAG-M-Cladribine is another purine analogue that inhibits RNR.

[0531] Combining the ADC, which targets First Target Protein (FTP) positive lymphomas and leukemias, with Fludarabine is advantageous, because on the one hand, the ADC will directly kill the FTP positive tumor cells via a mechanisms depending on DNA cross-linking resulting in apoptosis, while on the other hand the Fludarabine will inhibit the cells RNA and DNA polymerase, while also suppressing the DNA repair enzymes needed to resolve the DNA cross-links induced by the PBD dimer.

[0532] To show that the ADC works synergistically with Fludarabine, a panel of FTP(+) cell lines will be co-treated with a range of concentration of both the ADC and Fludarabine. As negative controls, the same panel of cell lines will be co-treated with a range of concentrations of Fludarabine and a non-targeted control ADC or with a range of concen-

tration of the ADC and vehicle. After incubation, two parameters will be measured: the amount of surface FTP (as determined by flow cytometry) and the in vitro cytotoxicity of the combinations (as determined by CellTiter-Glo® or MTS assays). Cytotoxic synergy is calculated by transforming the cell viability data into fraction affected, and calculating the combination index using the CalcuSyn analysis program.

[0533] CAS Number→21679-14-1

[0534] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0535] ii. NCBI Pubchem reference→657237

[0536] (see <https://pubchem.ncbi.nlm.nih.gov/>)

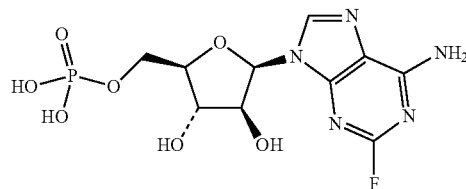
[0537] iii. IUPHAR/BPS reference→4802

[0538] (see <http://www.guidetopharmacology.org/>)

[0539] iv. Unique Ingredient Identifier (UNII) →1X9VK9O1SC

[0540] (see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

Formula I



Fludarabine: [(2R,3R,4S,5R)-5-(6-amino-2-fluoropyrimidin-9-yl)-3,4-dihydroxyoxolan-2-yl]methoxyphosphonic acid

Cytarabine

[0541] Cytarabine or cytosine arabinoside (Cytosar-U or Depocyt) is a antimetabolic chemotherapy drug used in the treatment of hematological malignancies such as acute myeloid leukemia (AML) and non-Hodgkin lymphoma. It is also known as ara-C (arabinofuranosyl cytidine). It kills cancer cells by interfering with DNA synthesis. It is actively metabolized to cytosine arabinoside triphosphate, which damages DNA when the cell cycle holds in the S phase (synthesis of DNA). Rapidly dividing cells, which require DNA replication for mitosis, are therefore most affected. Cytosine arabinoside also inhibits both DNA and RNA polymerases and nucleotide reductase enzymes needed for DNA synthesis.

[0542] Combining the ADC, which targets First Target Protein (FTP) positive lymphomas and leukemias, with Cytarabine is advantageous, because on the one hand, the ADC will directly kill the FTP positive tumor cells via a mechanisms depending on DNA cross-linking resulting in apoptosis, while on the other hand the Cytarabine will inhibit the cells RNA and DNA polymerase, while also suppressing DNA synthesis.

[0543] To show that the ADC works synergistically with Cytarabine, a panel of FTP(+) cell lines will be co-treated with a range of concentration of both the ADC and Cytarabine. As negative controls, the same panel of cell lines will be co-treated with a range of concentrations of Cytarabine and a non-targeted control ADC or with a range of concen-

tration of the ADC and vehicle. After incubation, two parameters will be measured: the amount of surface FTP (as determined by flow cytometry) and the in vitro cytotoxicity of the combinations (as determined by CellTiter-Glo® or MTS assays). Cytotoxic synergy is calculated by transforming the cell viability data into fraction affected, and calculating the combination index using the CalcuSyn analysis program (see example 4).

[0544] CAS Number→147-94-4

[0545] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0546] ii. NCBI Pubchem reference→6253

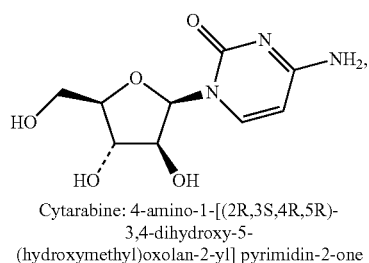
[0547] (see <https://pubchem.ncbi.nlm.nih.gov/>)

[0548] iii. IUPHAR/BPS reference→4827

[0549] (see <http://www.guidetopharmacology.org>)

[0550] iv. Unique Ingredient Identifier (UNII)→404079A1RDZ

[0551] (see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)



Formula II

Hypomethylating Agent

[0552] The term “hypomethylating agent” refers to a class of compounds that interfere with DNA methylation which is the addition of a methyl group to the 5-position of the cytosine pyrimidine ring or the nitrogen in position 6 of the adenine purine ring. DNA methylation stably alters the gene expression pattern in cells i.e. decrease gene expression (i.e. for the Vitamin D receptor). Hypomethylating agent are compounds that can inhibit methylation, resulting in the expression of the previously hypermethylated silenced genes. Cytidine analogs such as 5-azacytidine (azacitidine) and 5-aza-2'-deoxycytidine (decitabine) are the most commonly used Hypomethylating agent. These compounds work by binding to the enzymes that catalyse the methylation reaction, i.e. DNA methyltransferases.

[0553] To examine the extent of hypomethylation, samples or assays comprising a given, e.g., protein, gene, cell, or organism, are treated with a potential activating or inhibiting agent and are compared to control samples treated with an inactive control molecule. Control samples are assigned a relative activity value of 100%. Inhibition is achieved when the activity value relative to the control is about 90% or less, typically 85% or less, more typically 80% or less, most typically 75% or less, generally 70% or less, more generally 65% or less, most generally 60% or less, typically 55% or less, usually 50% or less, more usually 45% or less, most usually 40% or less, preferably 35% or less, more preferably 30% or less, still more preferably 25% or less, and most preferably less than 20%. Activation is

achieved when the activity value relative to the control is about 110%, generally at least 120%, more generally at least 140%, more generally at least 160%, often at least 180%, more often at least 2-fold, most often at least 2.5-fold, usually at least 5-fold, more usually at least 10-fold, preferably at least 20-fold, more preferably at least 40-fold, and most preferably over 40-fold higher.

[0554] Combining an ADC, which targets a first target protein (FTP) positive lymphomas and leukemias with a hypomethylating agent is advantageous, because on the one hand the ADC will directly kill the FTP positive tumor cells, while on the other hand the a hypomethylating agent will interfere with DNA methylation. This interference is by way of causing demethylation in that sequence, which adversely affects the way that cell regulatory proteins are able to bind to the DNA/RNA substrate. This activity synergises with the ADC because PBD dimers cross-link DNA in a covalent fashion, so combining them with other agents that interfere with DNA synthesis via a different mechanism provides a benefit.

[0555] Specific Hypomethylating agents suitable for use as secondary agents in the present disclosure include:

[0556] a) 5-azacytidine (azacitidine)

[0557] i. CAS Number→320-67-2

[0558] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0559] ii. NCBI Pubchem reference→9444

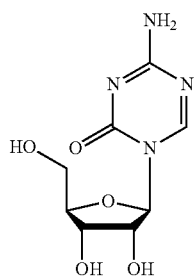
[0560] (see <https://pubchem.ncbi.nlm.nih.gov/>)

[0561] iii. IUPHAR/BPS reference→6796

[0562] (see <http://www.guidetopharmacology.org/>)

[0563] iv. Unique Ingredient Identifier (UNII)→M801H13NRU

[0564] (see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)



5-azacytidine: 4-Amino-1-β-D-ribofuranosyl-1,3,5-triazin-2(1H)-one

Formula III

[0565] b) 5-aza-2'-deoxycytidine (decitabine)

[0566] i. CAS Number→2353-33-5

[0567] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0568] ii. NCBI Pubchem reference→451668

[0569] (see <https://pubchem.ncbi.nlm.nih.gov/>)

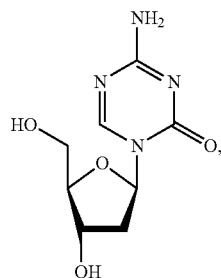
[0570] iii. IUPHAR/BPS reference→6805

[0571] (see <http://www.guidetopharmacology.org/>)

[0572] iv. Unique Ingredient Identifier (UNII)→776B62CQ27

[0573] (see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

b)



5-aza-2'-deoxycytidine: 4-Amino-1-(2-deoxy-β-D-erythro-pentofuranosyl)-1,3,5-triazin-2(1H)-one

Formula IV

PARP Inhibitors

[0574] Poly (adenosine diphosphate [ADP]) ribose polymerase (PARP) are a family of enzymes involved in a wide range of cellular functions including DNA transcription, DNA damage response, genomic stability maintenance, cell cycle regulation, and cell death. PARP-1 is the most abundant and best characterised protein of this group. In oncology, its integral role in the repair of single-strand DNA breaks (SSBs) via the base excision repair (BER) pathway has been a focus of high interest and several PARP-1 inhibitors (PARPi) have been developed (including but not limited to Olaparib, CEP-9722, talazoparib, Rucaparib, Iniparib, Veliparib and Niraparib) and are tested clinically. In cancer therapeutics, PARPi work predominantly by preventing the repair of DNA damage, ultimately causing cell death.

[0575] PARP is composed of four domains of interest: a DNA-binding domain, a caspase-cleaved domain, an auto-modification domain, and a catalytic domain. The DNA-binding domain is composed of two zinc finger motifs. In the presence of damaged DNA (base pair-excised), the DNA-binding domain will bind the DNA and induce a conformational shift. It has been shown that this binding occurs independent of the other domains. This is integral in a programmed cell death model based on caspase cleavage inhibition of PARP. The auto-modification domain is responsible for releasing the protein from the DNA after catalysis. Also, it plays an integral role in cleavage-induced inactivation.

[0576] PARP is found in the cell nucleus. The main role is to detect and initiate an immediate cellular response to metabolic, chemical, or radiation-induced single-strand DNA breaks (SSB) by signalling the enzymatic machinery involved in the SSB repair. Once PARP detects a SSB, it binds to the DNA, undergoes a structural change, and begins the synthesis of a polymeric adenosine diphosphate ribose (poly (ADP-ribose) or PAR) chain, which acts as a signal for the other DNA-repairing enzymes. Target enzymes include DNA ligase III (LigIII), DNA polymerase beta (polβ), and scaffolding proteins such as X-ray cross-complementing gene 1 (XRCC1). After repairing, the PAR chains are degraded via Poly(ADP-ribose) glycohydrolase (PARG).

[0577] NAD⁺ is required as substrate for generating ADP-ribose monomers. It has been thought that overactivation of PARP may deplete the stores of cellular NAD⁺ and induce a progressive ATP depletion and necrotic cell death, since glucose oxidation is inhibited. But more recently it was suggested that inhibition of hexokinase activity leads to defects in glycolysis. (see Andrabi, PNAS 2014). Note below that PARP is inactivated by caspase-3 cleavage during programmed cell death.

[0578] PARP enzymes are essential in a number of cellular functions, including expression of inflammatory genes: PARP1 is required for the induction of ICAM-1 gene expression by smooth muscle cells, in response to TNF.

[0579] PBDs are a class of naturally occurring anti-tumor antibiotics found in *Streptomyces*. PBD dimers exert their cytotoxic mode of action via cross-linking of two strands of DNA, which results in the blockade of replication and tumor cell death. Importantly, the cross-links formed by PBD dimers are relatively non-distorting of the DNA structure, making them hidden to DNA repair mechanisms, which are often impaired in human tumors as opposed to normal tissues.

[0580] Combining PBD-based ADCs with PARPi (including but not limited to Olaparib, CEP-9722, talazoparib, Rucaparib, Iniparib, Veliparib and Niraparib) is advantageous because repair of the DNA damaged caused by the PBD dimers is blocked by the PARP inhibition hence resulting in accumulation of DNA damage leading to cancer cell death.

[0581] To show that treatment of solid tumor-derived cell lines with PBD-based ADCs and PARPi has an additive or synergistic anti-tumor effect, a panel of solid tumor-derived cell lines will be treated with a range of concentration of each ADC and a PARPi. After incubation, the in vitro cytotoxicity of the combinations (as determined by CellTiter-Glo® or MTS assays) will be measured. Cytotoxic synergy is calculated by transforming the cell viability data into fraction affected, and calculating the combination index using the CalcuSyn analysis program.

[0582] "PARP inhibitor" means any chemical compound or biological molecule reduces PARP activity.

[0583] To examine the extent of inhibition of, e.g., PARP activity, samples or assays comprising a given, e.g., protein, gene, cell, or organism, are treated with a potential activating or inhibiting agent and are compared to control samples treated with an inactive control molecule. Control samples are assigned a relative activity value of 100%. Inhibition is achieved when the activity value relative to the control is about 90% or less, typically 85% or less, more typically 80% or less, most typically 75% or less, generally 70% or less, more generally 65% or less, most generally 60% or less, typically 55% or less, usually 50% or less, more usually 45% or less, most usually 40% or less, preferably 35% or less, more preferably 30% or less, still more preferably 25% or less, and most preferably less than 20%.

[0584] Specific PARPi suitable for use in the present disclosure include:

[0585] a) Olaparib

[0586] i. CAS Number→763113-22-0

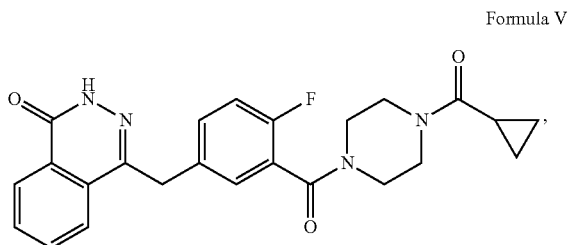
[0587] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0588] ii. NCBI Pubchem reference→23725625

[0589] (see <https://pubchem.ncbi.nlm.nih.gov/>)

[0590] iii. Unique Ingredient Identifier (UNII)
→WOH1JD9AR8

[0591] (see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

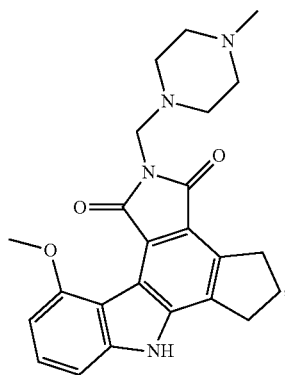


olaparib: 4-[(3-[(4-cyclopropylcarbonyl)piperazin-1-yl]carbonyl)-4-fluorophenyl]methyl(2H)phthalazin-1-one

[0592] b) CEP-9722

[0593] i. CAS Number→916574-83-9

[0594] (see <http://www.cas.org/content/chemical-substances/faqs>)



CEP-9722: 11-methoxy-2-((4-methylpiperazin-1-yl)methyl)-4,5,6,7-tetrahydro-1H-cyclopenta[a]pyrrolo[3,4-c]carbazole-1,3(2H)-dione

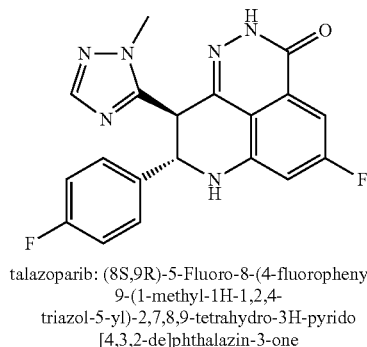
[0595] c) BMN-673/talazoparib

[0596] i. CAS Number→1207456-01-6

[0597] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0598] ii. Unique Ingredient Identifier (UNII)
→9QHX048FRV

Formula VII



talazoparib: (8S,9R)-5-Fluoro-8-(4-fluorophenyl)-9-(1-methyl-1H-1,2,4-triazol-5-yl)-2,7,8,9-tetrahydro-3H-pyrido[4,3,2-de]phthalazin-3-one

[0599] d) Rucaparib

[0600] i. CAS Number→283173-50-2

[0601] (see <http://www.cas.org/content/chemical-substances/faqs>)

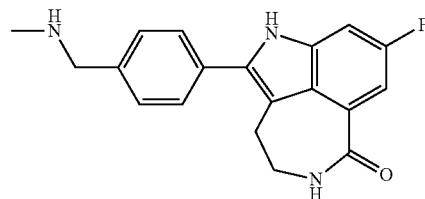
[0602] ii. NCBI Pubchem reference→9931954

[0603] (see <https://pubchem.ncbi.nlm.nih.gov/>)

[0604] iii. Unique Ingredient Identifier (UNII)
→8237F3U7EH

[0605] (see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

Formula VIII



Rucaparib: 8-Fluoro-2-((4-((methylamino)methyl)phenyl)-1,3,4,5-tetrahydro-6H-azepino[5,4,3-cd]indol-6-one

[0606] e) Iniparib/SAR24-550/BSI-201

[0607] i. CAS Number→160003-66-7

[0608] (see <http://www.cas.org/content/chemical-substances/faqs>)

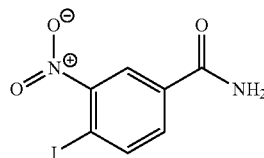
[0609] ii. NCBI Pubchem reference→9796068

[0610] (see <https://pubchem.ncbi.nlm.nih.gov/>)

[0611] iii. Unique Ingredient Identifier (UNII)
→2ZW17KHK8F

[0612] (see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

Formula IX



Iniparib: 4-Iodo-3-nitrobenzamide

[0613] f) Veliparib (ABT-888)

[0614] i. CAS Number→912444-00-9

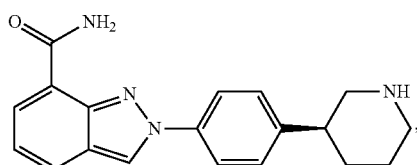
[0615] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0616] ii. NCBI Pubchem reference→11960529

[0617] (see <https://pubchem.ncbi.nlm.nih.gov/>)

[0618] iii. Unique Ingredient Identifier (UNII)→01O4K0631N

[0619] (see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)



Formula X

Veliparib: 2-((R)-2-Methylpyrrolidin-2-yl)-1H-benzimidazole-4-carboxamide

[0620] g) Niraparib/MK-4827

[0621] i. CAS Number→1038915-60-4

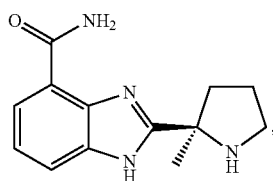
[0622] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0623] ii. NCBI Pubchem reference→24958200

[0624] (see <https://pubchem.ncbi.nlm.nih.gov/>)

[0625] iii. Unique Ingredient Identifier (UNII)→HMC2H89N35

[0626] (see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)



Formula XI

Niraparib: 2-[4-[(3S)-3-Piperidyl]phenyl]indazole-7-carboxamide

[0627] h) BGB-290

[0628] i. CAS Number→1820833-75-7

[0629] (see <http://www.cas.org/content/chemical-substances/faqs>)

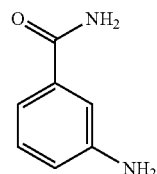
[0630] i) 3-aminobenzamide

[0631] i. CAS Number→3544-24-9

[0632] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0633] ii. NCBI Pubchem reference→1645

[0634] (see <https://pubchem.ncbi.nlm.nih.gov/>)



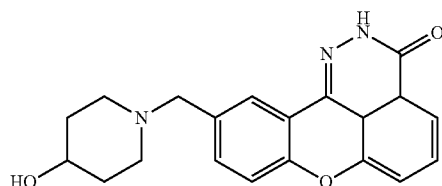
3-Aminobenzamide

Formula XII

[0635] j) E7016

[0636] i. CAS Number→902128-92-1

[0637] (see <http://www.cas.org/content/chemical-substances/faqs>)



E706: Benzopyrano(4,3,2-de)phthalazin-3(2H)-one, 10-((4-hydroxy-1-piperidinyl)methyl)-

Formula XIII

[0638] In some embodiments, PARP polypeptide is PARP1, which corresponds to Genbank accession no. AAA60137, version no. AAA60137.1, record update date: Jun. 23, 2010 08:48 AM. In one embodiment, the nucleic acid encoding PARP1 polypeptide corresponds to Genbank accession no. M18112, version no. M18112.1, record update date: Jun. 23, 2010 08:48 AM. In some embodiments, PARP1 polypeptide corresponds to Uniprot/Swiss-Prot accession No. P09874.

Agents that Upregulate HER2 Expression

[0639] An agent that “upregulates HER2 expression” means any chemical compound or biological molecule that increase the amount of HER2 protein on a tumour cell surface.

[0640] To examine the extent of enhancement samples or assays comprising a given, e.g., protein, gene, cell, or organism, are treated with a potential activating agent and are compared to control samples treated with an inactive control molecule. Control samples are assigned a relative expression value of 100%. Activation is achieved when the expression value relative to the control is about 110%, generally at least 120%, more generally at least 140%, more generally at least 160%, often at least 180%, more often at least 2-fold, most often at least 2.5-fold, usually at least 5-fold, more usually at least 10-fold, preferably at least 20-fold, more preferably at least 40-fold, and most preferably over 40-fold higher.

[0641] Specific agents that upregulate HER2 expression suitable for use as secondary agents in the present disclosure include:

[0642] a) gemcitabine

[0643] i. CAS Number→95058-81-4

[0644] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0645] ii. NCBI Pubchem reference→60750

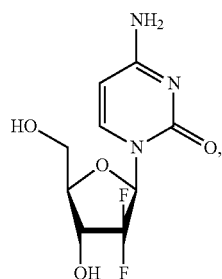
[0646] (see <https://pubchem.ncbi.nlm.nih.gov/>)

[0647] iii. DrugBank reference→DB00441

[0648] (see <https://www.drugbank.ca/>)

[0649] iv. Unique Ingredient Identifier (UNII)—B76N6SBZ8R

[0650] (see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)



Formula XIV

Gemcitabine: 4-Amino-1-(2-deoxy-2,2-difluoro-β-D-erythro-pentofuranosyl)pyrimidin-2(1H)-on

[0651] b) tamoxifen

[0652] i. CAS Number→10540-29-1

[0653] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0654] ii. NCBI Pubchem reference→2733526

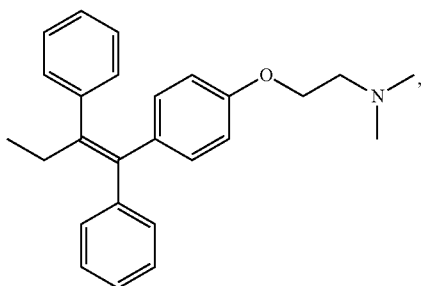
[0655] (see <https://pubchem.ncbi.nlm.nih.gov/>)

[0656] iii. DrugBank reference→DB00675

[0657] (see <https://www.drugbank.ca/>)

[0658] iv. Unique Ingredient Identifier (UNII)—→094Z181Y45

[0659] (see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)



Tamoxifen: (Z)-2-[4-(1,2-diphenylbut-1-enyl)phenoxy]-N,N-dimethylethanamine

[0660] Gemcitabine is the preferred agent that upregulated HER2.

AXLi

[0661] The secondary agent as described herein may be an AXL inhibitor.

[0662] “AXL inhibitor” means any chemical compound or biological molecule that reduces AXL signalling.

[0663] To examine the extent of inhibition of, e.g., AXL activity, samples or assays comprising a given, e.g., protein, gene, cell, or organism, are treated with a potential activating or inhibiting agent and are compared to control samples treated with an inactive control molecule. Control samples are assigned a relative activity value of 100%. Inhibition is achieved when the activity value relative to the control is about 90% or less, typically 85% or less, more typically 80% or less, most typically 75% or less, generally 70% or less, more generally 65% or less, most generally 60% or less, typically 55% or less, usually 50% or less, more usually 45% or less, most usually 40% or less, preferably 35% or less, more preferably 30% or less, still more preferably 25% or less, and most preferably less than 20%. Activation is achieved when the activity value relative to the control is about 110%, generally at least 120%, more generally at least 140%, more generally at least 160%, often at least 180%, more often at least 2-fold, most often at least 2.5-fold, usually at least 5-fold, more usually at least 10-fold, preferably at least 20-fold, more preferably at least 40-fold, and most preferably over 40-fold higher.

[0664] Inhibition of AXL with, for example, the AXL inhibitors TP0903 and BGB324 has been shown to decrease expression of DNA repair genes and to diminish the efficiency of the homologous recombination repair machinery. Consequently, AXL inhibition caused a state of HR-deficiency in the cells, making them sensitive to DNA damaging agents.

[0665] Combining ADC with AXLi including but not limited to BGB324 and TP0903 is advantageous because on the one hand, ADC will induce DNA damage in AXL-positive cancer cell lines, while on the other hand treatment with the AXLi will diminish the efficiency of the homologous recombination repair machinery making the cells more sensitive to the DNA damage induced by the PBD dimers hence resulting in accumulation of DNA damage leading to cancer cell death.

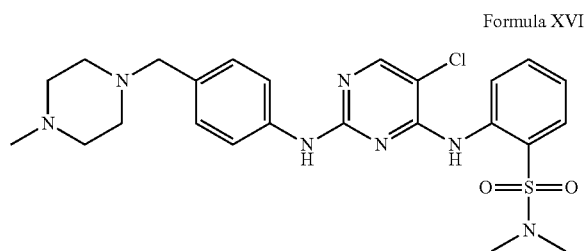
[0666] To show that co-treatment of AXL-positive cancer cell lines with ADC and the AXLi (including but not limited to BGB324 and TP0903) has an additive or synergistic anti-tumor effect, a panel of cell lines including, but not limited to MDA-MB-157 and SKLU1 will be co-treated with a range of concentration of both ADC and the AXLi BGB324 or TP-093. After incubation the in vitro cytotoxicity of the combinations (as determined by CellTiter-Glo® or MTS assays) will be measured.

[0667] Specific AXL inhibitors suitable for use as secondary agents in the present disclosure include:

[0668] c) TP0903

[0669] i. CAS Number→1341200-45-0

[0670] (see <http://www.cas.org/content/chemical-substances/faqs>)



2-((5-chloro-2-((4-((4-methylpiperazin-1-yl)methyl)phenyl)amino)pyrimidin-4-yl)amino)-N,N-dimethylbenzenesulfonamide [TP0903]

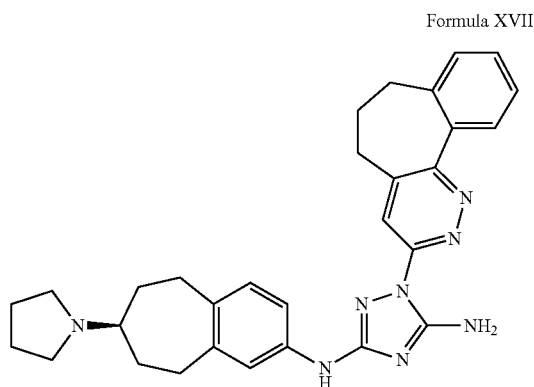
[0671] d) BGB324

[0672] i. CAS Number→1037624-75-1

[0673] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0674] ii. Unique Ingredient Identifier (UNII)→01CW2LX8AS

[0675] (see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

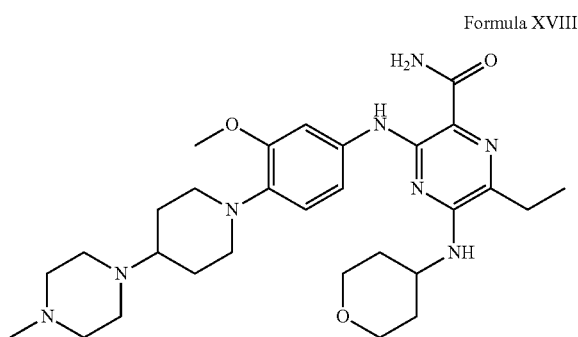


1-(6,7-dihydro-5H-benzo[2,3]cyclohepta[2,4-d]pyridazin-3-yl)-3-N-[(7S)-7-pyrrolidin-1-yl-6,7,8,9-tetrahydro-5H-benzo[7]annulen-3-yl]-1,2,4-triazole-3,5-diamine [BGB324]

[0676] e) Gilteritinib (ASP2215)

[0677] i. CAS Number→1254053-43-4

[0678] (see <http://www.cas.org/content/chemical-substances/faqs>)



6-ethyl-3-((3-methoxy-4-(4-(4-methylpiperazin-1-yl)piperidin-1-yl)phenyl)amino)-5-((tetrahydro-2H-pyran-4-yl)amino)pyrazine-2-carboxamide [Gilteritinib]

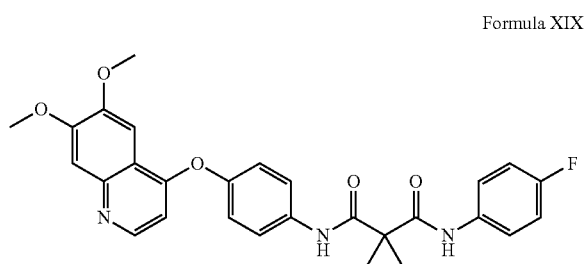
[0679] f) Cabozantinib

[0680] i. CAS Number→849217-68-1

[0681] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0682] ii. Unique Ingredient Identifier (UNII)→1C39JW444G

[0683] (see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

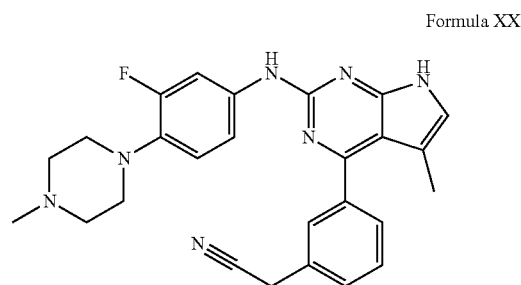


N-(4-((6,7-Dimethoxyquinolin-4-yl)oxy)phenyl)-N'-(4-fluorophenyl)cyclopropane-1,1-dicarboxamide [Cabozantinib]

[0684] g) SG17079

[0685] i. CAS Number→1239875-86-5

[0686] (see <http://www.cas.org/content/chemical-substances/faqs>)



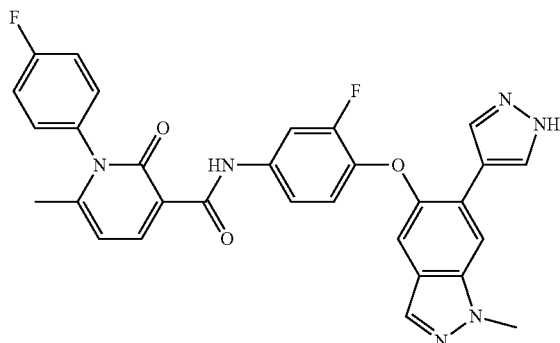
2-(3-(2-((3-fluoro-4-(4-methylpiperazin-1-yl)phenyl)amino)-5-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)acetonitrile [SG17079]

[0687] h) Merestinib

[0688] i. CAS Number→1206799-15-6

[0689] (see <http://www.cas.org/content/chemical-substances/faqs>)

Formula XXI



N-(3-Fluoro-4-([1-methyl-6-(1H-methyl-6-(1H-pyrazol-4-yl)-1H-indazol-5-yl]oxy)phenyl)-1-(4-fluorophenyl)-6-methyl-2-oxo-1,2-dihydropyridine-3-carboxamide [Merestinib]

[0690] i) amuvatinib (MP-470)

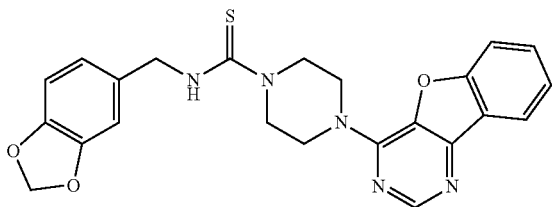
[0691] i. CAS Number→850879-09-3

[0692] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0693] ii. Unique Ingredient Identifier (UNII)→SO9S6QZB4R

[0694] (see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

Formula XXII



N-(1,3-benzodioxol-5-ylmethyl)-4-([1]benzofuro[3,2-d]pyrimidin-4-yl)piperazine-1-carbothioamide [Amuvatinib]

[0695] j) bosutinib (SKI-606)

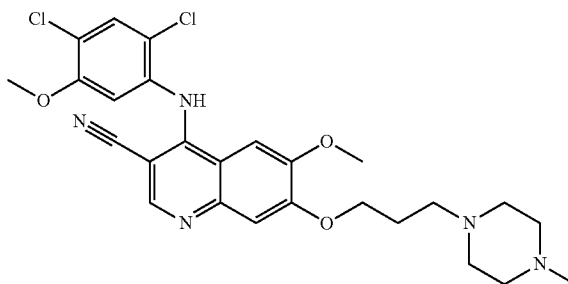
[0696] i. CAS Number→380843-75-4

[0697] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0698] ii. Unique Ingredient Identifier (UNII)→5018V4AEZO

[0699] (see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

Formula XXIII



4-[(2,4-dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(4-methylpiperazin-1-yl)propoxy]quinoline-3-carbonitrile [Bosutinib]

[0700] k) MGCD265

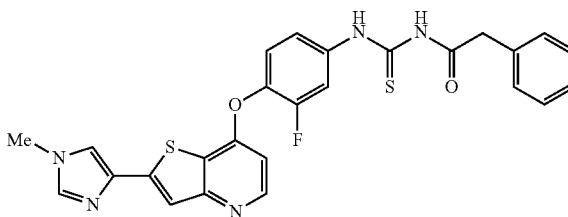
[0701] i. CAS Number→875337-44-3

[0702] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0703] ii. Unique Ingredient Identifier (UNII)→93M6577H9D

[0704] (see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

Formula XXIV



N-(3-fluoro-4-(2-(1-methyl-1H-imidazol-4-yl)thieno(3,2-b)pyridin-7-yloxy)phenyl)carbamothioyl)-2-phenylacetamide [MGCD265]

[0705] l) foretinib (GSK1363089/XL880)

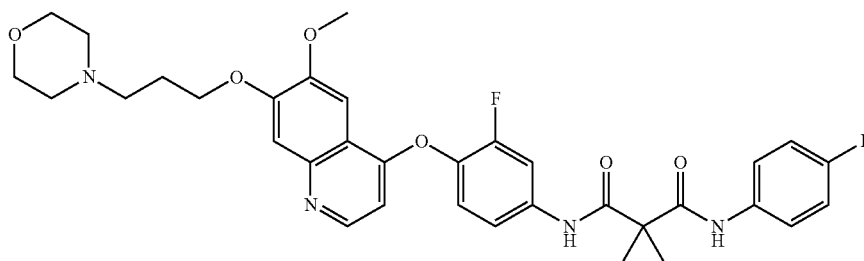
[0706] i. CAS Number→849217-64-7

[0707] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0708] ii. Unique Ingredient Identifier (UNII)→81FH7VK1C4

[0709] (see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

Formula XXV



N1'-[3-fluoro-4-[[6-methoxy-7-(3-morpholinopropoxy)-4-quinolyl]oxy]phenyl]-N1-(4-fluorophenyl)cyclopropane-1,1-dicarboxamide [Foretinib]

BRAF_i

[0710] The secondary agent as described herein may be an BRAF inhibitor.

[0711] “BRAF inhibitor” means any chemical compound or biological molecule that reduces BRAF activity.

[0712] To examine the extent of inhibition of, e.g., BRAF activity, samples or assays comprising a given, e.g., protein, gene, cell, or organism, are treated with a potential activating or inhibiting agent and are compared to control samples treated with an inactive control molecule. Control samples are assigned a relative activity value of 100%. Inhibition is achieved when the activity value relative to the control is about 90% or less, typically 85% or less, more typically 80% or less, most typically 75% or less, generally 70% or less, more generally 65% or less, most generally 60% or less, typically 55% or less, usually 50% or less, more usually 45% or less, most usually 40% or less, preferably 35% or less, more preferably 30% or less, still more preferably 25% or less, and most preferably less than 20%. Activation is achieved when the activity value relative to the control is about 110%, generally at least 120%, more generally at least 140%, more generally at least 160%, often at least 180%, more often at least 2-fold, most often at least 2.5-fold, usually at least 5-fold, more usually at least 10-fold, preferably at least 20-fold, more preferably at least 40-fold, and most preferably over 40-fold higher.

[0713] B-Raf (BRAF) is a member of the Raf kinase family of growth signal transduction protein kinases. This protein plays a role in regulating the MAP kinase/ERKs signaling pathway, which affects cell division, differentiation, and secretion.

[0714] B-Raf is a serine/threonine-specific protein kinase. As such, it catalyzes the phosphorylation of serine and threonine residues in a consensus sequence on target proteins by ATP, yielding ADP and a phosphorylated protein as products. Since it is a highly regulated signal transduction kinase, B-Raf must first bind Ras-GTP before becoming active as an enzyme. Once B-Raf is activated, a conserved protein kinase catalytic core phosphorylates protein substrates by promoting the nucleophilic attack of the activated substrate serine or threonine hydroxyl oxygen atom on the γ-phosphate group of ATP through bimolecular nucleophilic substitution.

[0715] Mutations in BRAF have been found in cancers, including non-Hodgkin lymphoma, colorectal cancer, malignant melanoma, papillary thyroid carcinoma, non-small-cell lung carcinoma, adenocarcinoma of the lung,

brain tumours including glioblastoma and pilocytic astrocytomas as well as inflammatory diseases like Erdheim-Chester disease. Mutation can lead to uncontrolled growth, especially in melanoma. For example, the V600E mutation in B-Raf is known to drive cell proliferation in melanoma mutated gene. Such mutations makes the mutant BRAF gene constitutively active, driving proliferation of the melanoma. By inhibiting mutated B-Raf, cell proliferation is blocked and apoptosis (controlled cell death) is induced.

[0716] Examples of such potential combinations are BRAF inhibitors such as vemurafenib and dabrafenib. These BRAF inhibitors inhibit the B-Raf protein directly.

[0717] Combining ADC, which targets AXL positive tumors, with BRAF_i is advantageous, because on the one hand, ADC will directly kill the AXL positive tumor cells via a mechanism depending on DNA cross-linking resulting in apoptosis, while on the other hand, BRAF_i will interfere with cell proliferation through inhibition of BRAF.

[0718] To show that ADC works synergistically with BRAF_i, a panel of AXL (+) cell lines including, but not limited to MDA-MB231, NCI-H1299 and SNU12 cells, will be co-treated with a range of concentrations of both ADC and BRAF_i. As negative controls, the same panel of cell lines will be co-treated with a range of concentrations of MEK_i or with a range of concentration of ADC and vehicle. After incubation, the in vitro cytotoxicity of the combinations will be determined by an MTS assay. To determine the cytotoxicity, Cell viability is measured by adding MTS per well and incubating for 4 hours at 37 °C.

[0719] Percentage cell viability is calculated compared to the untreated control. Cytotoxic synergy is calculated by transforming the cell viability data into fraction affected, and calculating the combination index (Table 1) using the CalcuSyn analysis program.

[0720] Specific BRAF inhibitors suitable for use as secondary agents in the present disclosure include:

[0721] a) vemurafenib

[0722] i. CAS Number→918504-65-1

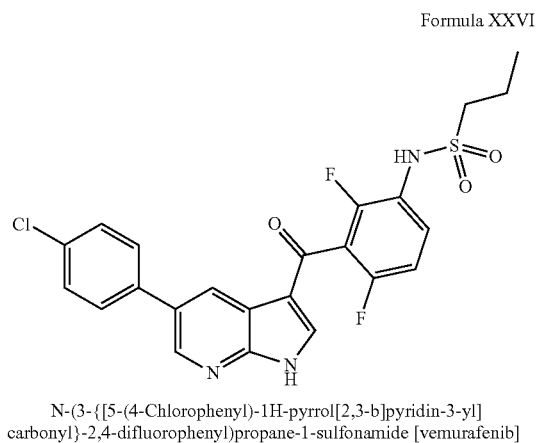
[0723] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0724] ii. DrugBank reference→DB08881

[0725] (see <https://www.drugbank.ca/>)

[0726] iii. Unique Ingredient Identifier (UNII)→207SMY3FQT

[0727] (see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)



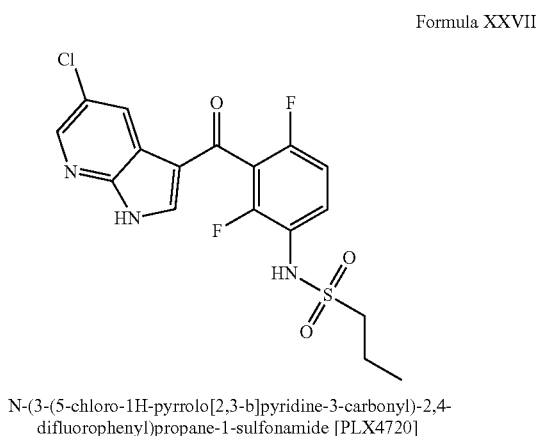
[0728] b) PLX4720

[0729] i. CAS Number→918505-84-7

[0730] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0731] ii. Unique Ingredient Identifier (UNII)→EQY31RO8HA

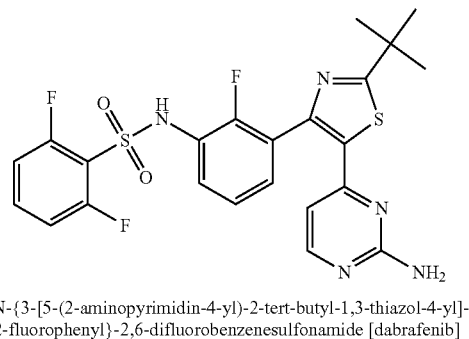
[0732] (see <http://www.fda.gov/ForIndustry/Data-Standards/SubstanceRegistrationSystem-Unique-IngredientIdentifierUNII/default.htm>)



[0733] c) dabrafenib

[0734] i. CAS Number→1195765-45-7

[0735] (see <http://www.cas.org/content/chemical-substances/faqs>)



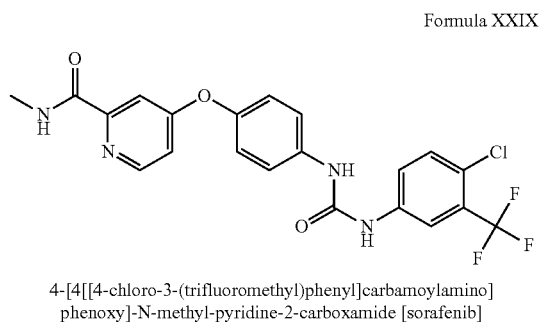
[0736] d) Sorafenib

[0737] i. CAS Number→284461-73-0

[0738] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0739] ii. Unique Ingredient Identifier (UNII)→9ZOQ3TZI87

[0740] (see <http://www.fda.gov/ForIndustry/Data-Standards/SubstanceRegistrationSystem-Unique-IngredientIdentifierUNII/default.htm>)



[0741] e) Encorafenib

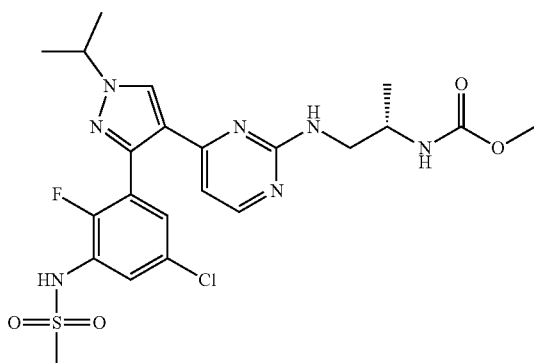
[0742] i. CAS Number→1269440-17-6

[0743] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0744] ii. Unique Ingredient Identifier (UNII)→8L7891MRB6

[0745] (see <http://www.fda.gov/ForIndustry/Data-Standards/SubstanceRegistrationSystem-Unique-IngredientIdentifierUNII/default.htm>)

Formula XXX

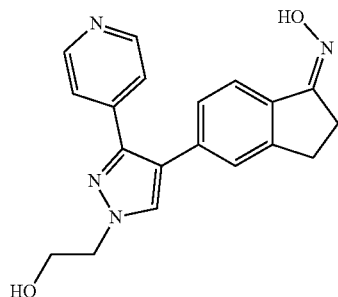


Methyl [(2S)-1-([4-(3-(5-chloro-2-fluoro-3-[(methylsulfonyl)amino]phenyl)-1-isopropyl-1H-pyrazol-4-yl)-2-pyrimidinyl]amino)-2-propanyl]carbamate [encorafenib]

[0746] f) GDC0879

[0747] i. CAS Number→905281-76-7

[0748] (see <http://www.cas.org/content/chemical-substances/faqs>)



(E)-2,3-Dihydro-5-[1-(2-hydroxyethyl)-3-(4-pyridinyl)-1H-pyrazol-4-yl]-1H-inden-1-one oxime [GDC0879]

MEKi

[0749] The secondary agent as described herein may be a MEK inhibitor.

[0750] “MEK inhibitor” means any chemical compound or biological molecule that reduces MEK1 and/or MEK2 activity.

[0751] MEK1 in humans is encoded by the MAP2K1 gene. MEK1 is a member of the dual-specificity protein kinase family that acts as a mitogen-activated protein (MAP) kinase kinase. MAP kinases, also known as extracellular signal-regulated kinases (ERKs), act as an integration point for multiple biochemical signals. This protein kinase lies upstream of MAP kinases and stimulates the enzymatic activity of MAP kinases upon activation by a wide variety of extra- and intracellular signals. As an essential component of the MAP kinase signal transduction pathway, this kinase is involved in many cellular processes such as proliferation, differentiation, transcription regulation and development.

[0752] MEK2 in humans is encoded by the MAP2K2 gene. The protein encoded by this gene is a dual specificity protein kinase that belongs to the MAP kinase kinase family.

This kinase is known to play a critical role in mitogen growth factor signal transduction. It phosphorylates and thus activates MAPK1/ERK2 and MAPK3/ERK1.

[0753] To examine the extent of inhibition of, e.g., MEK activity, samples or assays comprising a given, e.g., protein, gene, cell, or organism, are treated with a potential activating or inhibiting agent and are compared to control samples treated with an inactive control molecule. Control samples are assigned a relative activity value of 100%. Inhibition is achieved when the activity value relative to the control is about 90% or less, typically 85% or less, more typically 80% or less, most typically 75% or less, generally 70% or less, more generally 65% or less, most generally 60% or less, typically 55% or less, usually 50% or less, more usually 45% or less, most usually 40% or less, preferably 35% or less, more preferably 30% or less, still more preferably 25% or less, and most preferably less than 20%. Activation is achieved when the activity value relative to the control is about 110%, generally at least 120%, more generally at least 140%, more generally at least 160%, often at least 180%, more often at least 2-fold, most often at least 2.5-fold, usually at least 5-fold, more usually at least 10-fold, preferably at least 20-fold, more preferably at least 40-fold, and most preferably over 40-fold higher.

[0754] Examples of suitable MEK inhibitors are Trametinib, Cobimetinib, Binimetinib and Selumetinib. A MEK inhibitor inhibits the mitogen-activated protein kinase kinase enzymes MEK1 and/or MEK2. Defects in the MAP/ERK pathway can lead uncontrolled growth, especially in melanoma.

[0755] Some MEK inhibitors, such as Trametinib, inhibit MEK1 and MEK2 and are approved for the treatment of patients with BRAF V600E mutated metastatic melanoma. As described above, the V600E mutation makes the mutant BRAF gene constitutively active, driven proliferation of the melanoma. By inhibiting the MAP/ERK pathway, cell proliferation is blocked and apoptosis (controlled cell death) is induced.

[0756] Combining ADC, which targets AXL positive tumors, with MEKi is advantageous, because on the one hand, ADC will directly kill the AXL positive tumor cells via a mechanisms depending on DNA cross-linking resulting in apoptosis, while on the other hand, MEKi will interfere with cell proliferation through inhibition of the MAP/ERK cell signalling pathway.

[0757] To show that ADC works synergistically with MEKi, a panel of AXL (+) cell lines including, but not limited to MDA-MB231, H1299 and SNU12C cells, will be co-treated with a range of concentration of both ADC and MEKi. As negative controls, the same panel of cell lines will be co-treated with a range of concentrations of MEKi or with a range of concentration of ADC and vehicle. After incubation, the in vitro cytotoxicity of the combinations will be determined by an MTS assay. To determine the cytotoxicity, Cell viability is measured by adding MTS per well and incubating for 4 hours at 37 C. Percentage cell viability is calculated compared to the untreated control. Cytotoxic synergy is calculated by transforming the cell viability data into fraction affected, and calculating the combination index using the CalcuSyn analysis program.

[0758] Specific MEK inhibitors suitable for use as secondary agents in the present disclosure include:

[0759] a) Trametinib

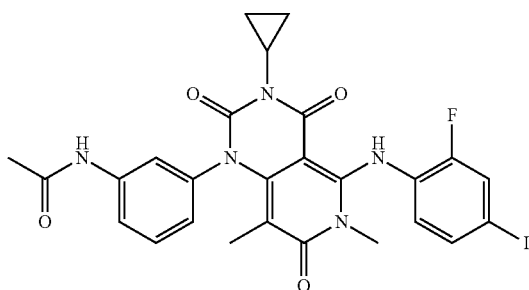
[0760] i. CAS Number→871700-17-3

[0761] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0762] ii. Unique Ingredient Identifier (UNII)→33E86K87QN

[0763] (see <http://www.fda.gov/ForIndustry/Data-Standards/SubstanceRegistrationSystem-Unique-IngredientIdentifierUNII/default.htm>)

Formula XXXII



N-(3-(3-Cyclopropyl-5-[(2-fluoro-4-iodophenyl)amino]-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydropyrido[4,3-d]pyrimidin-1(2H)-yl)phenyl)acetamide

[0764] b) Cobimetinib

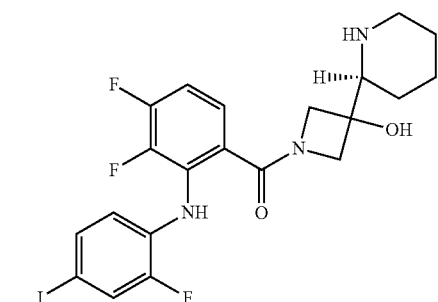
[0765] i. CAS Number→934660-93-2

[0766] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0767] ii. Unique Ingredient Identifier (UNII)→ER29L26N1X

[0768] (see <http://www.fda.gov/ForIndustry/Data-Standards/SubstanceRegistrationSystem-Unique-IngredientIdentifierUNII/default.htm>)

Formula XXXIII



(S)-[3,4-Difluoro-2-(2-fluoro-4-iodophenylamino)phenyl][3-hydroxy-3-(piperidin-2-yl)azetidin-1-yl] methanone

[0769] c) Binimetinib

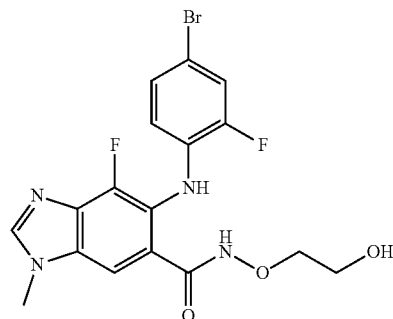
[0770] i. CAS Number→606143-89-9

[0771] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0772] ii. Unique Ingredient Identifier (UNII)→181R97MR71

[0773] (see <http://www.fda.gov/ForIndustry/Data-Standards/SubstanceRegistrationSystem-Unique-IngredientIdentifierUNII/default.htm>)

Formula XXIV



5-((4-bromo-2-fluorophenyl)amino)-4-fluoro-N-(2-hydroxyethoxy)-1-methyl-1H-benzo[d]imidazole-6-carboxamide

[0774] d) Selumetinib

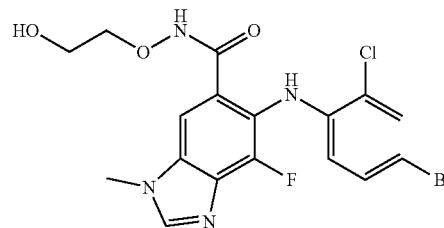
[0775] i. CAS Number→606143-52-6

[0776] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0777] ii. Unique Ingredient Identifier (UNII)→6UH91I579U

[0778] (see <http://www.fda.gov/ForIndustry/Data-Standards/SubstanceRegistrationSystem-Unique-IngredientIdentifierUNII/default.htm>)

Formula XXXV



6-(4-bromo-2-chloroanilino)-7-fluoro-N-(2-hydroxyethoxy)-3-methylbenzimidazole-5-carboxamide

[0779] e) PD-325901

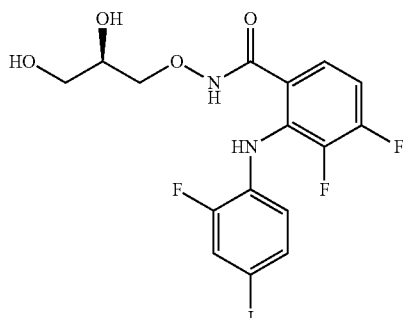
[0780] i. CAS Number→391210-10-9

[0781] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0782] ii. Unique Ingredient Identifier (UNII)→86K0J5AK6M

[0783] (see <http://www.fda.gov/ForIndustry/Data-Standards/SubstanceRegistrationSystem-Unique-IngredientIdentifierUNII/default.htm>)

Formula XXXVI



N-[(2R)-2,3-dihydroxypropoxy]-3,4-difluoro-2-(2-fluoro-4-iodoanilino)benzamide

[0784] f) CI-1040

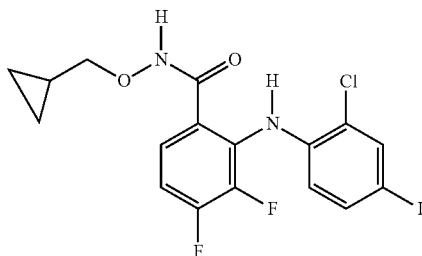
[0785] i. CAS Number→212631-79-3

[0786] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0787] ii. Unique Ingredient Identifier (UNII)→R3K9Y00J04

[0788] (see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

Formula XXXVII



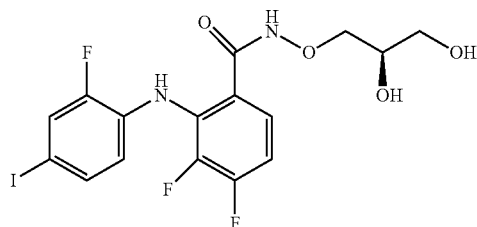
2-[(2-chloro-4-iodophenyl)amino]-N-(cyclopropylmethoxy)-3,4-difluorobenzamide

[0789] g) PD035901

[0790] i. CAS Number→391210-10-9

[0791] (see <http://www.cas.org/content/chemical-substances/faqs>)

Formula XXXVIII



PD035901

[0792] h) U0126

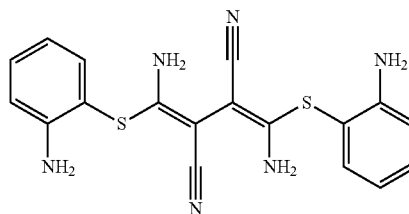
[0793] i. CAS Number→218601-62-8

[0794] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0795] ii. Unique Ingredient Identifier (UNII)→8027P94HLL

[0796] (see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

Formula XXXIX



1,4-diamino-2,3-dicyano-1,4-bis(2-aminophenylthio)butadiene

[0797] i) TAK-733

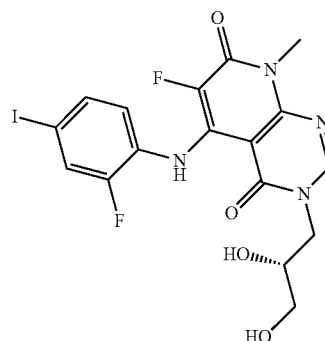
[0798] iii. CAS Number→1035555-63-5

[0799] (see <http://www.cas.org/content/chemical-substances/faqs>)

[0800] iv. Unique Ingredient Identifier (UNII)→5J61HSP0QJ

[0801] (see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

Formula XL



3-[(2R)-2,3-dihydroxypropyl]-6-fluoro-5-(2-fluoro-4-iodoanilino)-8-methylpyrido[2,3-d]pyrimidine-4,7-dione

[0802] In some embodiments, BRAF polypeptide corresponds to Genbank accession no. AAA35609, version no. AAA35609.2, record update date: Jun. 23, 2010 09:41 AM. In one embodiment, the nucleic acid encoding BRAF polypeptide corresponds to Genbank accession no. M95712, version no. M95712.2, record update date: Jun. 23, 2010 09:41 AM. In some embodiments, BRAF polypeptide corresponds to Uniprot/Swiss-Prot accession No. P15056.

[0803] In some embodiments, MEK1 polypeptide corresponds to Genbank accession no. AAA36318, version no. AAA36318.1, record update date: Jun. 23, 2010 08:48 AM. In one embodiment, the nucleic acid encoding MEK1 polypeptide corresponds to Genbank accession no. L05624,

version no. L05624.1, record update date: Jun. 23, 2010 08:48 AM. In some embodiments, MEK1 polypeptide corresponds to Uniprot/Swiss-Prot accession No. Q02750.

[0804] In some embodiments, MEK2 polypeptide corresponds to Genbank accession no. AAH00471, version no. AAH00471.1, record update date: Sep. 23, 2014 03:30 PM. In one embodiment, the nucleic acid encoding MEK2 polypeptide corresponds to Genbank accession no. BC000471, version no. BC000471.2, record update date: Sep. 23, 2014 03:30 PM. In some embodiments, MEK2 polypeptide corresponds to Uniprot/Swiss-Prot accession No. P36507.

Advantageous Properties of the Described Combinations

[0805] Both the ADC and secondary agent when used as a single agent in isolation have demonstrated clinical utility—for example, in the treatment of cancer. However, as described herein, combination of the ADC and secondary agent is expected to provide one or more of the following advantages over treatment with either ADC or secondary agent alone:

[0806] 1) effective treatment of a broader range of cancers;

[0807] 2) effective treatment of resistant or refractory forms of disorders such as cancer, and individuals with disorders such as cancer who have relapsed after a period of remission;

[0808] 3) increased response rate to treatment; and/or

[0809] 4) Increased durability of treatment.

[0810] Effective treatment of a broader range of cancers as used herein means that following treatment with the combination a complete response is observed with a greater range of recognised cancer types. That is, a complete response is seen from cancer types not previously reported to completely respond to either ADC or secondary agent alone.

[0811] Effective treatment of a resistant, refractory, or relapsed forms as used herein means that following treatment with the combination a complete response is observed in individuals that are either partially or completely resistant or refractory to treatment with either ADC or secondary agent alone (for example, individuals who show no response or only partial response following treatment with either agent alone, or those with relapsed disorder). In some embodiments, a complete response following treatment with the ADC/secondary agent combination is observed at least 10% of individuals that are either partially or completely resistant or refractory to treatment with either ADC or secondary agent alone. In some embodiments, a complete response following treatment with the ADC/secondary agent combination is observed at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% of individuals that are either partially or completely resistant or refractory to treatment with either ADC or secondary agent alone.

[0812] Increased response rate to treatment as used herein means that following treatment with the combination a complete response is observed in a greater proportion of individuals than is observed following treatment with either ADC or secondary agent alone. In some embodiments, a complete response following treatment with the ADC/secondary agent combination is observed at least 10% of treated individuals. In some embodiments, a complete response following treatment with the ADC/secondary agent combi-

nation is observed at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% of treated individuals.

[0813] Increased durability of treatment as used herein means that average duration of complete response in individuals treated with the combination is longer than in individuals who achieve complete response following treatment with either ADC or secondary agent alone. In some embodiments, the average duration of a complete response following treatment with the ADC/secondary agent combination is at least 6 months. In some embodiments, the average duration of a complete response following treatment with the ADC/secondary agent combination is at least 12 months, at least 18 months, at least 24 months, at least 3 years, at least 4 years, at least 5 years, at least 6 years, at least 7 years, at least 8 years, at least 9 years, at least 10 years, at least 15 years, or at least 20 years.

[0814] ‘Complete response’ is used herein to mean the absence of any clinical evidence of disease in an individual. Evidence may be assessed using the appropriate methodology in the art, for example CT or PET scanning, or biopsy where appropriate. The number of doses required to achieve complete response may be one, two, three, four, five, ten or more. In some embodiments the individuals achieve complete response no more than a year after administration of the first dose, such as no more than 6 months, no more than 3 months, no more than a month, no more than a fortnight, or no more than a week after administration of the first dose.

Treated Disorders

[0815] The combined therapies described herein include those with utility for anticancer activity.

[0816] In particular, in certain aspects the therapies include an antibody conjugated, i.e. covalently attached by a linker, to a PBD drug moiety, i.e. toxin. When the drug is not conjugated to an antibody, the PBD drug has a cytotoxic effect. The biological activity of the PBD drug moiety is thus modulated by conjugation to an antibody. The antibody-drug conjugates (ADC) of the disclosure selectively deliver an effective dose of a cytotoxic agent to tumor tissue whereby greater selectivity, i.e. a lower efficacious dose, may be achieved.

[0817] Thus, in one aspect, the present disclosure provides combined therapies comprising administering an ADC which binds a first target protein for use in therapy, wherein the method comprises selecting a subject based on expression of the target protein.

[0818] In one aspect, the present disclosure provides a combined therapy with a label that specifies that the therapy is suitable for use with a subject determined to be suitable for such use. The label may specify that the therapy is suitable for use in a subject has expression of the first target protein, such as overexpression of the first target protein. The label may specify that the subject has a particular type of cancer.

[0819] The first target protein is preferably AXL. The disorder may be a proliferative disease, for example a cancer such as breast, lung, gastric, head and neck, colorectal, renal, pancreatic, uterine, hepatic, bladder, endometrial and prostate cancers as well as lymphomas (e.g., non-Hodgkin's lymphoma, NHL) and leukemia (particularly acute myeloid leukemia, AML). The disorder may be an immune disorder, cardiovascular disorder, thrombosis, diabetes, immune

checkpoint disorder, or fibrotic disorder (fibrosis) such as strabismus, scleroderma, keloid, Nephrogenic systemic fibrosis, pulmonary fibrosis, idiopathic pulmonary fibrosis (IPF), cystic fibrosis (CF), systemic sclerosis, cardiac fibrosis, non-alcoholic steatohepatitis (NASH), other types of liver fibrosis, primary biliary cirrhosis, renal fibrosis, cancer, and atherosclerosis. The label may specify that the subject has a AXL+ cancer.

[0820] In a further aspect there is also provided a combined therapy as described herein for use in the treatment of a proliferative disease. Another aspect of the present disclosure provides the use of a conjugate compound in the manufacture of a medicament for treating a proliferative disease.

[0821] One of ordinary skill in the art is readily able to determine whether or not a candidate combined therapy treats a proliferative condition for any particular cell type. For example, assays which may conveniently be used to assess the activity offered by a particular compound are described below.

[0822] The combined therapies described herein may be used to treat a proliferative disease. The term “proliferative disease” pertains to an unwanted or uncontrolled cellular proliferation of excessive or abnormal cells which is undesired, such as, neoplastic or hyperplastic growth, whether in vitro or in vivo.

[0823] Examples of proliferative conditions include, but are not limited to, benign, pre-malignant, and malignant cellular proliferation, including but not limited to, neoplasms and tumours (e.g. histiocytoma, glioma, astrocytoma, osteoma), cancers (e.g. lung cancer, small cell lung cancer, gastrointestinal cancer, bowel cancer, colon cancer, breast carcinoma, ovarian carcinoma, prostate cancer, testicular cancer, liver cancer, kidney cancer, bladder cancer, pancreas cancer, brain cancer, sarcoma, osteosarcoma, Kaposi's sarcoma, melanoma), lymphomas, leukemias, psoriasis, bone diseases, fibroproliferative disorders (e.g. of connective tissues), and atherosclerosis. Cancers of particular interest include, but are not limited to, leukemias and ovarian cancers.

[0824] Any type of cell may be treated, including but not limited to, lung, gastrointestinal (including, e.g. bowel, colon), breast (mammary), ovarian, prostate, liver (hepatic), kidney (renal), bladder, pancreas, brain, and skin.

[0825] Disorders of particular interest include, but are not limited to cancers, including metastatic cancers and metastatic cancer cells, such as circulating tumour cells, which may be found circulating in body fluids such as blood or lymph. Cancers of particular interest include breast, lung, gastric, head and neck, colorectal, renal, pancreatic, uterine, hepatic, bladder, endometrial and prostate cancers as well as lymphomas (e.g., non-Hodgkin's lymphoma, NHL) and leukemia (particularly acute myeloid leukemia, AML).

[0826] Other disorders of interest include any condition in which Axl is overexpressed, or wherein Axl antagonism will provide a clinical benefit. These include immune disorders, cardiovascular disorders, thrombosis, diabetes, immune checkpoint disorders, fibrotic disorders (fibrosis), or proliferative diseases such as cancer, particularly metastatic cancer. Furthermore, Axl is known to play a role in many cancers of epithelial origin.

[0827] Fibrotic disorders of interest include strabismus, scleroderma, keloid, Nephrogenic systemic fibrosis, pulmonary fibrosis, idiopathic pulmonary fibrosis (IPF), cystic

fibrosis (CF), systemic sclerosis, cardiac fibrosis, non-alcoholic steatohepatitis (NASH), other types of liver fibrosis, primary biliary cirrhosis, renal fibrosis, cancer, and atherosclerosis. In these diseases, the chronic development of fibrosis in tissue leads to marked alterations in the architecture of the affected organs and subsequently cause defective organ function. As a result of this process of sustained attrition to organs, many diseases that involve fibrosis are often progressive conditions and have a poor long-term prognosis (see Rockey, D. C., Bell, P. D. and Hill, J. A. (2015), *N. Engl. Med.*, Vol. 372, pp. 1138-1149).

[0828] The proliferative disease may be characterised by the presence of a neoplasm comprising both AXL+ve and AXL-ve cells.

[0829] The proliferative disease may be characterised by the presence of a neoplasm composed of AXL-ve neoplastic cells, optionally wherein the AXL-ve neoplastic cells are associated with AXL+ve non-neoplastic cells.

[0830] The target neoplasm or neoplastic cells may be all or part of a solid tumour.

[0831] “Solid tumor” herein will be understood to include solid haematological cancers such as lymphomas (Hodgkin's lymphoma or non-Hodgkin's lymphoma) which are discussed in more detail herein.

[0832] Solid tumors may be neoplasms, including non-haematological cancers, comprising or composed of AXL+ve neoplastic cells. Solid tumors may be neoplasms, including non-haematological cancers, infiltrated with AXL+ve cells, such as AXL+ve immune suppressive dendritic cells, NK cells, or macrophages; such solid tumours may lack expression of AXL (that is, comprise or be composed of AXL-ve neoplastic cells).

[0833] It is contemplated that the combined therapies of the present disclosure may be used to treat various diseases or disorders, e.g. characterized by the overexpression of a tumor antigen. Exemplary conditions or hyperproliferative disorders include benign or malignant tumors; leukemia, haematological, and lymphoid malignancies. Others include neuronal, glial, astrocytal, hypothalamic, glandular, macrophagal, epithelial, stromal, blastocoeic, inflammatory, angiogenic and immunologic, including autoimmune disorders and graft-versus-host disease (GVHD).

[0834] Generally, the disease or disorder to be treated is a hyperproliferative disease such as cancer. Examples of cancer to be treated herein include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (e.g. epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, as well as head and neck cancer.

[0835] Autoimmune diseases for which the combined therapies may be used in treatment include rheumatologic disorders (such as, for example, rheumatoid arthritis, Sjögren's syndrome, scleroderma, lupus such as SLE and

lupus nephritis, polymyositis/dermatomyositis, cryoglobulinemia, anti-phospholipid antibody syndrome, and psoriatic arthritis), osteoarthritis, autoimmune gastrointestinal and liver disorders (such as, for example, inflammatory bowel diseases (e.g. ulcerative colitis and Crohn's disease), autoimmune gastritis and pernicious anemia, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, and celiac disease), vasculitis (such as, for example, ANCA-associated vasculitis, including Churg-Strauss vasculitis, Wegener's granulomatosis, and polyarteritis), autoimmune neurological disorders (such as, for example, multiple sclerosis, opsoclonus myoclonus syndrome, myasthenia gravis, neuromyelitis optica, Parkinson's disease, Alzheimer's disease, and autoimmune polyneuropathies), renal disorders (such as, for example, glomerulonephritis, Goodpasture's syndrome, and Berger's disease), autoimmune dermatologic disorders (such as, for example, psoriasis, urticaria, hives, pemphigus vulgaris, bullous pemphigoid, and cutaneous lupus erythematosus), hematologic disorders (such as, for example, thrombocytopenic purpura, thrombotic thrombocytopenic purpura, post-transfusion purpura, and autoimmune hemolytic anemia), atherosclerosis, uveitis, autoimmune hearing diseases (such as, for example, inner ear disease and hearing loss), Behcet's disease, Raynaud's syndrome, organ transplant, graft-versus-host disease (GVHD), and autoimmune endocrine disorders (such as, for example, diabetic-related autoimmune diseases such as insulin-dependent diabetes mellitus (IDDM), Addison's disease, and autoimmune thyroid disease (e.g. Graves' disease and thyroiditis)). More preferred such diseases include, for example, rheumatoid arthritis, ulcerative colitis, ANCA-associated vasculitis, lupus, multiple sclerosis, Sjögren's syndrome, Graves' disease, IDDM, pernicious anemia, thyroiditis, and glomerulonephritis.

[0836] In some aspects, the subject has a proliferative disorder selected from a cancer such as breast, lung, gastric, head and neck, colorectal, renal, pancreatic, uterine, hepatic, bladder, endometrial and prostate cancers as well as lymphomas (e.g., non-Hodgkin's lymphoma, NHL) and leukemia (particularly acute myeloid leukemia, AML). In some aspects, the subject has a disorder selected from an immune disorder, cardiovascular disorder, thrombosis, diabetes, immune checkpoint disorder, or fibrotic disorder (fibrosis) such as strabismus, scleroderma, keloid, Nephrogenic systemic fibrosis, pulmonary fibrosis, idiopathic pulmonary fibrosis (IPF), cystic fibrosis (CF), systemic sclerosis, cardiac fibrosis, non-alcoholic steatohepatitis (NASH), other types of liver fibrosis, primary biliary cirrhosis, renal fibrosis, cancer, and atherosclerosis. The label may specify that the subject has a AXL+ cancer. Breast cancer and AML are cancers of particular interest.

[0837] In some aspects, the subject has a proliferative disease may be characterised by the presence of a neoplasm comprising both AXL+ve and AXL-ve cells.

[0838] The proliferative disease may be characterised by the presence of a neoplasm composed of AXL-ve neoplastic cells, optionally wherein the AXL-ve neoplastic cells are associated with AXL+ve non-neoplastic cells.

[0839] The target neoplasm or neoplastic cells may be all or part of a solid tumour.

[0840] "Solid tumor" herein will be understood to include solid haematological cancers such as lymphomas (Hodgkin's lymphoma or non-Hodgkin's lymphoma) which are discussed in more detail herein.

[0841] Solid tumors may be neoplasms, including non-haematological cancers, comprising or composed of AXL+ve neoplastic cells. Solid tumors may be neoplasms, including non-haematological cancers, infiltrated with AXL+ve cells, such as AXL+ve immune suppressive dendritic cells, NK cells, or macrophages; such solid tumours may lack expression of AXL (that is, comprise or be composed of AXL-ve neoplastic cells).

Patient Selection

[0842] In certain aspects, the individuals are selected as suitable for treatment with the combined treatments before the treatments are administered.

[0843] As used herein, individuals who are considered suitable for treatment are those individuals who are expected to benefit from, or respond to, the treatment. Individuals may have, or be suspected of having, or be at risk of having cancer. Individuals may have received a diagnosis of cancer. In particular, individuals may have, or be suspected of having, or be at risk of having, lymphoma. In some cases, individuals may have, or be suspected of having, or be at risk of having, a solid cancer that has tumour associated non-tumor cells that express a first target protein, such as infiltrating cells that express a first target protein.

[0844] In some aspects, individuals are selected on the basis of the amount or pattern of expression of a first target protein. In some aspects, the selection is based on expression of a first target protein at the cell surface.

[0845] In certain aspects, the target is a second target protein. In some aspects, the selection is based on expression of a second target protein at the cell surface.

[0846] In some aspects, the selection is based on levels of both a first target protein and a second target protein at the cell surface.

[0847] In some cases, expression of the target in a particular tissue of interest is determined. For example, in a sample of lymphoid tissue or tumor tissue. In some cases, systemic expression of the target is determined. For example, in a sample of circulating fluid such as blood, plasma, serum or lymph.

[0848] In some aspects, the individual is selected as suitable for treatment due to the presence of target expression in a sample. In those cases, individuals without target expression may be considered not suitable for treatment.

[0849] In other aspects, the level of target expression is used to select a individual as suitable for treatment. Where the level of expression of the target is above a threshold level, the individual is determined to be suitable for treatment.

[0850] In some aspects, the presence of a first target protein and/or a second target protein in cells in the sample indicates that the individual is suitable for treatment with a combination comprising an ADC and a secondary agent. In other aspects, the amount of first target protein and/or a second target protein expression must be above a threshold level to indicate that the individual is suitable for treatment. In some aspects, the observation that first target protein and/or a second target protein localisation is altered in the sample as compared to a control indicates that the individual is suitable for treatment.

[0851] In some aspects, an individual is indicated as suitable for treatment if cells obtained from lymph node or extra nodal sites react with antibodies against first target protein and/or a second target protein as determined by IHC.

[0852] In some aspects, a patient is determined to be suitable for treatment if at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or more of all cells in the sample express a first target protein. In some aspects disclosed herein, a patient is determined to be suitable for treatment if at least at least 10% of the cells in the sample express a first target protein.

[0853] In some aspects, a patient is determined to be suitable for treatment if at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or more of all cells in the sample express a second target protein. In some aspects disclosed herein, a patient is determined to be suitable for treatment if at least at least 10% of the cells in the sample express a second target protein.

[0854] The first target protein is preferably AXL.

[0855] The second target protein may be PD1, PDL1, GITR, OX40, CTLA, PARP1, MEK1, MEK2, or BRAF. The second target protein is preferably PD-L1.

Samples

[0856] The sample may comprise or may be derived from: a quantity of blood; a quantity of serum derived from the individual's blood which may comprise the fluid portion of the blood obtained after removal of the fibrin clot and blood cells; a quantity of pancreatic juice; a tissue sample or biopsy; or cells isolated from said individual.

[0857] A sample may be taken from any tissue or bodily fluid. In certain aspects, the sample may include or may be derived from a tissue sample, biopsy, resection or isolated cells from said individual.

[0858] In certain aspects, the sample is a tissue sample. The sample may be a sample of tumor tissue, such as cancerous tumor tissue. The sample may have been obtained by a tumor biopsy. In some aspects, the sample is a lymphoid tissue sample, such as a lymphoid lesion sample or lymph node biopsy. In some cases, the sample is a skin biopsy.

[0859] In some aspects the sample is taken from a bodily fluid, more preferably one that circulates through the body. Accordingly, the sample may be a blood sample or lymph sample. In some cases, the sample is a urine sample or a saliva sample.

[0860] In some cases, the sample is a blood sample or blood-derived sample. The blood derived sample may be a selected fraction of an individual's blood, e.g. a selected cell-containing fraction or a plasma or serum fraction.

[0861] A selected cell-containing fraction may contain cell types of interest which may include white blood cells (WBC), particularly peripheral blood mononuclear cells (PBC) and/or granulocytes, and/or red blood cells (RBC). Accordingly, methods according to the present disclosure may involve detection of a first target polypeptide or nucleic acid in the blood, in white blood cells, peripheral blood mononuclear cells, granulocytes and/or red blood cells.

[0862] The sample may be fresh or archival. For example, archival tissue may be from the first diagnosis of an individual, or a biopsy at a relapse. In certain aspects, the sample is a fresh biopsy.

[0863] The first target polypeptide is preferably AXL.

Individual Status

[0864] The individual may be an animal, mammal, a placental mammal, a marsupial (e.g., kangaroo, wombat), a monotreme (e.g., duckbilled platypus), a rodent (e.g., a guinea pig, a hamster, a rat, a mouse), murine (e.g., a mouse), a lagomorph (e.g., a rabbit), avian (e.g., a bird), canine (e.g., a dog), feline (e.g., a cat), equine (e.g., a horse), porcine (e.g., a pig), ovine (e.g., a sheep), bovine (e.g., a cow), a primate, simian (e.g., a monkey or ape), a monkey (e.g., marmoset, baboon), an ape (e.g., gorilla, chimpanzee, orangutang, gibbon), or a human.

[0865] Furthermore, the individual may be any of its forms of development, for example, a foetus. In one preferred embodiment, the individual is a human. The terms "subject", "patient" and "individual" are used interchangeably herein.

[0866] In some cases the individual has, is suspected of having, or has received a diagnosis of, a proliferative disease characterised by the presence of a neoplasm comprising both AXL+ve and AXL-ve cells. The neoplasm may be composed of AXL-ve neoplastic cells, optionally wherein the AXL-ve neoplastic cells are associated with AXL+ve non-neoplastic cells. The target neoplasm or neoplastic cells may be all or part of a solid tumour. The solid tumour may be a neoplasm, including a non-haematological cancer, comprising or composed of AXL+ve neoplastic cells. The solid tumour may be a neoplasm, including a non-haematological cancer, infiltrated with AXL+ve cells, such as AXL+ve immune suppressive dendritic cells, NK cells, or macrophages; such solid tumours may lack expression of AXL (that is, comprise or be composed of AXL-ve neoplastic cells).

[0867] In some aspects disclosed herein, an individual has, or is suspected as having, or has been identified as being at risk of cancer. In some aspects disclosed herein, the individual has already received a diagnosis of cancer. The individual may have received a diagnosis of a cancer such as breast, lung, gastric, head and neck, colorectal, renal, pancreatic, uterine, hepatic, bladder, endometrial and prostate cancers as well as lymphomas (e.g., non-Hodgkin's lymphoma, NHL) and leukemia (particularly acute myeloid leukemia, AML). Breast cancer and AML are cancers of particular interest.

[0868] In some aspects disclosed herein, an individual has, or is suspected as having, or has been identified as being at risk of, or has received a diagnosis of an immune disorder, cardiovascular disorder, thrombosis, diabetes, immune checkpoint disorder, or fibrotic disorder (fibrosis) such as strabismus, scleroderma, keloid, Nephrogenic systemic fibrosis, pulmonary fibrosis, idiopathic pulmonary fibrosis (IPF), cystic fibrosis (CF), systemic sclerosis, cardiac fibrosis, non-alcoholic steatohepatitis (NASH), other types of liver fibrosis, primary biliary cirrhosis, renal fibrosis, cancer, and atherosclerosis.

[0869] In some cases, the individual has received a diagnosis of a solid cancer containing AXL+ expressing infiltrating cells.

[0870] The Individual may be undergoing, or have undergone, a therapeutic treatment for that cancer. The subject may, or may not, have previously received ADCxAXL. In some cases the cancer is breast cancer or AML.

Controls

[0871] In some aspects, target expression in the individual is compared to target expression in a control. Controls are useful to support the validity of staining, and to identify experimental artefacts.

[0872] In some cases, the control may be a reference sample or reference dataset. The reference may be a sample that has been previously obtained from a individual with a known degree of suitability. The reference may be a dataset obtained from analyzing a reference sample.

[0873] Controls may be positive controls in which the target molecule is known to be present, or expressed at high level, or negative controls in which the target molecule is known to be absent or expressed at low level.

[0874] Controls may be samples of tissue that are from individuals who are known to benefit from the treatment. The tissue may be of the same type as the sample being tested. For example, a sample of tumor tissue from a individual may be compared to a control sample of tumor tissue from a individual who is known to be suitable for the treatment, such as a individual who has previously responded to the treatment.

[0875] In some cases the control may be a sample obtained from the same individual as the test sample, but from a tissue known to be healthy. Thus, a sample of cancerous tissue from a individual may be compared to a non-cancerous tissue sample.

[0876] In some cases, the control is a cell culture sample.

[0877] In some cases, a test sample is analyzed prior to incubation with an antibody to determine the level of background staining inherent to that sample.

[0878] In some cases an isotype control is used. Isotype controls use an antibody of the same class as the target specific antibody, but are not immunoreactive with the sample. Such controls are useful for distinguishing non-specific interactions of the target specific antibody.

[0879] The methods may include hematopathologist interpretation of morphology and immunohistochemistry, to ensure accurate interpretation of test results. The method may involve confirmation that the pattern of expression correlates with the expected pattern. For example, where the amount of a first target protein and/or a second target protein expression is analyzed, the method may involve confirmation that in the test sample the expression is observed as membrane staining, with a cytoplasmic component. The method may involve confirmation that the ratio of target signal to noise is above a threshold level, thereby allowing clear discrimination between specific and non-specific background signals.

[0880] The first target protein is preferably AXL.

[0881] The second target protein may be PD1, PDL1, GITR, OX40, CTLA, PARPi, MEK1, MEK2, or BRAF. The second target protein is preferably PD-L1.

Methods of Treatment

[0882] The term “treatment,” as used herein in the context of treating a condition, pertains generally to treatment and therapy, whether of a human or an animal (e.g., in veterinary applications), in which some desired therapeutic effect is achieved, for example, the inhibition of the progress of the condition, and includes a reduction in the rate of progress, a halt in the rate of progress, regression of the condition,

amelioration of the condition, and cure of the condition. Treatment as a prophylactic measure (i.e., prophylaxis, prevention) is also included.

[0883] The term “therapeutically-effective amount” or “effective amount” as used herein, pertains to that amount of an active compound, or a material, composition or dosage from comprising an active compound, which is effective for producing some desired therapeutic effect, commensurate with a reasonable benefit/risk ratio, when administered in accordance with a desired treatment regimen.

[0884] Similarly, the term “prophylactically-effective amount,” as used herein, pertains to that amount of an active compound, or a material, composition or dosage from comprising an active compound, which is effective for producing some desired prophylactic effect, commensurate with a reasonable benefit/risk ratio, when administered in accordance with a desired treatment regimen.

[0885] Disclosed herein are methods of therapy. Also provided is a method of treatment, comprising administering to a subject in need of treatment a therapeutically-effective amount of an ADC and a secondary agent. The term “therapeutically effective amount” is an amount sufficient to show benefit to a subject. Such benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage, is within the responsibility of general practitioners and other medical doctors. The subject may have been tested to determine their eligibility to receive the treatment according to the methods disclosed herein. The method of treatment may comprise a step of determining whether a subject is eligible for treatment, using a method disclosed herein.

[0886] The ADC may comprise an anti-AXL antibody. The ADC may comprise a drug which is a PBD dimer. The ADC may be an anti-AXL-ADC, and in particular, ADCx-AXL. The ADC may be an ADC disclosed in GB1702029.8, GB1719906.8, or PCT/EP2018/053163.

[0887] The secondary agent may be:

[0888] (a) a PD1 antagonist, such as pembrolizumab, nivolumab, MEDI0680, PDR001 (spartalizumab), Camrelizumab, AUNP12, Pidilizumab, Cemiplimab (REGN-2810), AMP-224, BGB-A317 (Tisleizumab), or BGB-108;

[0889] (b) a PD-L1 antagonist, such as atezolizumab (Tecentriq), BMS-936559/MDX-1105, durvalumab/MEDI4736, or MSB0010718C (Avelumab);

[0890] (c) a GITR (Glucocorticoid-Induced TNFR-Related protein) agonist, such as MEDI1873, TRX518, GWN323, MK-1248, MK-4166, BMS-986156 or INCAGN1876;

[0891] (d) an OX40 agonist, such as MEDI0562, MEDI6383, MOXR0916, RG7888, OX40mAb24, INCAGN1949, GSK3174998, or PF-04518600;

[0892] (e) a CTLA-4 antagonist, such as ipilimumab (brand name Yervoy) or Tremelimumab (Originally developed by Pfizer, now Medimmune);

[0893] (f) Fludarabine or Cytarabine;

[0894] (g) a hypomethylating agent, such as cytidine analogs—for example, 5-azacytidine (azacitidine) and 5-aza-2'-deoxycytidine (decitabine); or

[0895] (h) a PARP inhibitor (PARPi), such as Olaparib, CEP-9722, BMN-673/talazoparib, Rucaparib, Iniparib/

- SAR24-550/BSI-201, Veliparib (ABT-888), Niraparib/MK-4827, BGB-290, 3-aminobenzamide, and E7016;
- [0896] (i) an agent that upregulates HER2 expression, such as gemcitabine and tamoxifen;
- [0897] (j) an AXL-kinase inhibitor (AXLi) such as BGB324 (bemcentinib), TP0903, Gilteritinib (ASP2215), Cabozantinib (XL184), SG17079, Meresitinib, amuvatinib (MP-470), bosutinib (SKI-606), MGCD265, and foretinib (GSK1363089/XL880);
- [0898] (k) a BRAF inhibitor (BRAFi), such as vemurafenib, PLX4720, dabrafenib, Sorafenib, Encorafenib, and GDC0879; or
- [0899] (l) a MEK inhibitor (MEKi), such as Trametinib, Cobimetinib, Binimetinib, Selumetinib, PD-325901, CI-1040, PD035901, U0126, and TAK-733.

[0900] The treatment may involve administration of the ADC/secondary agent combination alone or in further combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated. Examples of treatments and therapies include, but are not limited to, chemotherapy (the administration of active agents, including, e.g. drugs, such as chemotherapeutics); surgery; and radiation therapy.

[0901] A “chemotherapeutic agent” is a chemical compound useful in the treatment of cancer, regardless of mechanism of action. Classes of chemotherapeutic agents include, but are not limited to: alkylating agents, antimetabolites, spindle poison plant alkaloids, cytotoxic/antitumor antibiotics, topoisomerase inhibitors, antibodies, photosensitizers, and kinase inhibitors. Chemotherapeutic agents include compounds used in “targeted therapy” and conventional chemotherapy.

[0902] Examples of chemotherapeutic agents include: Lenalidomide (REVLIMID®, Celgene), Vorinostat (ZOLINZA®, Merck), Panobinostat (FARYDAK®, Novartis), Mocetinostat (MGCD0103), Everolimus (ZORTRESS®, CERTICAN®, Novartis), Bendamustine (TREAKISYM®, RIBOMUSTIN®, LEVACT®, TREANDA®, Mundipharma International), erlotinib (TARCEVA®, Genentech/OSI Pharm.), docetaxel (TAXOTERE®, Sanofi-Aventis), 5-FU (fluorouracil, 5-fluorouracil, CAS No. 51-21-8), gemcitabine (GEMZAR®, Lilly), PD-0325901 (CAS No. 391210-10-9, Pfizer), cisplatin (cis-diamine, dichloroplatinum(II), CAS No. 15663-27-1), carboplatin (CAS No. 41575-94-4), paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, N.J.), trastuzumab (HERCEPTIN®, Genentech), temozolomide (4-methyl-5-oxo-2,3,4,6,8-pentazabicyclo [4.3.0] nona-2,7,9-triene-9-carboxamide, CAS No. 85622-93-1, TEMODAR®, TEMODAL®, Schering Plough), tamoxifen ((Z)-2-[4-(1,2-diphenylbut-1-enyl)phenoxy]-N,N-dimethylethanamine, NOLVADEX®, ISTUBAL®, VALODEX®), and doxorubicin (ADRIAMYCIN®), Akti-1/2, HPPD, and rapamycin.

[0903] More examples of chemotherapeutic agents include: oxaliplatin (ELOXATIN®, Sanofi), bortezomib (VELCADE®, Millennium Pharm.), sunitinib (SUNITINIB®, SU11248, Pfizer), letrozole (FEMARA®, Novartis), imatinib mesylate (GLEEVEC®, Novartis), XL-518 (MEK inhibitor, Exelixis, WO 2007/044515), ARRY-886 (MEK inhibitor, AZD6244, Array BioPharma, Astra Zeneca), SF-1126 (PI3K inhibitor, Semafore Pharmaceuticals), BEZ-235 (PI3K inhibitor, Novartis), XL-147 (PI3K inhibitor, Exelixis), PTK787/ZK 222584 (Novartis), fulvestrant

(FASLODEX®, AstraZeneca), leucovorin (folinic acid), rapamycin (sirolimus, RAPAMUNE®, Wyeth), lapatinib (TYKERB®, GSK572016, Glaxo Smith Kline), lonafamib (SARASAR™, SCH 66336, Schering Plough), sorafenib (NEXAVAR®, BAY43-9006, Bayer Labs), gefitinib (IRESSA®, AstraZeneca), irinotecan (CAMPTOSAR®, CPT-11, Pfizer), tipifamib (ZARNESTRA™, Johnson & Johnson), ABRAXANE™ (Cremophor-free), albumin-engineered nanoparticle formulations of paclitaxel (American Pharmaceutical Partners, Schaumburg, IL), vandetanib (rINN, ZD6474, ZACTIMA®, AstraZeneca), chloranmbucil, AG1478, AG1571 (SU 5271; Sugen), temsirolimus (TORISEL®, Wyeth), pazopanib (GlaxoSmithKline), canfosamide (TELCYTA®, Telik), thiotepa and cyclophosphamide (CYTOXAN®, NEOSAR®); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylmelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analog topotecan); bryostatins; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogs); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogs, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, chlorophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (e.g. calicheamicin, calicheamicin gamma11, calicheamicin omega11 (*Angew Chem. Intl. Ed. Engl.* (1994) 33:183-186); dynemicin, dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, anthramycin, azaserine, bleomycins, cactinomycin, carabacin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, nemorubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogs such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmo-fur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitostanol, mepitostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as folinic acid; aceglutamate; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; len-

tinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepe; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine (NAVELBINE®); novantrone; teniposide; edatrexate; daunomycin; aminopterin; capecitabine (XELODA®, Roche); ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylomithine (DMFO); retinoids such as retinoic acid; and pharmaceutically acceptable salts, acids and derivatives of any of the above. Combinations of agents may be used, such as CHP (doxorubicin, prednisone, cyclophosphamide), or CHOP (doxorubicin, prednisone, cyclophosphamide, vincristine).

[0904] Also included in the definition of "chemotherapeutic agent" are: (i) anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX®; tamoxifen citrate), raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON® (toremifene citrate); (ii) aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® (megestrol acetate), AROMASIN® (exemestane; Pfizer), formestane, fadrozole, RIVISOR® (vorozole), FEMARA® (letrozole; Novartis), and ARIMIDEX® (anastrozole; AstraZeneca); (iii) anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); (iv) protein kinase inhibitors such as MEK inhibitors (WO 2007/044515); (v) lipid kinase inhibitors; (vi) antisense oligonucleotides, particularly those which inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, for example, PKC- α , Raf and H-Ras, such as oblimersen (GENASENSE®, Genta Inc.); (vii) ribozymes such as VEGF expression inhibitors (e.g., ANGIOZYME®) and HER2 expression inhibitors; (viii) vaccines such as gene therapy vaccines, for example, ALLOVECTIN®, LEUVECTIN®, and VAXID®; PROLEUKIN® rIL-2; topoisomerase 1 inhibitors such as LURTOTECAN®, ABARELIX® rmRH; (ix) anti-angiogenic agents such as bevacizumab (AVASTIN®, Genentech); and pharmaceutically acceptable salts, acids and derivatives of any of the above.

[0905] Also included in the definition of "chemotherapeutic agent" are therapeutic antibodies such as alemtuzumab (Campath), bevacizumab (AVASTIN®, Genentech); cetuximab (ERBITUX®, Imclone); panitumumab (VECTIBIX®, Amgen), rituximab (RITUXAN®, Genentech/Biogen Idec), ofatumumab (ARZERRA®, GSK), pertuzumab (PERTJETA™, OMNITARG™, 2C4, Genentech), trastuzumab (HERCEPTIN®, Genentech), tositumomab (Bexxar,

Corixa), MDX-060 (Medarex) and the antibody drug conjugate, gemtuzumab ozogamicin (MYLOTARG®, Wyeth).

[0906] Humanized monoclonal antibodies with therapeutic potential as chemotherapeutic agents in combination with the conjugates of the disclosure include: alemtuzumab, apolizumab, aselizumab, atlizumab, bapineuzumab, bevacizumab, bivatuzumab mertansine, cantuzumab mertansine, cedelizumab, certolizumab pegol, cidfusituzumab, cidtuzumab, daclizumab, eculizumab, efalizumab, epratuzumab, erlizumab, felvizumab, fontolizumab, gemtuzumab ozogamicin, inotuzumab ozogamicin, ipilimumab, labetuzumab, lintuzumab, matuzumab, mepolizumab, motavizumab, motovizumab, natalizumab, nimotuzumab, nolovizumab, numavizumab, ocrelizumab, omalizumab, palivizumab, pascolizumab, pecfusituzumab, pectuzumab, pertuzumab, pexelizumab, ralvizumab, ranibizumab, reslivizumab, reslizumab, resyvizumab, rovelizumab, ruplizumab, sibrotuzumab, sipilizumab, sontuzumab, tacatuzumab tetraxetan, tadocizumab, talizumab, tefibazumab, tocilizumab, toralizumab, trastuzumab, tucotuzumab celmoleukin, tucusituzumab, umavizumab, urtoxazumab, and visilizumab.

[0907] Compositions according to the present disclosure are preferably pharmaceutical compositions. Pharmaceutical compositions according to the present disclosure, and for use in accordance with the present disclosure, may comprise, in addition to the active ingredient, i.e. a conjugate compound, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, e.g. cutaneous, subcutaneous, or intravenous.

[0908] Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may comprise a solid carrier or an adjuvant. Liquid pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included. A capsule may comprise a solid carrier such as a gelatin.

[0909] For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

Dosage

[0910] It will be appreciated by one of skill in the art that appropriate dosages of the ADC and/or the secondary agent, and compositions comprising these active elements, can vary from subject to subject. Determining the optimal dosage will generally involve the balancing of the level of therapeutic benefit against any risk or deleterious side effects. The selected dosage level will depend on a variety of factors including, but not limited to, the activity of the

particular compound, the route of administration, the time of administration, the rate of excretion of the compound, the duration of the treatment, other drugs, compounds, and/or materials used in combination, the severity of the condition, and the species, sex, age, weight, condition, general health, and prior medical history of the subject. The amount of compound and route of administration will ultimately be at the discretion of the physician, veterinarian, or clinician, although generally the dosage will be selected to achieve local concentrations at the site of action which achieve the desired effect without causing substantial harmful or deleterious side-effects.

[0911] In certain aspects, the dosage of ADC is determined by the expression of a first target protein observed in a sample obtained from the subject. Thus, the level or localization of expression of the first target protein in the sample may be indicative that a higher or lower dose of ADC is required. For example, a high expression level of the first target protein may indicate that a higher dose of ADC would be suitable. In some cases, a high expression level of the first target protein may indicate the need for administration of another agent in addition to the ADC. For example, administration of the ADC in conjunction with a chemotherapeutic agent. A high expression level of the first target protein may indicate a more aggressive therapy.

[0912] In certain aspects, the dosage of the secondary agent is determined by the expression of a second target protein observed in a sample obtained from the subject. Thus, the level or localisation of expression of the second target protein in the sample may be indicative that a higher or lower dose of secondary agent is required. For example, a high expression level of the second target protein may indicate that a higher dose of secondary agent would be suitable. In some cases, a high expression level of the second target protein may indicate the need for administration of another agent in addition to the secondary agent. For example, administration of the secondary agent in conjunction with a chemotherapeutic agent. A high expression level of the second target protein may indicate a more aggressive therapy.

[0913] In certain aspects, the dosage level is determined by the expression of a first target protein on neoplastic cells in a sample obtained from the subject. For example, when the target neoplasm is composed of, or comprises, neoplastic cells expressing the first target protein.

[0914] In certain aspects, the dosage level is determined by the expression of a first target protein on cells associated with the target neoplasm. For example, the target neoplasm may be a solid tumour composed of, or comprising, neoplastic cells that express the first target protein. For example, the target neoplasm may be a solid tumour composed of, or comprising, neoplastic cells that do not express the first target protein. The cells expressing the first target protein may be neoplastic or non-neoplastic cells associated with the target neoplasm. For example, the cells expressing the first target protein may be cells infiltrating a solid tumour comprising or composed of neoplastic cells which do not express the first target protein.

[0915] Administration can be effected in one dose, continuously or intermittently (e.g., in divided doses at appropriate intervals) throughout the course of treatment. Methods of determining the most effective means and dosage of administration are well known to those of skill in the art and will vary with the formulation used for therapy, the purpose

of the therapy, the target cell(s) being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician, veterinarian, or clinician.

[0916] In general, a suitable dose of each active compound is in the range of about 100 ng to about 25 mg (more typically about 1 pg to about 10 mg) per kilogram body weight of the subject per day. Where the active compound is a salt, an ester, an amide, a prodrug, or the like, the amount administered is calculated on the basis of the parent compound and so the actual weight to be used is increased proportionately.

[0917] In one embodiment, each active compound is administered to a human subject according to the following dosage regime: about 100 mg, 3 times daily.

[0918] In one embodiment, each active compound is administered to a human subject according to the following dosage regime: about 150 mg, 2 times daily.

[0919] In one embodiment, each active compound is administered to a human subject according to the following dosage regime: about 200 mg, 2 times daily.

[0920] However in one embodiment, each conjugate compound is administered to a human subject according to the following dosage regime: about 50 or about 75 mg, 3 or 4 times daily.

[0921] In one embodiment, each conjugate compound is administered to a human subject according to the following dosage regime: about 100 or about 125 mg, 2 times daily.

[0922] For the ADC, where it is a PBD bearing ADC, the dosage amounts described above may apply to the conjugate (including the PBD moiety and the linker to the antibody) or to the effective amount of PBD compound provided, for example the amount of compound that is releasable after cleavage of the linker.

[0923] The first target protein is preferably AXL. The ADC may comprise an anti-AXL antibody. The ADC may comprise a drug which is a PBD dimer. The ADC may be an anti-AXL-ADC, and in particular, ADCxAXL. The ADC may be an ADC disclosed in GB1702029.8, GB1719906.8, and PCT/EP2018/053163.

[0924] The secondary agent may a PD1 antagonist. Suitable PD1 antagonists include pembrolizumab, nivolumab, MEDI0680, PDR001, Camrelizumab, AUNP12, Pidilizumab REGN-2810, and BGB-108.

Antibodies

[0925] The term “antibody” herein is used in the broadest sense and specifically covers monoclonal antibodies, polyclonal antibodies, dimers, multimers, multispecific antibodies (e.g., bispecific antibodies), intact antibodies (also described as “full-length” antibodies) and antibody fragments, so long as they exhibit the desired biological activity, for example, the ability to bind a first target protein (Miller et al (2003) *Jour. of Immunology* 170:4854-4861). Antibodies may be murine, human, humanized, chimeric, or derived from other species such as rabbit, goat, sheep, horse or camel.

[0926] An antibody is a protein generated by the immune system that is capable of recognizing and binding to a specific antigen. (Janeway, C., Travers, P., Walport, M., Shlomchik (2001) *Immuno Biology*, 5th Ed., Garland Publishing, New York). A target antigen generally has numerous binding sites, also called epitopes, recognized by Complementarity Determining Regions (CDRs) on multiple anti-

bodies. Each antibody that specifically binds to a different epitope has a different structure. Thus, one antigen may have more than one corresponding antibody. An antibody may comprise a full-length immunoglobulin molecule or an immunologically active portion of a full-length immunoglobulin molecule, i.e., a molecule that contains an antigen binding site that immunospecifically binds an antigen of a target of interest or part thereof, such targets including but not limited to, cancer cell or cells that produce autoimmune antibodies associated with an autoimmune disease. The immunoglobulin can be of any type (e.g. IgG, IgE, IgM, IgD, and IgA), class (e.g. IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass, or allotype (e.g. human G1m1, G1m2, G1m3, non-G1m1 [that, is any allotype other than G1m1], G1m17, G2m23, G3m21, G3m28, G3m11, G3m5, G3m13, G3m14, G3m10, G3m15, G3m16, G3m6, G3m24, G3m26, G3m27, A2m1, A2m2, Km1, Km2 and Km3) of immunoglobulin molecule. The immunoglobulins can be derived from any species, including human, murine, or rabbit origin.

[0927] “Antibody fragments” comprise a portion of a full length antibody, generally the antigen binding or variable region thereof. Examples of antibody fragments include Fab, Fab', F(ab')₂, and scFv fragments; diabodies; linear antibodies; fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, CDR (complementary determining region), and epitope-binding fragments of any of the above which immunospecifically bind to cancer cell antigens, viral antigens or microbial antigens, single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

[0928] The term “monoclonal antibody” as used herein refers to 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 site. Furthermore, in contrast to polyclonal antibody preparations which include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present disclosure may be made by the hybridoma method first described by Kohler et al (1975) *Nature* 256:495, or may be made by recombinant DNA methods (see, U.S. Pat. No. 4,816,567). The monoclonal antibodies may also be isolated from phage antibody libraries using the techniques described in Clackson et al (1991) *Nature*, 352:624-628; Marks et al (1991) *J. Mol. Biol.*, 222:581-597 or from transgenic mice carrying a fully human immunoglobulin system (Lonberg (2008) *Curr. Opinion* 20(4):450-459).

[0929] The monoclonal antibodies herein specifically include “chimeric” antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical

with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; and Morrison et al (1984) *Proc. Natl. Acad. Sci. USA*, 81:6851-6855). Chimeric antibodies include “primatized” antibodies comprising variable domain antigen-binding sequences derived from a non-human primate (e.g. Old World Monkey or Ape) and human constant region sequences.

[0930] An “intact antibody” herein is one comprising VL and VH domains, as well as a light chain constant domain (CL) and heavy chain constant domains, CH1, CH2 and CH3. The constant domains may be native sequence constant domains (e.g. human native sequence constant domains) or amino acid sequence variant thereof. The intact antibody may have one or more “effector functions” which refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody. Examples of antibody effector functions include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; and down regulation of cell surface receptors such as B cell receptor and BCR.

[0931] Depending on the amino acid sequence of the constant domain of their heavy chains, intact antibodies can be assigned to different “classes.” There are five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into “subclasses” (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains that correspond to the different classes of antibodies are called α , δ , ϵ , γ , and μ , respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

[0932] Anti-PD-L1 antibodies are known in the art and are useful in the methods disclosed herein. These antibodies include Atezolizumab (MPDL3280; CAS number 1380723-44-3), Avelumab (MSB0010718C; CAS number 1537032-82-8), and Durvalumab (CAS number 1428935-60-7).

BRIEF DESCRIPTION OF THE FIGURES

[0933] Embodiments and experiments illustrating the principles of the disclosure will now be discussed with reference to the accompanying figures in which:

[0934] FIG. 1. Sequences

[0935] FIG. 2. Binding of a conjugate according to the invention to AXL

[0936] FIG. 3. in vivo efficacy of a conjugate according to the invention

[0937] FIG. 4. in vitro synergy between ADCxAXL and Cytarabine in SN12C cells

[0938] FIG. 5. in vitro synergy between ADCxAXL and Fludarabine in SN12C cells

[0939] FIG. 6. in vitro synergy between ADCxAXL and Decitabine in SN12C cells

[0940] FIG. 7. in vitro synergy between ADCxAXL and Olaparib in SN12C cells

[0941] FIG. 8. in vitro synergy between ADCxAXL and Gemcitabine in SN12C cells

[0942] The disclosure includes the combination of the aspects and preferred features described except where such a combination is clearly impermissible or expressly avoided.

[0943] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0944] Aspects and embodiments of the present disclosure will now be illustrated, by way of example, with reference to the accompanying figures. Further aspects and embodiments will be apparent to those skilled in the art. All documents mentioned in this text are incorporated herein by reference.

[0945] Throughout this specification, including the claims which follow, unless the context requires otherwise, the word “comprise,” and variations such as “comprises” and “comprising,” will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

[0946] It must be noted that, as used in the specification and the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. Ranges may be expressed herein as from “about” one particular value, and/or to “about” another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by the use of the antecedent “about,” it will be understood that the particular value forms another embodiment.

Some Embodiments

[0947] The following paragraphs describe some specific embodiments of the present disclosure:

[0948] 1. A method for treating cancer in an individual, the method comprising administering to the individual an effective amount of ADCxAXL and a secondary agent.

[0949] 2. A first composition comprising ADCxAXL for use in a method of treating cancer in an individual, wherein the treatment comprises administration of the first composition in combination with a second composition comprising a secondary agent.

[0950] 3. A first composition comprising a secondary agent for use in a method of treating a disorder in an individual, wherein the treatment comprises administration of the first composition in combination with a second composition comprising ADCxAXL.

[0951] 4. Use of ADCxAXL in the manufacture of a medicament for treating cancer in an individual, wherein the medicament comprises ADCxAXL, and wherein the treatment comprises administration of the medicament in combination with a composition comprising a secondary agent.

[0952] 5. Use of a secondary agent in the manufacture of a medicament for treating cancer in an individual, wherein the medicament comprises a secondary agent, and wherein the treatment comprises administration of the medicament in combination with a composition comprising ADCxAXL.

[0953] 6. A kit comprising:

[0954] a first medicament comprising ADCxAXL;

[0955] a second medicament comprising a secondary agent; and, optionally, a package insert comprising instructions for administration of the first medicament to an individual in combination with the second medicament for the treatment of cancer.

[0956] 7. A kit comprising a medicament comprising ADCxAXL and a package insert comprising instructions for

administration of the medicament to an individual in combination with a composition comprising a secondary agent for the treatment of cancer.

[0957] 8. A kit comprising a medicament comprising a secondary agent and a package insert comprising instructions for administration of the medicament to an individual in combination with a composition comprising ADCxAXL for the treatment of cancer.

[0958] 9. A pharmaceutical composition comprising ADCxAXL and a secondary agent.

[0959] 10. A method of treating cancer in an individual, the method comprising administering to the individual an effective amount of the composition of paragraph 9.

[0960] 11. The composition of paragraph 9 for use in a method of treating cancer in an individual.

[0961] 12. The use of the composition of paragraph 9 in the manufacture of a medicament for treating cancer in an individual.

[0962] 13. A kit comprising the composition of paragraph 9 and a set of instructions for administration of the medicament to an individual for the treatment of cancer.

[0963] 14. The composition, method, use, or kit according to any previous paragraph, wherein the treatment comprises administering ADCxAXL before the secondary agent, simultaneous with the secondary agent, or after the secondary agent.

[0964] 15. The composition, method, use, or kit according to any previous paragraph, wherein the treatment further comprises administering a chemotherapeutic agent.

[0965] 16. The composition, method, use, or kit according to any previous paragraph, wherein the individual is human.

[0966] 17. The composition, method, use, or kit according to any previous paragraph, wherein the individual has a disorder or has been determined to have cancer.

[0967] 18. The composition, method, use, or kit according to any previous paragraph, wherein the individual has, or has been has been determined to have, a cancer characterised by the presence of a neoplasm comprising both AXL+ve and AXL-ve cells.

[0968] 19. The composition, method, use, or kit according to any previous paragraph, wherein the individual has, or has been has been determined to have, a cancer characterised by the presence of a neoplasm comprising, or composed of, AXL-ve neoplastic cells.

[0969] 20. The composition, method, use, or kit according to any previous paragraph, wherein the cancer or neoplasm is all or part of a solid tumour.

[0970] 21. The composition, method, use, or kit according to any previous paragraph, wherein the individual has, or has been has been determined to have, a cancer which expresses AXL or AXL+ tumour-associated non-tumour cells, such as AXL+ infiltrating cells.

[0971] 22. The composition, method, use, or kit according to paragraph 21, wherein the AXL+ infiltrating cells are dendritic cells, NK cells, or macrophages.

[0972] 23. The composition, method, use, or kit according to any preceding paragraph, wherein the individual has, or has been has been determined to have, a cancer which expresses PD-L1.

[0973] 24. The composition, method, use, or kit according to any one of the preceding paragraphs, wherein the treatment:

[0974] a) effectively treats a broader range of disorders,

[0975] b) effectively treats resistant, refractory, or relapsed disorders,

[0976] c) has an increased response rate, and/or

[0977] d) has increased durability;

[0978] as compared to treatment with either ADCxAXL or the secondary agent alone.

[0979] 25. The composition, method, use, or kit according to any one of the preceding paragraphs, wherein the cancer is selected from the group comprising: breast cancer, lung cancer, gastric cancer, head and neck cancer, colorectal cancer, renal cancer, pancreatic cancer, uterine cancer, hepatic cancer, bladder cancer, endometrial cancer, prostate cancer, non-Hodgkin's lymphoma, NHL, AML), an immune disorder, cardiovascular disorder, thrombosis, diabetes, immune checkpoint disorder, and fibrotic disorder.

[0980] 26. A composition, method, use, or kit according to any one of paragraphs 1 to 25, wherein the secondary agent is a PD1 antagonist.

[0981] 27. A composition, method, use, or kit according to paragraph 26, wherein the PD1 antagonist is selected from pembrolizumab, nivolumab, MEDI0680, PDR001 (spartalizumab), Camrelizumab, AUNP12, Pidilizumab Cemiplimab (REGN-2810), AMP-224, BGB-A317 (Tisleizumab), and BGB-108.

[0982] 28. A composition, method, use, or kit according to any one of paragraphs 1 to 25, wherein the secondary agent is a PD-L1 antagonist.

[0983] 29. A composition, method, use, or kit according to paragraph 28, wherein the PD-L1 antagonist is selected from atezolizumab (Tecentriq), BMS-936559/MDX-1105, durvalumab/MEDI4736, and MSB0010718C (Avelumab).

[0984] 30. A composition, method, use, or kit according to any one of paragraphs 1 to 25, wherein the secondary agent is a G1TR (Glucocorticoid-Induced TNFR-Related protein) agonist.

[0985] 31. A composition, method, use, or kit according to paragraph 30, wherein the G1TR (Glucocorticoid-Induced TNFR-Related protein) agonist is selected from MEDI1873, TRX518, GWN323, MK-1248, MK 4166, BMS-986156 and INCAGN1876.

[0986] 32. A composition, method, use, or kit according to any one of paragraphs 1 to 25, wherein the secondary agent is a OX40 agonist.

[0987] 33. A composition, method, use, or kit according to paragraph 32, wherein the OX40 agonist is selected from MEDI0562, MEDI6383, MOXR0916, RG7888, OX40mAb24, INCAGN1949, GSK3174998, and PF-04518600.

[0988] 34. A composition, method, use, or kit according to any one of paragraphs 1 to 25, wherein the secondary agent is a CTLA-4 antagonist.

[0989] 35. A composition, method, use, or kit according to paragraph 34, wherein the CTLA-4 antagonist is selected from ipilimumab and Tremelimumab.

[0990] 36. A composition, method, use, or kit according to any one of paragraphs 1 to 25, wherein the secondary agent is Fludarabine.

[0991] 37. A composition, method, use, or kit according to any one of paragraphs 1 to 25, wherein the secondary agent is Cytarabine.

[0992] 38. A composition, method, use, or kit according to any one of paragraphs 1 to 25, wherein the secondary agent is a hypomethylating agent.

[0993] 39. A composition, method, use, or kit according to paragraph 38, wherein the hypomethylating agent is azacitidine.

[0994] 40. A composition, method, use, or kit according to paragraph 38, wherein the hypomethylating agent is decitabine.

[0995] 41. A composition, method, use, or kit according to any one of paragraphs 1 to 25, wherein the secondary agent is a PARP inhibitor (PARPi).

[0996] 42. A composition, method, use, or kit according to paragraph 41, wherein the PARPi is selected from Olaparib, CEP-9722, BMN-673/talazoparib, Rucaparib, Iniparib/SAR24-550/BSI-201, Veliparib (ABT-888), Nirapanb/MK-4827, BGB-290, 3-aminobenzamide, and E7016.

[0997] 43. A composition, method, use, or kit according to any one of paragraphs 1 to 25, wherein the secondary agent is an agent that upregulates HER2 expression.

[0998] 44. A composition, method, use, or kit according to paragraph 41, wherein the agent that upregulates HER2 expression is selected from gemcitabine and tamoxifen.

[0999] 45. A composition, method, use, or kit according to any one of paragraphs 1 to 25, wherein the secondary agent is an AXL-kinase inhibitor (AXLi).

[1000] 46. A composition, method, use, or kit according to paragraph 45, wherein the AXLi is selected from BGB324 (bemcentinib), TP0903, Gilteritinib (ASP2215), Cabozantinib (XL184), SG17079, Merestinib, amuvatinib (MP-470), bosutinib (SKI-606), MGCD265, and foretinib (GSK1363089/XL880).

[1001] 47. A composition, method, use, or kit according to any one of paragraphs 1 to 25, wherein the secondary agent is a BRAF inhibitor (BRAFi).

[1002] 48. A composition, method, use, or kit according to paragraph 47, wherein the BRAFi is selected from vemurafenib, PLX4720, dabrafenib, Sorafenib, Encorafenib, and GDC0879.

[1003] 49. A composition, method, use, or kit according to any one of paragraphs 1 to 25, wherein the secondary agent is a MEK inhibitor (MEKi).

[1004] 50. A composition, method, use, or kit according to paragraph 49, wherein the AXLi is selected from Trametinib, Cobimetinib, Binimetinib, Selumetinib, PD-325901, CI-1040, PD035901, U0126, and TAK-733.

STATEMENTS OF INVENTION

[1005] 1. A method for treating a disorder in an individual, the method comprising administering to the individual an effective amount of an ADC and a secondary agent.

[1006] 2. A first composition comprising an ADC for use in a method of treating a disorder in an individual, wherein the treatment comprises administration of the first composition in combination with a second composition comprising a secondary agent.

[1007] 3. A first composition comprising a secondary agent for use in a method of treating a disorder in an individual, wherein the treatment comprises administration of the first composition in combination with a second composition comprising an ADC.

[1008] 4. Use of an ADC in the manufacture of a medicament for treating a disorder in an individual, wherein the medicament comprises an ADC, and wherein the treatment comprises administration of the medicament in combination with a composition comprising a secondary agent.

[1009] 5. Use of a secondary agent in the manufacture of a medicament for treating a disorder in an individual, wherein the medicament comprises a secondary agent, and wherein the treatment comprises administration of the medicament in combination with a composition comprising an ADC.

[1010] 6. A kit comprising:

[1011] a first medicament comprising an ADC;

[1012] a second medicament comprising a secondary agent; and, optionally,

[1013] a package insert comprising instructions for administration of the first medicament to an individual in combination with the second medicament for the treatment of a disorder.

[1014] 7. A kit comprising a medicament comprising an ADC and a package insert comprising instructions for administration of the medicament to an individual in combination with a composition comprising a secondary agent for the treatment of a disorder.

[1015] 8. A kit comprising a medicament comprising a secondary agent and a package insert comprising instructions for administration of the medicament to an individual in combination with a composition comprising an ADC for the treatment of a disorder.

[1016] 9. A pharmaceutical composition comprising an ADC and a secondary agent.

[1017] 10. A method of treating a disorder in an individual, the method comprising administering to the individual an effective amount of the composition of paragraph 9.

[1018] 11. The composition of paragraph 9 for use in a method of treating a disorder in an individual.

[1019] 12. The use of the composition of paragraph 9 in the manufacture of a medicament for treating a disorder in an individual.

[1020] 13. A kit comprising the composition of paragraph 9 and a set of instructions for administration of the medicament to an individual for the treatment of a disorder.

[1021] 14. The composition, method, use, or kit according to any previous paragraph, wherein the treatment comprises administering the ADC before the secondary agent, simultaneous with the secondary agent, or after the secondary agent.

[1022] 15. The composition, method, use, or kit according to any previous paragraph, wherein the treatment further comprises administering a chemotherapeutic agent.

[1023] 16. The composition, method, use, or kit according to any previous paragraph, wherein the individual is human.

[1024] 17. The composition, method, use, or kit according to any preceding paragraph, wherein the individual has a disorder or has been determined to have a disorder.

[1025] 18. The composition, method, use, or kit according to paragraph 17, wherein the individual has, or has been has been determined to have, a cancer which expresses a first target protein (FTP) or FTP+ tumour-associated non-tumour cells, such as FTP+ infiltrating cells.

[1026] 19. The composition, method, use, or kit according to any preceding paragraph, wherein the individual has, or has been has been determined to have, a cancer which expresses a second target protein (STP).

[1027] 20. The composition, method, use, or kit according to any one of the preceding paragraphs, wherein the treatment:

[1028] a) effectively treats a broader range of disorders,

[1029] b) effectively treats resistant, refractory, or relapsed disorders,

[1030] c) has an increased response rate, and/or

[1031] d) has increased durability;

[1032] as compared to treatment with either the ADC or the secondary agent alone.

[1033] 21. A composition, method, use, or kit according to any previous paragraph, wherein the ADC is an anti-AXL ADC.

[1034] 22. A composition, method, use, or kit according to paragraph 21, wherein the anti-AXL ADC is ADCxAXL.

[1035] 23. A composition, method, use, or kit according to any previous paragraph, wherein the FTP is AXL.

[1036] 24. A composition, method, use, or kit according to any previous paragraph, wherein the disorder is a proliferative disease.

[1037] 25. The composition, method, use, or kit of paragraph 24, wherein the disorder is cancer.

[1038] 26. The composition, method, use, or kit according to any previous paragraph, wherein the individual has, or has been has been determined to have, a cancer characterised by the presence of a neoplasm comprising both AXL+ve and AXL-ve cells.

[1039] 27. The composition, method, use, or kit according to any previous paragraph, wherein the individual has, or has been has been determined to have, a cancer characterised by the presence of a neoplasm comprising, or composed of, AXL-ve neoplastic cells.

[1040] 28. The composition, method, use, or kit according to any previous paragraph, wherein the cancer or neoplasm is all or part of a solid tumour.

[1041] 29. The composition, method, use, or kit of any previous paragraph, wherein the disorder is selected from the group comprising: breast cancer, lung cancer, gastric cancer, head and neck cancer, colorectal cancer, renal cancer, pancreatic cancer, uterine cancer, hepatic cancer, bladder cancer, endometrial cancer, prostate cancer, non-Hodgkin's lymphoma, NHL, AML), an immune disorder, cardiovascular disorder, thrombosis, diabetes, immune checkpoint disorder, and fibrotic disorder.

[1042] A composition, method, use, or kit according to any previous paragraph, wherein the STP is PD-L1.

[1043] 31. A composition, method, use, or kit according to any one of paragraphs 1 to 30, wherein the secondary agent is a PD1 antagonist.

[1044] 32. A composition, method, use, or kit according to paragraph 31, wherein the PD1 antagonist is selected from pembrolizumab, nivolumab, MEDI0680, PDR001 (spartalizumab), Camrelizumab, AUNP12, Pidilizumab Cemiplimab (REGN-2810), AMP-224, BGB-A317 (Tislelizumab), and BGB-108.

[1045] 33. A composition, method, use, or kit according to any one of paragraphs 1 to 30, wherein the secondary agent is a PD-L1 antagonist.

[1046] 34. A composition, method, use, or kit according to paragraph 33, wherein the PD-L1 antagonist is selected from atezolizumab (Tecentriq), BMS-936559/MDX-1105, durvalumab/MEDI4736, and MSB0010718C (Avelumab).

[1047] 35. A composition, method, use, or kit according to any one of paragraphs 1 to 30, wherein the secondary agent is a G1TR (Glucocorticoid-induced TNFR-Related protein) agonist.

[1048] 36. A composition, method, use, or kit according to paragraph 35, wherein the G1TR (Glucocorticoid-Induced

TNFR-Related protein) agonist is selected from MEDI1873, TRX518, GWN323, MK-1248, MK 4166, BMS-986156 and INCAGN1876.

[1049] 37. A composition, method, use, or kit according to any one of paragraphs 1 to 30, wherein the secondary agent is a OX40 agonist.

[1050] 38. A composition, method, use, or kit according to paragraph 37, wherein the OX40 agonist is selected from MEDI0562, MEDI6383, MOXR0916, RG7888, OX40mAb24, INCAGN1949, GSK3174998, and PF-04518600.

[1051] 39. A composition, method, use, or kit according to any one of paragraphs 1 to 30, wherein the secondary agent is a CTLA-4 antagonist.

[1052] 40. A composition, method, use, or kit according to paragraph 39, wherein the CTLA-4 antagonist is selected from ipilimumab and Tremelimumab.

[1053] 41. A composition, method, use, or kit according to any one of paragraphs 1 to 30, wherein the secondary agent is Fludarabine.

[1054] 42. A composition, method, use, or kit according to any one of paragraphs 1 to 30, wherein the secondary agent is Cytarabine.

[1055] 43. A composition, method, use, or kit according to any one of paragraphs 1 to 30, wherein the secondary agent is a hypomethylating agent.

[1056] 44. A composition, method, use, or kit according to paragraph 43, wherein the hypomethylating agent is azacitidine.

[1057] 45. A composition, method, use, or kit according to paragraph 43, wherein the hypomethylating agent is decitabine.

[1058] 46. A composition, method, use, or kit according to any one of paragraphs 1 to 30, wherein the secondary agent is a PARP inhibitor (PARPi).

[1059] 47. A composition, method, use, or kit according to paragraph 46, wherein the PARPi is selected from Olaparib, CEP-9722, BMN-673/talazoparib, Rucaparib, Iniparib/SAR24-550/BSI-201, Veliparib (ABT-888), Nirapadb/MK-4827, BGB-290, 3-aminobenzamide, and E7016.

[1060] 48. A composition, method, use, or kit according to any one of paragraphs 1 to 30, wherein the secondary agent is an agent that upregulates HER2 expression.

[1061] 49. A composition, method, use, or kit according to paragraph 48, wherein the agent that upregulates HER2 expression is selected from gemcitabine and tamoxifen.

[1062] 50. A composition, method, use, or kit according to any one of paragraphs 1 to 30, wherein the secondary agent is an AXL-kinase inhibitor (AXLi).

[1063] 51. A composition, method, use, or kit according to paragraph 50, wherein the AXLi is selected from BGB324 (bemcentinib), TP0903, Gilteritinib (ASP2215), Cabozantinib (XL184), SG17079, Merestinib, amuvatinib (MP-470), bosutinib (SKI-606), MGCD265, and foretinib (GSK1363089/XL880).

[1064] 52. A composition, method, use, or kit according to any one of paragraphs 1 to 30, wherein the secondary agent is a BRAF inhibitor (BRAFi).

[1065] 53. A composition, method, use, or kit according to paragraph 52, wherein the BRAFi is selected from vemurafenib, PLX4720, dabrafenib, Sorafenib, Encorafenib, and GDC0879.

[1066] 54. A composition, method, use, or kit according to any one of paragraphs 1 to 30, wherein the secondary agent is a MEK inhibitor (MEKi).

[1067] 55. A composition, method, use, or kit according to paragraph 54, wherein the AXLi is selected from Trametinib, Cobimetinib, Binimetinib, Selumetinib, PD-325901, CI-1040, PD035901, U0126, and TAK-733.

EXAMPLES

[1068] In the following examples:

[1069] the FTP is preferably AXL.

[1070] Cell lines expressing AXL suitable for use in the examples include MDA-MB231, NCI-H1299 and SN12C.

[1071] Disease A—Colorectal

[1072] Disease B—Gastric cancer

[1073] Disease C—Pancreatic cancer

Example 1

[1074] To show that a PBD-ADC can induce ICD and therefore can be a suitable combination agent with immunology (IO) drugs, cell lines expressing a first target protein (FTP), will be incubated for 0, 6, 24 and 48 hours with etoposide (negative control) and oxaliplatin (positive control), 1 µg/mL ADC, 1 µg/mL anti-FTP (the antibody in ADC) and 1 µg/mL of B12-SG3249 (a non-binding control ADC with the same PBD payload as ADC).

[1075] After Incubation, the amount of AnnexinV-/PI+ (early apoptotic cells) will be measured by Flow cytometry together with the upregulation of surface calreticulin and HSP-70. ER stress will be measured by Northern blot analyses of IRE1 phosphorylation, ATF4 and JNK phosphorylation.

Example 2

[1076] In a separate experiment, cell lines expressing FTPs will be incubated for 0, 6, 24 and 48 hours with etoposide (negative control) and oxaliplatin (positive control), 1 µg/mL ADC (ADC targeting FTP with a PBD dimer warhead), 1 µg/mL anti-FTP (the antibody in ADC) and 1 µg/mL of B12-SG3249 (a non-binding control ADC with the same PBD payload as ADC).

[1077] After incubation, the cells are washed, and fed to human Dendritic cells (DCs) for an additional 24 h. Activation of the DCs is subsequently measured by increased surface expression of CD86 on the DC population (as determined by Flow cytometry) and by measuring DC mediated release of IL-8 and MIP2.

Example 3

[1078] The purpose of this study is to preliminarily assess the safety, tolerability, pharmacological and clinical activity of this combination

[1079] The following cancer types have been chosen for study: Disease A, Disease B, and Disease C

[1081] Evidence for efficacy as single agents exists for both drugs:

[1082] ADC (see, for example, GB1702029.8, GB1719906.8, and PCT/EP2018/053163.)

[1083] Secondary agent (see KS Peggs et al. 2009, Clinical and Experimental Immunology, 157: 9-19 [doi: 10.1111/j.1365-2249.2009.03912.x])

[1084] This primary purpose of this study is to explore whether these agents can be safely combined, and if so, will identify the dose(s) and regimens appropriate for further study. The study will also assess whether each combination induces pharmacologic changes in tumor that would suggest potential clinical benefit.

[1085] In addition, it will provide preliminary evidence that a combination may increase the response rate and durability of response compared with published data for treatment with single agent ADC or secondary agent.

[1086] Each disease group may include a subset of patients previously treated with the secondary agent to explore whether combination therapy might overcome resistance to secondary agent therapy. For each disease, it is not intended to apply specific molecular selection as the data available at present generally do not support excluding patients on the basis of approved molecular diagnostic tests.

Rationale for ADC Starting Dose

[1087] The RDE for already established for ADC (in ug/kg administered every three weeks) will be used for all patients in this study. To ensure patient safety, a starting dose below the RDE will be used; the starting dose level will be one where patient benefit could still be demonstrated in study ADC1, suggesting that patients enrolled at such dose level will gain at least some benefit by taking part.

Rationale for Secondary Agent Starting Dose

[1088] The RDE for already established for the secondary agent (in ug/kg administered every three weeks) will be used for all patients in this study. To ensure patient safety, a starting dose below the RDE will be used; the starting dose level will be one where patient benefit could still be demonstrated in study SA1, suggesting that patients enrolled at such dose level will gain at least some benefit by taking part.

Objectives and Related Endpoints

[1089]

Objective	Endpoint
Primary Objective	
To characterize the safety and tolerability of ADC in combination with the secondary agent, and to identify the	Frequency and severity of treatment-emergent AEs and SAEs Changes between baseline and post-baseline laboratory parameters and

-continued

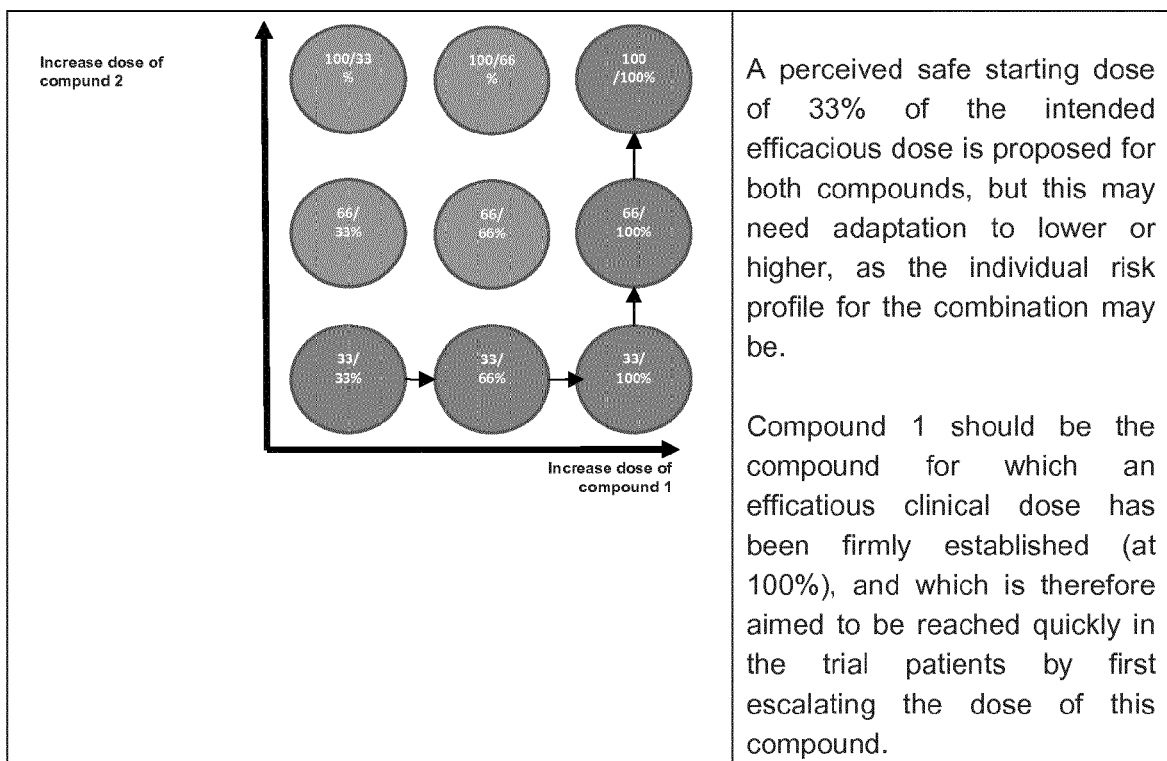
Objective	Endpoint
recommended dose and schedules for future studies	vital signs Incidence of dose limiting toxicities (DLTs) during the first cycle of treatment (dose escalation only) Frequency of dose interruptions and dose reductions
Secondary Objectives	
To evaluate the clinical activity of the combination of ADC with the secondary agent	ORR, DOR, PFS, OS
To characterize the pharmacokinetic (PK) profile of each of the two compounds ADC and the secondary agent	AUC and Cmax for each compound
Evidence for immunogenicity and ADAs to ADC	Anti-Drug-Antibodies (ADAs) before, during and after treatment with ADC
Exploratory Objectives	
To examine potential correlation of PK profiles with safety/tolerability and efficacy	Correlation coefficients between AUC and/or Cmax of each compound or a compound measure and any of the safety or efficacy variables
To characterize changes in the immune infiltrate in tumors	Immunohistochemistry of pre- and on-treatment tumor biopsies,
To characterize changes in circulating levels of cytokines in plasma and markers of activation in circulating immune cells	Measurements (e.g. via ELISA) of immunologically relevant cytokines in plasma or serum; staining levels for activation markers of circulating immune cells (e.g. FACS)

Study Design

[1090] This phase Ib, multi-center, open-label study to characterize the safety, tolerability, pharmacokinetics (PK), pharmacodynamics (PD) and antitumor activity of the ADC in combination with the secondary agent, in patients with disease A, disease B, and disease C.

[1091] The study is comprised of a dose escalation part followed by a dose expansion part.

[1092] Dose escalation will start with reduced starting doses (compared to their respective recommended phase 2 or licensed dose levels), for both ADC and the secondary agent, to guarantee patient safety. Starting doses will be 33% (or 50%) of the RDE for each compound. Subsequently, doses will be first escalated for the secondary agent until the RDE or licensed dose has been reached, or a lower dose if necessary for tolerability reasons. Then, the dose for ADC will be escalated, until the RDE for combination treatment is reached. This is visualized in the below diagram:



[1093] If the dose combination is determined to be safe, it may be tested in additional patients to confirm the safety and tolerability at that dose level. Further tailoring of the dose of each compound may be conducted, and/or the regimen may be modified.

[1094] The dose escalation of the combination will be guided by a Bayesian Logistic Regression Model (BLRM) based on any Dose Limiting Toxicities (DLTs) observed in the first (or first two, TBC) cycles of therapy. Use of a BLRM is a well-established method to estimate the maximum tolerated dose (MTD)/recommended dose for expansion (RDE) in cancer patients. The adaptive BLRM will be guided by the Escalation With Overdose Control (EWOC) principle to control the risk of DLT in future patients on the study. The use of Bayesian response adaptive models for small datasets has been accepted by FDA and EMEA ("Guideline on clinical trials in small populations", Feb. 1, 2007) and endorsed by numerous publications (Babb et al. 1998, Neuenschwander et al. 2008).

[1095] The decisions on new dose combinations are made by the Investigators and sponsor study personnel in a dose escalation safety call (DESC) based upon the review of patient tolerability and safety information (including the BLRM summaries of DLT risk, if applicable) along with PK, PD and preliminary activity information available at the time of the decision.

[1096] Once the MTD(s)/RDE is determined for the combination, the expansion part of the study may be initiated to further assess the safety, tolerability and preliminary efficacy.

[1097] For combinations with IO, changes in the immune infiltrate in tumors will also be characterized following combination treatment in the target disease indications.

[1098] Given the available prior clinical experience with the agents in this study, it is expected that in most cases a combination dose can be identified without testing a large number of dose levels or schedules. To assess the pharmacodynamic activity of the combinations, patients will be asked to undergo a tumor biopsy at baseline and again after approximately two cycles of therapy.

[1099] For IO combo: The extent of the change in tumor infiltration by immune cells including lymphocytes and macrophages will contribute to a decision on any potential benefit.

Dose Escalation Part

[1100] During the dose escalation part of the study, patients will be treated with a fixed dose of ADC administered i.v., and increasing doses of the secondary agent until the RDE for the secondary agent has been reached. Subsequently, doses of ADC are increased (in different cohorts) while the dose for the secondary agent is kept constant.

[1101] Two to approximately 3 or 4 patients with disease A, disease B or disease C will be treated in each escalation cohort until the determination of MTD(s)/RDE(s) is determined.

[1102] There will be a 24-hour observation before enrolling the second patient at Dose Level 1. The DLT observation period at each dose level is either 1 cycle (3 weeks) or 2 cycles (6 weeks) as mandated by the appropriate authorities for 10 therapies, after which it will be determined whether to escalate to the next dose level, stay at the current dose level, or de-escalate to the previous dose level for the next

cohort. There will be no de-escalation from Dose Level 1. Inpatient dose escalation is not permitted.

[1103] Dose escalation is not permitted unless 2 or more patients have complete DLT information through the first cycle in any given dose level. Dose escalation will be determined by using a mCRM with a target DLT rate of 30% and an equivalence interval of 20% to 35%, and with dose escalation-with-overdose-control (EWOC) and no dose skipping.

[1104] Patients will be assigned to a cohort that is actively enrolling. Dose escalation will be performed in each combination following the completion of one cycle of treatment. Safety assessments including adverse events (AEs) and laboratory values will be closely monitored for all enrolled patients in order to identify any DLTs. A single MTD/RDE will be defined; a disease-specific MTD/RDE will not be established.

[1105] The mCRM will be implemented for DE under the oversight of a Dose Escalation Steering Committee (DESC). The DESC will confirm each escalating dose level after reviewing all available safety data. PK data from patients in that dose level and prior dose levels may also inform decision making. The DESC may halt dose escalation prior to determining the MTD based on emerging PK, PD, toxicity or response data.

[1106] Additional patients may be included at any dose level to further assess the safety and tolerability if at least 1 patient in the study has achieved a partial response or better, or if further evaluation of PK or PD data is deemed necessary by the DESC to determine the RDE.

[1107] Dose Escalation will be stopped after 3 cohorts (or at least 6 patients) are consecutively assigned to the same dose level. If the MTD is not reached, the recommended dose for expansion (RDE) will be determined. Prior to the determination of the MTD/RDE a minimum of 6 patients must have been treated with the combination.

[1108] It is intended that paired tumor biopsies will be obtained from patients during dose escalation. Analysis of these biopsies will contribute to a better understanding of the relationship between the dose and the pharmacodynamic activity of the combination.

Safety Oversight by the Dose Escalation Steering Committee

[1109] A DESC comprised of ADC Therapeutics and the investigators will review patient safety on an ongoing basis during the DE to determine if the dose escalation schedule prescribed by the mCRM warrants modification. In addition to safety observations, PK and/or PD data may also inform decision making. Intermediate doses may be assigned after agreement between ADC Therapeutics and investigators. The DESC may continue to provide oversight during Part 2. No formal Data Safety Monitoring Board (DSMB) will be used.

Dose Expansion Part

[1110] Once the MTD/RDE has been declared, dose expansion part may begin. The main objective of the expansion part is to further assess the safety and tolerability of the study treatment at the MTD/RDE and to gain a preliminary understanding of the efficacy of the combination compared to historical single agent efficacy data.

[1111] An important exploratory objective is to assess changes in the immune infiltrate in tumor in response to treatment. This will be assessed in paired tumor biopsies collected from patients, with a minimum of ten evaluable biopsy pairs (biopsy specimens must contain sufficient tumor for analysis) in patients treated at the MTD/RDE. If this is not feasible, collection of these biopsies may be stopped. A minimum of 10 to 20 patients are planned to be treated in each investigational arm,

[1112] Several different investigational arms will open, one per disease. A total of nine investigational arms may be run in the dose expansion. Should enrollment for any of these groups not be feasible, then enrollment to that group may be closed before the 10 to 20 patients target is met.

[1113] In each treatment group a maximum of approximately six patients who have received and progressed on prior single administration (i.e. not in combination) secondary agent therapy will be allowed to be treated. This number may be increased if a combination shows promise of overcoming resistance to prior treatment with single administration secondary agent.

Patient Population

[1114] The study will be conducted in adult patients with advanced Disease A, Disease B or Disease C as outlined above. The investigator or designee must ensure that only patients who meet all the following inclusion and none of the exclusion criteria are offered treatment in the study.

Inclusion Criteria

[1115] Patients eligible for inclusion in this study have to meet all of the following criteria:

[1116] 1. Written informed consent must be obtained prior to any procedures

[1117] 2. Age 18 years.

[1118] 3. Patients with advanced/metastatic cancer, with measurable disease as determined by RECIST version 1.1, who have progressed despite standard therapy or are intolerant to standard therapy, or for whom no standard therapy exists. Patients must fit into one of the following groups:

[1119] Disease A

[1120] Disease B

[1121] Disease C

[1122] 4. ECOG Performance Status 0-1 (or 2 TBC)

[1123] 5. TBC: Patient must have a site of disease amenable to biopsy, and be a candidate for tumor biopsy according to the treating institution's guidelines. Patient must be willing to undergo a new tumor biopsy at baseline, and again during therapy on this study.

[1124] 6. Prior therapy with the secondary agent or related compounds (i.e. same MOA) is allowed

Exclusion Criteria

[1125] Patients eligible for this study must not meet any of the following criteria:

[1126] 1. History of severe hypersensitivity reactions to other mAbs (OR to same backbone mAb as in ADC OR to same 10 mAb if applicable)

[1127] 2. Known history of positive serum human ADA to backbone of mAb as in ADC

[1128] 3. Central Nervous System (CNS) disease only (if applicable)

[1129] 4. Symptomatic CNS metastases or evidence of leptomeningeal disease (brain MRI or previously documented cerebrospinal fluid (CSF) cytology)

[1130] Previously treated asymptomatic CNS metastases are permitted provided that the last treatment (systemic anticancer therapy and/or local radiotherapy) was completed ≥ 8 weeks prior to 1st day of dosing, except usage of low dose steroids on a taper is allowed)

[1131] Patients with discrete dural metastases are eligible.

[1132] 5. Patient having out of range laboratory values defined as:

[1133] Serum creatinine $\leq 1.5 \times \text{ULN}$. If serum creatinine > 1.5 , the creatinine clearance (calculated using Cockcroft-Gault formula, or measured) must be $> 60 \text{ mL/min/1.73m}^2$ for a patient to be eligible

[1134] Total bilirubin $> 1.5 \times \text{ULN}$, except for patients with Gilbert's syndrome who are excluded if total bilirubin $> 3.0 \times \text{ULN}$ or direct bilirubin $> 1.5 \times \text{ULN}$

[1135] Alanine aminotransferase (ALT) $> 3 \times \text{ULN}$, except for patients that have tumor involvement of the liver, who are excluded if ALT $> 5 \times \text{ULN}$

[1136] Aspartate aminotransferase (AST) $> 3 \times \text{ULN}$, except for patients that have tumor involvement of the liver, who are excluded if AST $> 5 \times \text{ULN}$

[1137] Absolute neutrophil count $< 1.0 \times 10^9/\text{L}$

[1138] Platelet count $< 75 \times 10^9/\text{L}$

[1139] Hemoglobin (Hgb) $< 8 \text{ g/dL}$

[1140] Potassium, magnesium, calcium or phosphate abnormality $> \text{CTCAE grade 1}$ despite appropriate replacement therapy

[1141] 6. Impaired cardiac function or clinically significant cardiac disease, including any of the following:

[1142] Clinically significant and/or uncontrolled heart disease such as congestive heart failure requiring treatment (NYHA grade III or IV) or uncontrolled hypertension defined by a Systolic Blood Pressure (SBP) 160 mm Hg and/or Diastolic Blood Pressure (DBP) 100 mm Hg, with or without anti-hypertensive medication.

[1143] QTcF $> 470 \text{ msec}$ for females or $> 450 \text{ msec}$ for males on screening ECG using Fridericia's correction, congenital long QT syndrome

[1144] Acute myocardial infarction or unstable angina pectoris < 3 months (months prior to study entry)

[1145] Clinically significant valvular disease with documented compromise in cardiac function

[1146] Symptomatic pericarditis

[1147] History of or ongoing documented cardiomyopathy

[1148] Left Ventricular Ejection Fraction (LVEF) $< 40\%$, as determined by echocardiogram (ECHO) or Multi gated acquisition (MUGA) scan

[1149] History or presence of any clinically significant cardiac arrhythmias, e.g. ventricular, supraventricular, nodal arrhythmias, or conduction abnormality (TBC qualifier . . . requiring a pacemaker or not controlled with medication)

[1150] Presence of unstable atrial fibrillation (ventricular response rate $> 100 \text{ bpm}$).

[1151] NOTE: Patients with stable atrial fibrillation can be enrolled provided they do not meet other cardiac exclusion criteria.

[1152] Complete left bundle branch block (LBBB), bifascicular block

- [1153] Any clinically significant ST segment and/or T-wave abnormalities
- [1154] 7. Toxicity attributed to prior 10 therapy that led to discontinuation of therapy. Adequately treated patients for drug-related skin rash or with replacement therapy for endocrinopathies are not excluded, provided these toxicities did not lead to the discontinuation of prior treatment.
- [1155] 8. Patients with active, known or suspected autoimmune disease. Subjects with vitiligo, type 1 diabetes mellitus, residual hypothyroidism due to autoimmune condition only requiring hormone replacement, psoriasis not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger are permitted to enroll, provided the trigger can be avoided.
- [1156] 9. Human Immunodeficiency Virus (HIV), or active Hepatitis B (HBV) or Hepatitis C (HCV) virus infection
- [1157] Testing is not mandatory to be eligible. Testing for HCV should be considered if the patient is at risk for having undiagnosed HCV (e.g. history of injection drug use).
- [1158] 10. Malignant disease, other than that being treated in this study. Exceptions to this exclusion include the following: malignancies that were treated curatively and have not recurred within 2 years prior to study treatment; completely resected basal cell and squamous cell skin cancers; any malignancy considered to be indolent and that has never required therapy; and completely resected carcinoma in situ of any type.
- [1159] 11. Systemic anti-cancer therapy within 2 weeks of the first dose of study treatment. For cytotoxic agents that have major delayed toxicity, e.g. mitomycin C and nitrosoureas, 4 weeks is indicated as washout period. For patients receiving anticancer immunotherapies such as CTLA-4 antagonists, 6 weeks is indicated as the washout period.
- [1160] 12. Active diarrhea CTCAE grade 2 or a medical condition associated with chronic diarrhea (such as irritable bowel syndrome, inflammatory bowel disease)
- [1161] 13. Presence of 2: CTCAE grade 2 toxicity (except alopecia, peripheral neuropathy and ototoxicity, which are excluded if \geq CTCAE grade 3) due to prior cancer therapy.
- [1162] 14. Active infection requiring systemic antibiotic therapy.
- [1163] 15. Active ulceration of the upper GI tract or GI bleeding
- [1164] 16. Active bleeding diathesis or on oral anti-vitamin K medication (except low-dose warfarin and aspirin or equivalent, as long as the INR \leq 2.0)
- [1165] 17. Active autoimmune disease, motor neuropathy considered of autoimmune origin, and other CNS autoimmune disease
- [1166] 18. Patients requiring concomitant immunosuppressive agents or chronic treatment with corticoids except:
- [1167] replacement dose steroids in the setting of adrenal insufficiency
- [1168] topical, inhaled, nasal and ophthalmic steroids are allowed
- [1169] 19. Use of any live vaccines against infectious diseases (e.g. influenza, varicella, pneumococcus) within 4 weeks of initiation of study treatment (NB the use of live vaccines is not allowed through the whole duration of the study)
- [1170] 20. Use of hematopoietic colony-stimulating growth factors (e.g. G-CSF, GM-CSF, M-CSF) $<$ 2 weeks prior start of study drug. An erythroid stimulating agent is allowed as long as it was initiated at least 2 weeks prior to the first dose of study treatment.
- [1171] 21. Major surgery within 2 weeks of the first dose of study treatment (NB mediastinoscopy, insertion of a central venous access device, or insertion of a feeding tube are not considered major surgery).
- [1172] 22. Radiotherapy within 2 weeks of the first dose of study drug, except for palliative radiotherapy to a limited field, such as for the treatment of bone pain or a focally painful tumor mass. To allow for assessment of response to treatment, patients must have remaining measurable disease that has not been irradiated
- [1173] 23. Participation in an interventional, investigational study within 2 weeks of the first dose of study treatment.
- [1174] 24. Any medical condition that would, in the investigator's judgment, prevent the patient's participation in the clinical study due to safety concerns, compliance with clinical study procedures or interpretation of study results.
- [1175] 25. Sexually active males unless they use a condom during intercourse while taking drug and for 90 days after stopping study treatment and should not father a child in this period. A condom is required to be used also by vasectomized men in order to prevent delivery of the drug via seminal fluid.
- [1176] 26. Pregnant or lactating women, where pregnancy is defined as the state of a female after conception and until the termination of gestation, confirmed by a positive hCG laboratory test. In rare cases of an endocrine-secreting tumor, hCG levels may be above normal limits but with no pregnancy in the patient. In these cases, there should be a repeat serum hCG test (with a non-rising result) and a vaginal/pelvic ultrasound to rule out pregnancy. Upon confirmation of results and discussion with the Medical representative, these patients may enter the study.
- [1177] 27. Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, unless they are using highly effective methods of contraception during study treatment and for 90 days after the last any dose of study treatment. Highly effective contraception methods include:
- [1178] Total abstinence (when this is in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception)
- [1179] Female sterilization (have had surgical bilateral oophorectomy with or without hysterectomy), total hysterectomy or tubal ligation at least 6 weeks before taking study treatment. In case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment
- [1180] Male sterilization (at least 6 months prior to screening). For female patients on the study the vasectomized male partner should be the sole partner for that patient.

[1181] Use of oral (estrogen and progesterone), injected or implanted combined hormonal methods of contraception or placement of an intrauterine device (IUD) or intrauterine system (IUS) or other forms of hormonal contraception that have comparable efficacy (failure rate <1%), for example hormone vaginal ring or transdermal hormone contraception.

[1182] In case of use of oral contraception, women should have been stable on the same pill for a minimum of 3 months before taking study treatment.

[1183] Women are considered post-menopausal and not of child bearing potential if they have had 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (e.g. age appropriate, history of vasomotor symptoms) or have had surgical bilateral oophorectomy (with or without hysterectomy) or tubal ligation at least 6 weeks ago. In the case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment is she considered not of child bearing potential.

Dose-Limiting Toxicities and Dose Modification Guidelines

[1184] A dose-limiting toxicity (DLT) is defined as any of the following events thought to be at least possibly related to ADC per investigator judgment that occurs during the 21-day DLT evaluation period. Toxicity that is clearly and directly related to the primary disease or to another etiology is excluded from this definition.

DLT Definitions

[1185] A hematologic DLT is defined as:

[1186] Grade 3 or 4 febrile neutropenia or neutropenic infection

[1187] Grade 4 neutropenia lasting >7 days

[1188] Grade 4 thrombocytopenia

[1189] Grade 3 thrombocytopenia with clinically significant bleeding, or Grade 3 thrombocytopenia requiring a platelet transfusion

[1190] Grade 3 anemia that requires transfusion

[1191] Grade 4 anemia

[1192] A non-hematologic DLT is defined as:

[1193] Grade 4 non-hematologic toxicity

[1194] Grade 3 non-hematologic toxicity lasting >3 days despite optimal supportive care or medical intervention

[1195] A case of Hy's law (AST and/or ALT >3×ULN and bilirubin >2×ULN, and without initial findings of cholestasis (serum alkaline phosphatase (ALP) activity <2×ULN) and no other reason that could explain the combination of increased transaminases and serum total bilirubin, such as viral hepatitis A, B, or C, preexisting or acute liver disease, or another drug capable of causing the observed injury)

[1196] Grade 3 or higher hypersensitivity/infusion-related reaction (regardless of premedication). A grade 3

hypersensitivity I infusion-related reaction that resolves within 8 hours after onset with appropriate clinical management does not qualify as a DLT.

[1197] LVEF decrease to <40% or >20% decrease from baseline

[1198] Grade 4 tumor lysis syndrome (Grade 3 TLS will not constitute DLT unless it leads to irreversible end-organ damage)

[1199] The following conditions are not considered non-hematologic DLT:

[1200] Grade 3 fatigue for 5-7 days

[1201] Grade 3 diarrhea, nausea, or vomiting in the absence of premedication that responds to therapy and improves by at least 1 grade within 3 days for Grade 3 events or to Grade 1 within 7 days.

[1202] AST or ALT elevation $\geq 5 \times \text{ULN}$ but $\leq 8 \times \text{ULN}$, without concurrent elevation in bilirubin, that downgrades to \leq Grade 2 within 5 days after onset.

[1203] Grade 3 serum lipase or serum amylase for ≤ 7 days if without clinical signs or symptoms of pancreatitis

[1204] Patients who experience a DLT that resolves or stabilizes with appropriate medical management may continue treatment at the discretion of the investigator in consultation with the sponsor.

Dose Modifications

[1205] Guidelines for management of specific toxicities are detailed in the table below. For management of events not specified in the tables, the following may serve as a guidance to investigators:

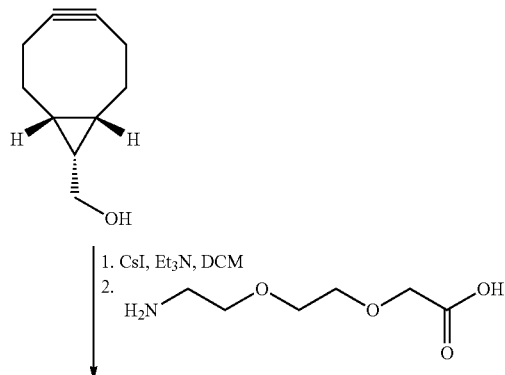
AE	
Grade	ADC Management Guideline
1	No dose adjustment is required.
2	First occurrence: Consider holding one or both drugs until improvement to \leq Grade 1 or baseline. Up to 1 dose of one or both drugs may be skipped to permit improvement. If improvement to Grade 1 or baseline occurs within 21 days from the last scheduled (but missed) dose of one or both drugs, continue one or both drugs at the original assigned dose level in subsequent treatment cycles. If improvement to \leq Grade 1 or baseline does not occur within 21 days from the last scheduled (but missed) dose, permanently discontinue one or both drugs. Second occurrence: Hold one or both drugs until improvement to Grade 1 or baseline.

-continued

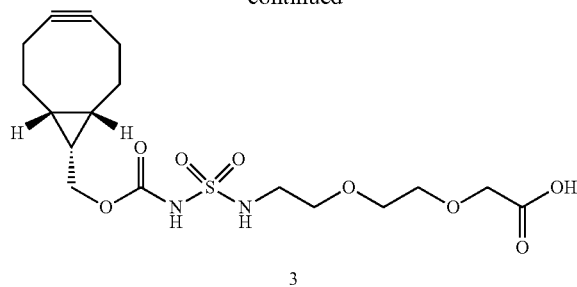
AE Grade	ADC Management Guideline
	Up to 1 dose of one or both drugs may be skipped to permit resolution. If improvement to Grade 1 or baseline occurs within 21 days from the last scheduled (but missed) dose, continue one or both drugs at 1 dose level below the original assigned dose in subsequent treatment cycles. If improvement to \leq Grade 1 or baseline does not occur within 21 days from the last scheduled (but missed) dose, permanently discontinue one or both drugs.
	Third occurrence: Permanently discontinue one or both drugs.
3	First occurrence: Hold one or both drugs until improvement to \leq Grade 1 or baseline. Up to 1 dose of one or both drugs may be skipped to permit improvement, then continue at 1 dose level below the original assigned dose in subsequent treatment cycles.
	Second occurrence: Permanently discontinue one or both drugs
4	Permanently discontinue one or both drugs.

Example 4: Synthesis of Intermediate 3

[1206]



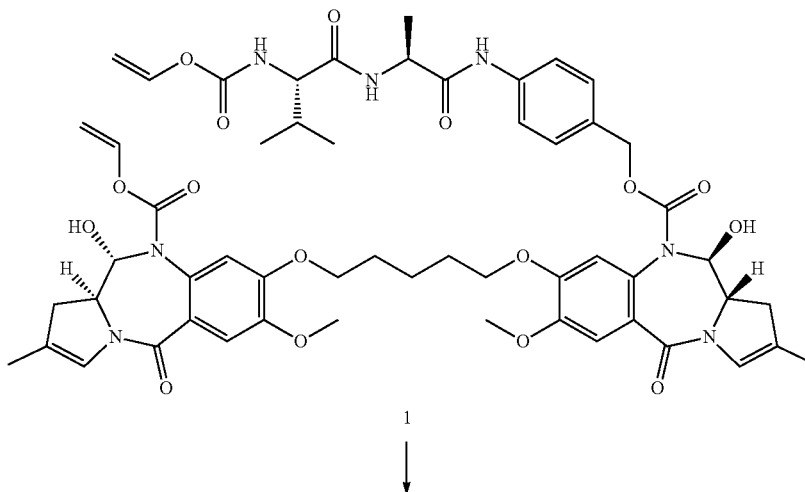
-continued



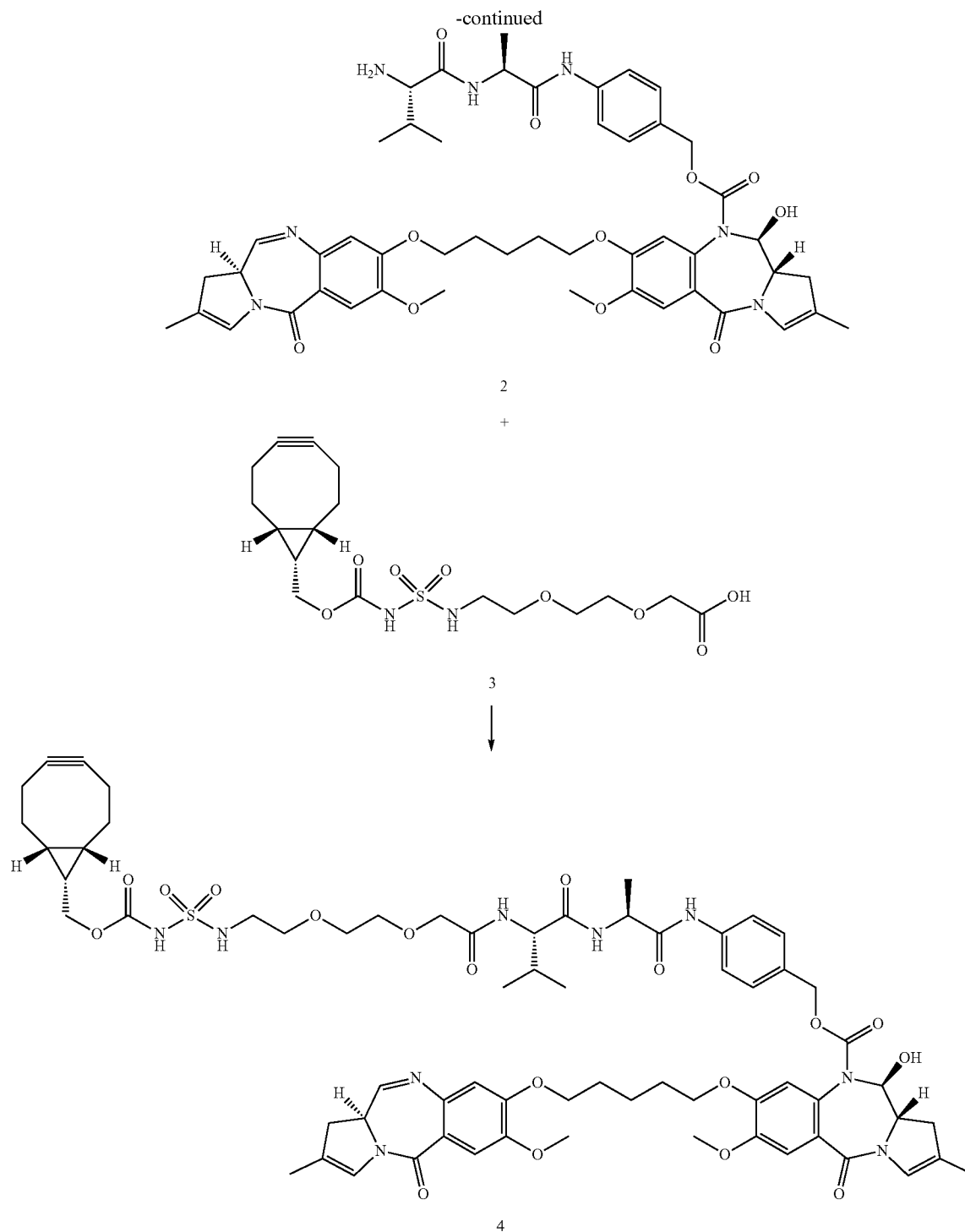
[1207] A solution of BCN alcohol (0.384 g, 2.55 mmole) in MeCN (25 mL) under a N_2 atmosphere was cooled to $0^\circ C$, and chlorosulfonyl isocyanate was added (CSI) was added dropwise (0.255 mL, 415 mg, 2.93 mmole, 1.15 equiv.). After stirring for 15 minutes, Et_3N was added dropwise (1.42 mL, 1.03 g, 10.2 mmole, 4 equiv.) and stirring was continued for another 10 minutes. Next, a solution of 2-(2-(2-aminoethoxy)ethoxy)acetic acid (1.0 g, 6.1 mmole, 2.4 equiv.) in H_2O (5 mL) was added and the reaction mixture was stirred to room temperature for 2 h. After this time, $CHCl_3$ (50 mL) and H_2O (100 mL) were added, and the layers were separated. To the aqueous layer in a separatory funnel was added CH_2Cl_2 (100 mL) and the pH was adjusted to 4 with 1 N HCl, before separation of layers. The water layer was extracted twice with CH_2Cl_2 (2 \times 100 mL), the organic layers were combined and dried (Na_2SO_4), filtered and concentrated. The residue was purified by flask column chromatography on silica, elution with CH_2Cl_2 to 20% MeOH in CH_2Cl_2 . Yield 0.42 g (1.0 mmole, 39%) of 3 as a colorless sticky wax.

Example 5: Synthesis of Drug Linker

[1208]



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[1209] Compound 1 can be synthesised as described in WO2014/057074—see compound 22.

[1210] (a) Palladium tetrakis(triphenylphosphine) ($\text{Pd}(\text{PPh}_3)_4$, 4.8 mg, 4.15 μmol) is weighed and put under an inert atmosphere. A solution of pyrrolidine (5.0 μL , 4.3 mg, 60 μmol) in DCM (1 mL) is degassed by bubbling N_2 through the solution. A solution of 1 (27 mg, 24 μmol) in

DCM (6 mL) is degassed by bubbling N_2 through the solution. While N_2 is still bubbled through the solution, the degassed solution of pyrrolidine is added. The weighed $\text{Pd}(\text{PPh}_3)_4$ is dissolved in DCM (1 mL) and 0.9 mL of this solution is added. After 50 min of bubbling of N_2 , DCM (25 mL) is added and the mixture is washed with aqueous saturated NH_4Cl (25 mL). After separation, the aqueous

layer is extracted with DCM (2×25 mL). The combined organic layers are dried (Na₂SO₄) and concentrated. The residue is purified by RP-HPLC (30-90% MeCN (0.1% formic acid) in H₂O (0.1% formic acid)). The combined fractions are passed through SPE (HCO₃⁻) columns and concentrated. After addition of MeCN (50 mL) the mixture is again concentrated. The resulting residue 2 is used in the next step.

[1211] The conversion of the reaction can be monitored through LCMS analysis. Column: XBridge BEH C18 Intelligent Speed (IS) Column, 130A, 3.5 μm (4.6 mm×20 mm). Mobile phase A: Water (0.1% formic acid), Mobile phase B (0.1% formic acid). Detection with PDA and ESI+. Samples can be prepared by diluting the reaction mixture with MeCN.

[1212] (b) To a solution of the above residue 2 in CHCl₃ (5 mL) is added a solution of 3 (15 mg, 36 μmol, mw 418 g/mole) in CHCl₃ (0.8 mL). The resulting mixture is added to solid EDC.HCl (4.7 mg, 25 μmol), CHCl₃ (5 mL) was added and the mixture stirred for 30 minutes. DCM (30 mL) is added and the resulting mixture is washed with water (30 mL). After separation, the aqueous phase is extracted with DCM (30 mL). The combined organic layers are dried (Na₂SO₄) and concentrated. The residue is purified by RP-HPLC (30-90% MeCN (no acid) in H₂O (0.01% formic acid)). The HPLC collection tubes are filled with 5% aqueous (NH₄)HCO₃ before collection. The combined HPLC fractions are extracted with DCM (3×20 mL). The combined organic layers are dried (Na₂SO₄) and concentrated. The product 4 is obtained as slightly yellow/white oil (21 mg, 16 μmol, mw 1323 g/mole, 67% over two steps).

[1213] The conversion of the reaction can be monitored through LCMS analysis. Column: XBridge BEH C18 Intelligent Speed (IS) Column, 130A, 3.5 μm (4.6 mm×20 mm). Mobile phase A: Water (0.1% formic acid), Mobile phase B (0.1% formic acid). Detection with PDA and ESI+.

Example 6: Antibody Modification

Reaction Conditions

[1214] The reaction conditions for the one-pot glycan remodelling are:

[1215] 15 mg/ml AXL Antibody (~0.1 mM)

[1216] 0.15 mg/mL EndoSH (1% w/w) from *Streptococcus pyogenes*

[1217] 1.13 mg/mL His-TnGalNAcT (7.5% w/w) Galactose-N-acetyl Transferase (GalNAcT) enzyme

[1218] 2.5 mM 6-N₃GalNAc-UDP (25 eq. compared to IgG)

[1219] 10 mM MnCl₂

[1220] 25 mM TrisHCl pH 8.0

[1221] 150 mM NaCl

[1222] Incubate 16 hours at 30° C.

Procedure

[1223] This example is on a 25 mg-scale, which may be altered as necessary. The individual components are added in order and mixed:

[1224] 106.5 μL 25 mM Tris pH 8.0, 150 mM NaCl (to obtain a final volume of 1667 μL)

[1225] 1 mL 25 mg/mL AXL Antibody in 25 mM Tris pH 8.0, 150 mM NaCl

[1226] 71.4 μL 3.5 mg/mL EndoSH in 25 mM Tris pH 8.0

[1227] 389 μL 4.82 mg/mL His-TnGalNAcT in 25 mM Tris pH 8.0

[1228] 16.7 μL 1M MnCl₂ in MQ

[1229] 83.4 μL 0.1 M 6-N₃GalNAc-UDP in MQ

[1230] This mixture for approximately 16 hours at 30° C. Completion of the modified galactose residue may be assessed by subjecting a sample to MS analysis. After protein A affinity purification, a small sample of the product may be reduced with DTT and subsequently subjected to MS analysis. A typical mass spectrum of a successful transfer reaction shows the formation of a one major product of (90% of total heavy chain), resulting from modified galactose transfer to core GlcNAc(Fuc) substituted Ab, and a minor product (±10% of total heavy chain), resulting from modified galactose transfer to core GlcNAc (without Fucose) substituted Ab.

Purification Procedure

[1231] Buffers

[1232] Binding/wash buffer (TBS pH 7.5):

[1233] 20 mM TrisHCl pH 7.5

[1234] 150 mM NaCl

[1235] Wash buffer for endotoxin removal (TBS pH 7.5+ Triton-X100):

[1236] 20 mM TrisHCl pH 7.5

[1237] 150 mM NaCl

[1238] 0.2% Triton X-100

[1239] Elution buffer:

[1240] 0.1 M Glycine pH 2.7

[1241] CIP buffer:

[1242] 0.5 M NaOH

Procedure

[1243] 1. Wash the MabSelectSure 5 mL column (5 mL/min) with the following buffers in order to clean the column before applying the sample:

[1244] Wash column with at least 5 column volumes (CV) TBS pH 7.5

[1245] Wash column with 15 CV 0.5 M NaOH

[1246] Wash column with 5 CV TBS pH 7.5

[1247] Wash column with 5 CV Glycine pH 2.7

[1248] Wash column with TBS pH 7.5 until a natural pH is obtained

[1249] 2. Remove precipitation from reaction mixture by centrifugation (5 min at 4000 g) or by filtration (0.22 or 0.45 μm filter)

[1250] 3. Load sample at 2 mL/min and perform the following steps with 5 mL/min:

[1251] Wash with at least 20 CV TBS=0.2% Triton X-100

[1252] Wash with at least 20 CV TBS

[1253] Elute with 0.1 M Glycine pH 2.7

[1254] 4. Immediately neutralize fractions by adding 1/5 volume of 1 M Tris-HCl pH 8.0 and mixing

[1255] 5. Dialyze sample against 3×≥50 volumes of PBS pH 7.4 at 4° C. (3×≥1 hour)

[1256] 6. Concentrate sample using spinfilter devices to ~20 mg/mL

Example 7: Conjugation of 4 to Modified Antibody to Produce ConjA

Reaction Conditions

- [1257] 15 mg/ml azido-modified AXL antibody (0.1 M IgG)
 [1258] 0.5 mM 4 (5 eq. compared to IgG=2.5 eq per azide)
 [1259] 10% DMF or 25% propyleneglycol
 [1260] PBS pH7.4

Procedure

- [1261] 1. Add 9 vols of 16.67 mg/ml azido-modified antibody in PBS pH7
 [1262] 2. Add 1 vol of 5 mM 4 in DMF and mix immediately.
 [1263] 3. Incubate overnight.
 [1264] 4. Measure conversion by RP-HPLC and MS.

Example 8: Purification of ADC

Sample Preparation

- [1265] The following requirements should be met before loading onto the column:
 [1266] Organic solvent $\leq 5\%$
 [1267] Total sample volume $\leq 3\%$ of the CV ($\leq 720 \mu\text{L}$ for Superdex 200 10/300 GL, and $\leq 10 \text{ ml}$ for Superdex 200 HiLoad 26/600)
 [1268] No precipitants
 [1269] The above requirements can be accomplished using the following procedure:
 [1270] 1. Dilute sample with PBS pH7.4 to a final organic solvent concentration of 55%
 [1271] 2. If volume exceeds 3% of the CV, the sample was concentrated using Amicon Ultra centrifugal filters (MWCO 10 kDa)
 [1272] 3. Potential precipitation is removed by centrifugation (10 min at 13000 rpm in a table top centrifuge)

Purification

- [1273] The purification was carried out using a Superdex 200 10/300 GL column (CV=23 ml, GE healthcare) on an Akta Purifier-10. The following washing steps are performed with a flow rate of 0.5 ml/min:
 [1274] Wash column with 1 CV water
 [1275] Wash column with 1 CV 0.5 M NaOH.
 [1276] Equilibrate column with PBS pH 7.4 (Sigma, D8537) until neutral pH is obtained.
 [1277] The sample is injected with 0.5 ml/min PBS pH7.4 and 1 ml fractions are collected (total run=1.5CV). Monomer fractions are pooled and dialysed at 4° C. against 3x1 L of formulation buffer (30 mM histidine, 200 mM sorbitol, 0.02% (w/v) tween-20, pH 6.0). Samples are filter-sterilized using 0.22 μm filter, snapfrozen using liquid nitrogen and stored at -80° C.
 [1278] Mass spectral analysis of the fabricator-digested sample showed one major product (observed mass 25691 Da, approximately 90% of total Fc/2 fragment), corresponding to the conjugated Fc/2 fragment. RP-HPLC analysis of the reduced sample indicated an average DAR of 1.98.

Example 9: In Vitro Cytotoxicity

[1279] H1299 cells were obtained from ATCC (ATCC number CRL-5803). H1299 medium was Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Gibco FBS. Cells were grown at 37° C., 5% CO₂ in a humidified incubator. Cell suspensions were dispensed into 96-well flat bottomed plates (104 cells per well). A set of 8x10-fold dilutions of stock ADC were prepared in cell culture medium. Each ADC dilution (50 μL per well) was dispensed into 4 replicate wells of the 96-well plate containing cell suspension. Control wells were prepared by adding the same volume of culture medium only. After incubation for 96 hours, cell viability was measured by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay (Promega, catalogue number G5421) following manufacturer's instructions. Absorbance was measured at 490 nm. Cell survival (%) was calculated from the mean absorbance in the 4 ADC-treated wells compared to the mean absorbance in the 4 control wells (100%). Dose response curves were generated from the mean data of 3 replicate experiments and the EC₅₀ values were determined by fitting data to a sigmoidal dose-response curve with variable slope using Prism (GraphPad, San Diego, Calif.). Error bars indicate standard deviation (SD).
 [1280] The EC₅₀ of ConjA was found to be 0.0554 $\mu\text{g/mL}$.

Example 10: Antigen Binding Study

[1281] Maxisorp plates were coated at +4° C. overnight with human Ax1 antigen (50 ng/well; batch in PBS. Non-reactive sites were blocked with SuperBlock buffer (overnight at +4° C. or room temperature). A set of 8x3-fold or 5-fold dilutions of stock ADC were prepared in sample buffer/PBS/Tween20. Each ADC dilution (60 μL /well) was dispensed into 4 replicate wells of the coated plate. Control wells were prepared by adding the same volume of sample buffer/PBS/Tween20. Anti-human kappa IgG-horseradish peroxidase (HRP) conjugate was used as secondary antibody (1:5000, 1 hour at room temperature). HRP was detected with 1-Step Ultra TMB-ELISA substrate solution (75 μL /well; 5 minutes at room temperature). Substrate reaction was stopped with 0.6 M HCl (75 μL /well). Optical density was measured at 450 nm on Envision using 450 nm Peroxidase program. Antigen binding curves were generated from the mean data of 3 replicate experiments using Prism (GraphPad, San Diego, Calif.). FIG. 2 shows the results obtained, where \blacktriangle is ConjA. Error bars indicate standard error of the mean (SEM). ConjA bound with high affinity to the extracellular domain of AXL coated on plates.

Example 11: In Vivo Efficacy Study

[1282] 5×10^6 MDA-MB-231 tumor cells were subcutaneously implanted to female athymic nude mice. ADC dosing with vehicle or test item was initiated when tumor volumes reached 88-172 mm³. ConjA was administered intravenously (i.v.) via tail vein injection once at a dose level of 1 mg/kg. The dosing volume was 10 mL/kg of body weight and was escalated to the body weight of each individual animal. Animals were euthanized if their tumor volume reached the endpoint volume of 1500 mm³ or at the end of the study, whichever came first. Animals weight, signs of any adverse, treatment-related side effects and clinical signs were monitored during the study period. For the calculation of mean tumour volume of the group, the following rule was

applied: when an animal exited the study due to tumour size, the final tumour volume recorded for the animal was included with the data used to calculate the mean volume at subsequent time points. Tumour volume and body weight values were not used to calculate a group mean tumour volumes/body weight when fewer than 50% of the animals in a group remained in the study. Prism (GraphPad, San Diego, Calif.) was used for graphical presentations and statistical analyses. FIG. 3 shows the results obtained, where ▲ is ConjA, and ○ is the vehicle alone. Error bars indicate SEM. A single dose of 1 mg/kg of ConjA strongly inhibited tumor growth with 10/10 mice being tumor-free 60 days after dosing.

Example 12: Rat Toxicology Study

Method

[1283] ConjA was evaluated in a single intravenous dose rat tolerability study. Male sprague-dawley rats (n=3/group) were dosed 3 & 6 mg/kg for ConjA on day 1, with necropsy on day 21 following dosing. Bodyweights and food consumption were monitored frequently with in-life sampling for clinical pathology (blood on days 8 and 21) and repeated sampling for pharmacokinetics. At necropsy, macroscopic observations were taken with selected organs weighed and retained for possible histopathology.

[1284] ConjA was clinically well tolerated at 3 & 6 mg/kg. Bodyweight gain was reduced by 11 and 21% in the 3 and 6 mg/kg groups respectively, consistent with reduced food consumption. Several haematology parameters were reduced on day 8, mainly in the 6 mg/kg dose group (reticulocytes (−76%), haemoglobin (−29%) white blood cells (−66%) and platelets (−37%)), with some evidence of recovery by day 21. At necropsy, reduced thymus weight was observed in all animals. Therefore, the maximum tolerated dose (MTD) for ConjA was 6 mg/kg.

Example 13: Synergy in SN12C Cells (AXL High-Expressing) Between ADCxAXL and Each of Cytarabine, Decitabine, Gemcitabine, Olaparib, and Fludarabine

[1285] Cells were plated on day 1 at 10,000 cells/well in 96-well plates, three replicates per experiment and total n of 3. Combination drug was added on day 2 at various doses (see figures) and incubated for 24 hours at 37° C., 5% CO₂. Drug only control was added in the following dosage range at the same time, all at a 10 fold dilution.

[1286] On day 3 ADCxAXL was added to cells containing drug, or media only as a control in the dosage range 0.001 pM-100 nM at a 10 fold dilution and incubated for a further 5 days (3xcell doubling time). Absorption was analysed at 492 nM on a Thermo Labsystems Multiscan Ascent plate reader using the MTT assay.

[1287] Data was analysed using Graphpad Prism v5.02, and synergism was plotted using Calcsyn v2.11. Strong synergism is indicative of a CI value of <0.7—moderate synergism carries a CI value of >0.7 and <1.

[1288] Results are shown in FIG. 4 (Cytarabine), FIG. 5 (Fludarabine), FIG. 6 (Decitabine), FIG. 7 (Olaparib), and FIG. 8 (Gemcitabine).

Example 14: Synergy Between ADCxAXL and Each of MEKi and BRAFi

MEKi

[1289] Examples of suitable MEK inhibitors such as Trametinib, Cobimetinib, Binimetinib, Selumetinib, U0126 or PD325901.

[1290] MEK inhibitors (MEKi) inhibit the mitogen-activated protein kinase enzymes MEK1 and/or MEK2. They can be used to affect the MAPK/ERK pathway which is often overactive in some cancers. Defects in the MAP/ERK pathway can lead uncontrolled growth, especially in melanoma. Hence MEK inhibitors have potential for treatment of some cancers especially BRAF-mutated melanoma and KRAS/BRAF mutated colorectal cancer (Wang et al., *Biochim. Biophys. Acta* 1773(8): 1248-1255 (2007). Interestingly, according to Miller et al (*Cancer Discovery* 6(4):382-399, 2016) incubation of tumor cells with MEKi (U0126 or PD325901) induced a strong accumulation of AXL on the membrane of tumor cells.

Trametinib

[1291] Trametinib (trade name Mekinist) is a MEK inhibitor, inhibiting MEK1 and MEK2. It is approved for the treatment of patients with BRAF V600E mutated metastatic melanoma. The V600E mutation makes the mutant BRAF gene constitutively active, driven proliferation of the melanoma. By inhibiting the MAP/ERK pathway, cell proliferation is blocked and apoptosis (controlled cell death) is induced.

[1292] Combining ADCxAXL, which targets AXL positive tumors, with MEKi is advantageous, because on the one hand, ADCxAXL will directly kill the AXL positive tumor cells via a mechanism depending on DNA cross-linking resulting in apoptosis, while on the other hand, MEKi will induce apoptosis through interference with cell proliferation through inhibition of the MAP/ERK cell signalling pathway. Also, combining ADCxAXL with MEKi is advantageous because the upregulation of AXL by MEKi will increase the uptake of ADCxAXL into the tumor cells resulting in higher accumulation of PBD dimer and subsequent DNA damage leading to higher cancer cell death.

[1293] To show that co-treatment of AXL-positive cancer cell lines with ADCxAXL and MEKi has an additive or synergistic anti-tumor effect, a panel of cell lines including, but not limited to MDA-MB231, SN12C, MDA-MB-157 and SKLU1 will be pre-incubated with cells with MEKi for up to 24h (1 or 10 uM) and then serial dilutions of ADCxAXL (or B12-PL1601 as control) will be added. After incubation the in vitro cytotoxicity of the combinations (as determined by CellTiter-Glo® or MTS assays) will be measured.

[1294] Alternatively, a panel of cell lines including, but not limited to MDA-MB231, SN12C, MDA-MB-157 and SKLU1 will be co-treated with a range of concentrations of both ADCxAXL and MEKi.

[1295] As negative controls, the same panel of cell lines will be co-treated with a range of concentrations of Trametinib or with a range of concentration of ADCxAXL and vehicle.

[1296] After incubation the in vitro cytotoxicity of the combinations (as determined by CellTiter-Glo® or MTS assays) will be measured. Percentage cell viability is calcu-

lated compared to the untreated control. Cytotoxic synergy is calculated by transforming the cell viability data into fraction affected, and calculating the combination index using the CalcuSyn analysis program.

BRAFⁱ

[1297] Examples of suitable BRAF inhibitors such as vemurafenib and dabrafenib. A BRAF inhibitor inhibits the (mutated) B-RAF protein directly. Mutations in BRAF can lead to uncontrolled growth, especially in melanoma.

Vemurafenib

[1298] Vemurafenib (trade name Zelboraf) directly inhibits B-RAF. It is approved for the treatment of patients with late-stage melanoma driven by a V600E B-RAF mutated gene. Mutations makes the mutant BRAF gene constitutively active, driving proliferation of the melanoma. By inhibiting mutated B-RAF, cell proliferation is blocked and apoptosis (controlled cell death) is induced.

[1299] Combining ADCxAXL, which targets AXL positive tumors, with Vemurafenib is advantageous, because on the one hand, ADCxAXL will directly kill the AXL positive tumor cells via a mechanisms depending on DNA cross-linking resulting in apoptosis, while on the other hand, Vemurafenib will induce apoptosis via interference with cell proliferation through inhibition of BRAF.

[1300] To show that ADCxAXL works synergistically with Vemurafenib, a panel of AXL (+) cell lines including, but not limited to MDA-MB231, NCI-H1299 and SNU12 cells, will be co-treated with a range of concentrations of both ADCxAXL and Vemurafenib.

[1301] As negative controls, the same panel of cell lines will be co-treated with a range of concentrations of Trametinib or with a range of concentration of ADCxAXL and vehicle.

[1302] After incubation, the in vitro cytotoxicity of the combinations will be determined by an MTS assay. To determine the cytotoxicity, Cell viability is measured by adding MTS per well and incubating for 4 hours at 37 C. Percentage cell viability is calculated compared to the untreated control. Cytotoxic synergy is calculated by transforming the cell viability data into fraction affected, and calculating the combination index using the CalcuSyn analysis program.

Example 15: Synergy Against AXL+Ve Neoplastic Cells Between ADCxAXL and Each of the Immunooncology (I/O) Secondary Agents PD1 Antagonists, PDL1 Antagonists, CTLA4 Antagonists, OX40 Agonists, and GITR Agonists

PD1 Antagonists

[1303] To test whether a PBD-based ADC against AXL combined with a PD1 antagonist shows additive or synergistic effect, the combination is tested in vivo in a syngeneic tumor model in immunocompetent mice (for AXL, potentially suitable models include 4T1, EMT-6, EMT-6-BRCA1 (-/-), EMT-6-BRCA1 (+/-), 4T1-BRCA1 (+/-), KLN 205, Lewis Lung, Madison109 Colon26, CT26, MC38, GL261, B16F10, CloudmanS91, Pan02, Renca, and MBT-2). For this purpose, an antibody cross reactive with mouse AXL is conjugated to a PBD warhead and this ADC is administered with the PD1 antagonist to mice grafted with a mouse tumor

cell line expressing AXL. The ADC is administered before the PD1 antagonist, concomitantly with the PD1 antagonist, or after the PD1 antagonist, as decided by the experimenter.

[1304] Typically, the ADC is dosed as a single dose between 0.1 and 1 mg/kg, while the PD1 antagonist is dosed Q3d×3 at doses between 1 and 10 mg/kg. Control groups include the ADC or PD1 antagonist alone. Tumor volumes and body weight is subsequently measured up to 60 days for all groups and the number of partially responding (PR), completely responding (CR) tumor free surviving (TFS mice is determined in each group.

[1305] Statistical analysis (typically a log-rank test) is performed to determine whether the mice treated with the combination have outperformed the mice treated with either ADC or PD1 antagonist alone.

PDL1 Antagonists

[1306] To test whether a PBD-based ADC against AXL combined with a PDL1 antagonist shows additive or synergistic effect, the combination is tested in vivo in a syngeneic tumor model in immunocompetent mice. For this purpose, an antibody cross reactive with mouse AXL is conjugated to a PBD warhead and this ADC is administered with the PDL1 antagonist to mice grafted with a mouse tumor cell line expressing AXL. The ADC is administered before the PDL1 antagonist, concomitantly with the PDL1 antagonist, or after the PDL1 antagonist, as decided by the experimenter.

[1307] Typically, the ADC is dosed as a single dose between 0.1 and 1 mg/kg, while the PD1 antagonist is dosed Q3d×3 at doses between 1 and 10 mg/kg. Control groups include the ADC or PDL1 antagonist alone. Tumor volumes and body weight is subsequently measured up to 60 days for all groups and the number of partially responding (PR), completely responding (CR) tumor free surviving (TFS mice is determined in each group.

[1308] Statistical analysis (typically a log-rank test) is performed to determine whether the mice treated with the combination have outperformed the mice treated with either ADC or PDL1 antagonist alone.

CTLA4 Antagonists

[1309] To test whether a PBD-based ADC against AXL combined with a CTLA4 antagonist shows additive or synergistic effect, the combination is tested in vivo in a syngeneic tumor model in immunocompetent mice. For this purpose, an antibody cross reactive with mouse AXL is conjugated to a PBD warhead and this ADC is administered with the CTLA4 antagonist to mice grafted with a mouse tumor cell line expressing AXL. The ADC is administered before the CTLA4 antagonist, concomitantly with the CTLA4 antagonist, or after the CTLA4 antagonist, as decided by the experimenter.

[1310] Typically, the ADC is dosed as a single dose between 0.1 and 1 mg/kg, while the CLTA4 antagonist is dosed Q3d×3 at doses between 1 and 10 mg/kg. Control groups include the ADC or CTLA4 antagonist alone. Tumor volumes and body weight is subsequently measured up to 60 days for all groups and the number of partially responding (PR), completely responding (CR) tumor free surviving (TFS mice is determined in each group.

[1311] Statistical analysis (typically a log-rank test) is performed to determine whether the mice treated with the

combination have outperformed the mice treated with either ADC or CTLA4 antagonist alone.

OX40 Agonists

[1312] To test whether a PBD-based ADC against AXL combined with a OX40 agonist shows additive or synergistic effect, the combination is tested in vivo in a syngeneic tumor model in immunocompetent mice. For this purpose, an antibody cross reactive with mouse AXL is conjugated to a PBD warhead and this ADC is administered with the OX40 agonist to mice grafted with a mouse tumor cell line expressing AXL. The ADC is administered before the OX40 agonist, concomitantly with the OX40 agonist, or after the OX40 agonist, as decided by the experimenter.

[1313] Typically, the ADC is dosed as a single dose between 0.1 and 1 mg/kg, while the OX40 agonist is dosed Q3d×3 at doses between 1 and 10 mg/kg. Control groups include the ADC or OX40 agonist alone. Tumor volumes and body weight is subsequently measured up to 60 days for all groups and the number of partially responding (PR), completely responding (CR) tumor free surviving (TFS) mice is determined in each group.

[1314] Statistical analysis (typically a log-rank test) is performed to determine whether the mice treated with the combination have outperformed the mice treated with either ADC or OX40 agonist alone.

GITR Agonists

[1315] To test whether a PBD-based ADC against AXL combined with a GITR agonist shows additive or synergistic effect, the combination is tested in vivo in a syngeneic tumor model in immunocompetent mice. For this purpose, an antibody cross reactive with mouse AXL is conjugated to a PBD warhead and this ADC is administered with the GITR agonist to mice grafted with a mouse tumor cell line expressing AXL. The ADC is administered before the GITR agonist, concomitantly with the GITR agonist, or after the GITR agonist, as decided by the experimenter.

[1316] Typically, the ADC is dosed as a single dose between 0.1 and 1 mg/kg, while the GITR agonist is dosed Q3d×3 at doses between 1 and 10 mg/kg. Control groups include the ADC or GITR agonist alone. Tumor volumes and body weight is subsequently measured up to 60 days for all groups and the number of partially responding (PR), completely responding (CR) tumor free surviving (TFS) mice is determined in each group.

[1317] Statistical analysis (typically a log-rank test) is performed to determine whether the mice treated with the combination have outperformed the mice treated with either ADC or GITR agonist alone.

Example 16: Synergy Against AXL-Ve Neoplastic Cells Between ADCxAXL and Each of the Immunooncology (I/O) Secondary Agents PD1 Antagonists, PDL1 Antagonists, CTLA4 Antagonists, OX40 Agonists, and GITR Agonists

[1318] AXL is also expressed on immune cells that infiltrate the local tumor environment and which can have a suppressive impact on the innate immune response against the tumor. Examples of such cells are NK cells, DC cells or macrophages. ADCxAXL can be used to target these immune cells, which will kill the immune suppressive cells, boosting the immune response.

[1319] In addition to this 'release of immune suppression' effect, killing of the immune cells by ADCxAXL will release local PBD warhead will kill neighboring neoplastic cells via bystander kill.

[1320] Accordingly, through these two mechanisms, tumors not expressing AXL can be killed by targeting immune cells in the local tumor environment. Also, AXL-ve tumor cells killed by PBD released from neighboring immune cells will induce additional immunogenic cell death, further strengthening the anti-tumor immune response.

PD1 Antagonists

[1321] To test whether a PBD-based ADC against AXL combined with a PD1 antagonist shows additive or synergistic effect against tumors not expressing AXL, the combination is tested in vivo in a syngeneic tumor model in immunocompetent mice. For this purpose, an antibody cross reactive with mouse AXL is conjugated to a PBD warhead and this ADC is administered with the PD1 antagonist to mice grafted with a mouse tumor cell line known to have high levels of infiltrating cells (e.g dendritic cells, NK cells, or macrophages), such as but not limited to MC38 and CT26. The ADC is administered before the PD1 antagonist, concomitantly with the PD1 antagonist, or after the PD1 antagonist, as decided by the experimenter.

[1322] Typically, the ADC is dosed as a single dose between 0.1 and 1 mg/kg, while the PD1 antagonist is dosed Q3d×3 at doses between 1 and 10 mg/kg. Control groups include the ADC or PD1 antagonist alone. Tumor volumes and body weight is subsequently measured up to 60 days for all groups and the number of partially responding (PR), completely responding (CR) tumor free surviving (TFS) mice is determined in each group.

[1323] Statistical analysis (typically a log-rank test) is performed to determine whether the mice treated with the combination have outperformed the mice treated with either ADC or PD1 antagonist alone.

PD-L1 Antagonists

[1324] To test whether a PBD-based ADC against AXL combined with a PDL1 antagonist shows additive or synergistic effect against tumors not expressing AXL, the combination is tested in vivo in a syngeneic tumor model in immunocompetent mice. For this purpose, an antibody cross reactive with mouse AXL is conjugated to a PBD warhead and this ADC is administered with the PDL1 antagonist to mice grafted with a mouse tumor cell line known to have high levels of infiltrating cells (e.g dendritic cells, NK cells, or macrophages), such as but not limited to MC38 and CT26. The ADC is administered before the PDL1 antagonist, concomitantly with the PDL1 antagonist, or after the PDL1 antagonist, as decided by the experimenter.

[1325] Typically, the ADC is dosed as a single dose between 0.1 and 1 mg/kg, while the PDL1 antagonist is dosed Q3d×3 at doses between 1 and 10 mg/kg. Control groups include the ADC or PDL1 antagonist alone. Tumor volumes and body weight is subsequently measured up to 60 days for all groups and the number of partially responding (PR), completely responding (CR) tumor free surviving (TFS) mice is determined in each group.

[1326] Statistical analysis (typically a log-rank test) is performed to determine whether the mice treated with the

combination have outperformed the mice treated with either ADC or PDL1 antagonist alone.

CTLA4 Antagonists

[1327] To test whether a PBD-based ADC against AXL combined with a CTLA4 antagonist shows additive or synergistic effect against tumors not expressing AXL, the combination is tested in vivo in a syngeneic tumor model in immunocompetent mice. For this purpose, an antibody cross reactive with mouse AXL is conjugated to a PBD warhead and this ADC is administered with the CTLA4 antagonist to mice grafted with a mouse tumor cell line know to have high levels of infiltrating cells (e.g dendritic cells, NK cells, or macrophages), such as but not limited to MC38 and CT26. The ADC is administered before the CTLA4 antagonist, concomitantly with the CTLA4 antagonist, or after the CTLA4 antagonist, as decided by the experimenter.

[1328] Typically, the ADC is dosed as a single dose between 0.1 and 1 mg/kg, while the CTLA4 antagonist is dosed Q3d×3 at doses between 1 and 10 mg/kg. Control groups include the ADC or CTLA4 antagonist alone. Tumor volumes and body weight is subsequently measured up to 60 days for all groups and the number of partially responding (PR), completely responding (CR) tumor free surviving (TFS mice is determined in each group.

[1329] Statistical analysis (typically a log-rank test) is performed to determine whether the mice treated with the combination have outperformed the mice treated with either ADC or CTLA4 antagonist alone.

OX40 Agonists

[1330] To test whether a PBD-based ADC against AXL combined with a OX40 agonist shows additive or synergistic effect against tumors not expressing AXL, the combination is tested in vivo in a syngeneic tumor model in immunocompetent mice. For this purpose, an antibody cross reactive with mouse AXL is conjugated to a PBD warhead and this ADC is administered with the OX40 agonist to mice grafted with a mouse tumor cell line know to have high levels of infiltrating cells (e.g dendritic cells, NK cells, or macrophages), such as but not limited to MC38 and CT26. The

ADC is administered before the OX40 agonist, concomitantly with the OX40 agonist, or after the OX40 agonist, as decided by the experimenter.

[1331] Typically, the ADC is dosed as a single dose between 0.1 and 1 mg/kg, while the OX40 agonist is dosed Q3d×3 at doses between 1 and 10 mg/kg. Control groups include the ADC or OX40 agonist alone. Tumor volumes and body weight is subsequently measured up to 60 days for all groups and the number of partially responding (PR), completely responding (CR) tumor free surviving (TFS mice is determined in each group.

[1332] Statistical analysis (typically a log-rank test) is performed to determine whether the mice treated with the combination have outperformed the mice treated with either ADC or OX40 agonist alone.

GITR Agonists

[1333] To test whether a PBD-based ADC against AXL combined with a GITR agonist shows additive or synergistic effect against tumors not expressing AXL, the combination is tested in vivo in a syngeneic tumor model in immunocompetent mice. For this purpose, an antibody cross reactive with mouse AXL is conjugated to a PBD warhead and this ADC is administered with the GITR agonist to mice grafted with a mouse tumor cell line know to have high levels of infiltrating cells (e.g dendritic cells, NK cells, or macrophages), such as but not limited to MC38 and CT26. The ADC is administered before the GITR agonist, concomitantly with the GITR agonist, or after the GITR agonist, as decided by the experimenter.

[1334] Typically, the ADC is dosed as a single dose between 0.1 and 1 mg/kg, while the GITR agonist is dosed Q3d×3 at doses between 1 and 10 mg/kg. Control groups include the ADC or GITR agonist alone. Tumor volumes and body weight is subsequently measured up to 60 days for all groups and the number of partially responding (PR), completely responding (CR) tumor free surviving (TFS mice is determined in each group.

[1335] Statistical analysis (typically a log-rank test) is performed to determine whether the mice treated with the combination have outperformed the mice treated with either ADC or GITR agonist alone.

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35 40 45
Ile Tyr Arg Thr Ser Asn Leu Ala Ser Gly Ile Pro Ala Arg Phe Ser
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Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu
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Ala Thr Ile Ser Ser Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
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Ala Arg His Pro Ile Tyr Tyr Thr Tyr Asp Asp Thr Met Asp Tyr Trp
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 115 120 125
 Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
 130 135 140
 Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
 145 150 155 160
 Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
 165 170 175
 Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
 180 185 190
 Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
 195 200 205
 His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
 210 215 220
 Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
 225 230 235 240
 Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
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 Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
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 His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
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 Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Xaa Ser Thr
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 Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
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 Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
 325 330 335
 Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
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 Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
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 Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
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 Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
 405 410 415
 Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
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<223> OTHER INFORMATION: 1H12 Light Chain

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Asn Phe His Trp Tyr Gln Gln Lys Pro Gly Leu Ala Pro Arg Leu Leu
35 40 45
Ile Tyr Arg Thr Ser Asn Leu Ala Ser Gly Ile Pro Ala Arg Phe Ser
50 55 60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu
65 70 75 80
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Trp Ser Gly Tyr Pro
85 90 95
Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala
100 105 110
Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
115 120 125
Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
130 135 140
Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145 150 155 160
Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
165 170 175
Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
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Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
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Gly

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<210> SEQ ID NO 9
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<210> SEQ ID NO 11
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Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Asn Pro Asp Ser Ser Thr Ile Asn Tyr Thr Pro Ser Leu
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Lys Asp Lys Phe Ile Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Ser Lys Val Arg Ser Glu Asp Thr Ala Leu Tyr Tyr Cys

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Leu Val Thr Val Ser Ser		
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20	25	30
Gly Thr Ser Leu Met His Trp Tyr Gln Gln Lys Pro Gly Gln Gln Pro		
35	40	45
Lys Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ala Gly Phe Pro Thr		
50	55	60
Arg Phe Ser Gly Ser Gly Ser Arg Thr Asp Phe Thr Leu Asn Ile His		
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Glu Tyr Pro Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Val Lys		
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<400> SEQUENCE: 13

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<210> SEQ ID NO 14
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 <220> FEATURE:
 <223> OTHER INFORMATION: 5F11 VH CDR2

<400> SEQUENCE: 14

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Asp

<210> SEQ ID NO 15
 <211> LENGTH: 9
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<400> SEQUENCE: 15

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<210> SEQ ID NO 16
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<210> SEQ ID NO 17
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<212> TYPE: PRT
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<400> SEQUENCE: 17

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<210> SEQ ID NO 18
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Gln Gln Ser Arg Glu Tyr Pro Arg Thr
1 5

<210> SEQ ID NO 19
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 5F11 RHA

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Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Tyr
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Glu Ile Asn Pro Asp Ser Ser Thr Ile Asn Tyr Thr Pro Ser Leu
50 55 60

Lys Asp Arg Phe Ala Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ser Pro Tyr Tyr Tyr Gly Pro Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser
115

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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 5F11 RHB

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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Tyr
20 25 30
Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Glu Ile Asn Pro Asp Ser Ser Thr Ile Asn Tyr Thr Pro Ser Leu
50 55 60
Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Ser Pro Tyr Tyr Tyr Gly Pro Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110
Leu Val Thr Val Ser
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<220> FEATURE:
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Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Tyr
20 25 30
Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ser Glu Ile Asn Pro Asp Ser Ser Thr Ile Asn Tyr Thr Pro Ser Leu
50 55 60
Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Ser Pro Tyr Tyr Tyr Gly Pro Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110
Leu Val Thr Val Ser
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Glu Ile Val Leu Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15
Glu Pro Ala Ser Ile Ser Cys Lys Ala Ser Gln Ser Val Ser Phe Ala
20 25 30
Gly Thr Ser Leu Met His Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro
35 40 45
Gln Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ala Gly Val Pro Asp
50 55 60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser
65 70 75 80
Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Gln Gln Ser Arg
85 90 95
Glu Tyr Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105 110

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<213> ORGANISM: Homo sapiens

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Met Ala Trp Arg Cys Pro Arg Met Gly Arg Val Pro Leu Ala Trp Cys
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Leu Ala Leu Cys Gly Trp Ala Cys Met Ala Pro Arg Gly Thr Gln Ala
20 25 30
Glu Glu Ser Pro Phe Val Gly Asn Pro Gly Asn Ile Thr Gly Ala Arg
35 40 45
Gly Leu Thr Gly Thr Leu Arg Cys Gln Leu Gln Val Gln Gly Glu Pro
50 55 60
Pro Glu Val His Trp Leu Arg Asp Gly Gln Ile Leu Glu Leu Ala Asp
65 70 75 80
Ser Thr Gln Thr Gln Val Pro Leu Gly Glu Asp Glu Gln Asp Asp Trp
85 90 95
Ile Val Val Ser Gln Leu Arg Ile Thr Ser Leu Gln Leu Ser Asp Thr
100 105 110
Gly Gln Tyr Gln Cys Leu Val Phe Leu Gly His Gln Thr Phe Val Ser
115 120 125
Gln Pro Gly Tyr Val Gly Leu Glu Gly Leu Pro Tyr Phe Leu Glu Glu
130 135 140
Pro Glu Asp Arg Thr Val Ala Ala Asn Thr Pro Phe Asn Leu Ser Cys
145 150 155 160
Gln Ala Gln Gly Pro Pro Glu Pro Val Asp Leu Leu Trp Leu Gln Asp
165 170 175
Ala Val Pro Leu Ala Thr Ala Pro Gly His Gly Pro Gln Arg Ser Leu
180 185 190
His Val Pro Gly Leu Asn Lys Thr Ser Ser Phe Ser Cys Glu Ala His
195 200 205
Asn Ala Lys Gly Val Thr Thr Ser Arg Thr Ala Thr Ile Thr Val Leu
210 215 220
Pro Gln Gln Pro Arg Asn Leu His Leu Val Ser Arg Gln Pro Thr Glu
225 230 235 240

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Leu	Glu	Val	Ala	Trp	Thr	Pro	Gly	Leu	Ser	Gly	Ile	Tyr	Pro	Leu	Thr	245	250	255
His	Cys	Thr	Leu	Gln	Ala	Val	Leu	Ser	Asp	Asp	Gly	Met	Gly	Ile	Gln	260	265	270
Ala	Gly	Glu	Pro	Asp	Pro	Pro	Glu	Glu	Pro	Leu	Thr	Ser	Gln	Ala	Ser	275	280	285
Val	Pro	Pro	His	Gln	Leu	Arg	Leu	Gly	Ser	Leu	His	Pro	His	Thr	Pro	290	295	300
Tyr	His	Ile	Arg	Val	Ala	Cys	Thr	Ser	Ser	Gln	Gly	Pro	Ser	Ser	Trp	305	310	315
Thr	His	Trp	Leu	Pro	Val	Glu	Thr	Pro	Glu	Gly	Val	Pro	Leu	Gly	Pro	325	330	335
Pro	Glu	Asn	Ile	Ser	Ala	Thr	Arg	Asn	Gly	Ser	Gln	Ala	Phe	Val	His	340	345	350
Trp	Gln	Glu	Pro	Arg	Ala	Pro	Leu	Gln	Gly	Thr	Leu	Leu	Gly	Tyr	Arg	355	360	365
Leu	Ala	Tyr	Gln	Gly	Gln	Asp	Thr	Pro	Glu	Val	Leu	Met	Asp	Ile	Gly	370	375	380
Leu	Arg	Gln	Glu	Val	Thr	Leu	Glu	Leu	Gln	Gly	Asp	Gly	Ser	Val	Ser	385	390	395
Asn	Leu	Thr	Val	Cys	Val	Ala	Ala	Tyr	Thr	Ala	Ala	Gly	Asp	Gly	Pro	405	410	415
Trp	Ser	Leu	Pro	Val	Pro	Leu	Glu	Ala	Trp	Arg	Pro	Gly	Gln	Ala	Gln	420	425	430
Pro	Val	His	Gln	Leu	Val	Lys	Glu	Pro	Ser	Thr	Pro	Ala	Phe	Ser	Trp	435	440	445
Pro	Trp	Trp	Tyr	Val	Leu	Leu	Gly	Ala	Val	Val	Ala	Ala	Ala	Cys	Val	450	455	460
Leu	Ile	Leu	Ala	Leu	Phe	Leu	Val	His	Arg	Arg	Lys	Lys	Glu	Thr	Arg	465	470	475
Tyr	Gly	Glu	Val	Phe	Glu	Pro	Thr	Val	Glu	Arg	Gly	Glu	Leu	Val	Val	485	490	495
Arg	Tyr	Arg	Val	Arg	Lys	Ser	Tyr	Ser	Arg	Arg	Thr	Thr	Glu	Ala	Thr	500	505	510
Leu	Asn	Ser	Leu	Gly	Ile	Ser	Glu	Glu	Leu	Lys	Glu	Lys	Leu	Arg	Asp	515	520	525
Val	Met	Val	Asp	Arg	His	Lys	Val	Ala	Leu	Gly	Lys	Thr	Leu	Gly	Glu	530	535	540
Gly	Glu	Phe	Gly	Ala	Val	Met	Glu	Gly	Gln	Leu	Asn	Gln	Asp	Asp	Ser	545	550	555
Ile	Leu	Lys	Val	Ala	Val	Lys	Thr	Met	Lys	Ile	Ala	Ile	Cys	Thr	Arg	565	570	575
Ser	Glu	Leu	Glu	Asp	Phe	Leu	Ser	Glu	Ala	Val	Cys	Met	Lys	Glu	Phe	580	585	590
Asp	His	Pro	Asn	Val	Met	Arg	Leu	Ile	Gly	Val	Cys	Phe	Gln	Gly	Ser	595	600	605
Glu	Arg	Glu	Ser	Phe	Pro	Ala	Pro	Val	Val	Ile	Leu	Pro	Phe	Met	Lys	610	615	620
His	Gly	Asp	Leu	His	Ser	Phe	Leu	Leu	Tyr	Ser	Arg	Leu	Gly	Asp	Gln	625	630	635

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Pro	Val	Tyr	Leu	Pro	Thr	Gln	Met	Leu	Val	Lys	Phe	Met	Ala	Asp	Ile	645	650	655
Ala	Ser	Gly	Met	Glu	Tyr	Leu	Ser	Thr	Lys	Arg	Phe	Ile	His	Arg	Asp	660	665	670
Leu	Ala	Ala	Arg	Asn	Cys	Met	Leu	Asn	Glu	Asn	Met	Ser	Val	Cys	Val	675	680	685
Ala	Asp	Phe	Gly	Leu	Ser	Lys	Lys	Ile	Tyr	Asn	Gly	Asp	Tyr	Tyr	Arg	690	695	700
Gln	Gly	Arg	Ile	Ala	Lys	Met	Pro	Val	Lys	Trp	Ile	Ala	Ile	Glu	Ser	705	710	715
Leu	Ala	Asp	Arg	Val	Tyr	Thr	Ser	Lys	Ser	Asp	Val	Trp	Ser	Phe	Gly	725	730	735
Val	Thr	Met	Trp	Glu	Ile	Ala	Thr	Arg	Gly	Gln	Thr	Pro	Tyr	Pro	Gly	740	745	750
Val	Glu	Asn	Ser	Glu	Ile	Tyr	Asp	Tyr	Leu	Arg	Arg	Gly	Asn	Arg	Leu	755	760	765
Lys	Gln	Pro	Ala	Asp	Cys	Leu	Asp	Gly	Leu	Tyr	Ala	Leu	Met	Ser	Arg	770	775	780
Cys	Trp	Glu	Leu	Asn	Pro	Gln	Asp	Arg	Pro	Ser	Phe	Thr	Glu	Leu	Arg	785	790	795
Glu	Asp	Leu	Glu	Asn	Thr	Leu	Lys	Ala	Leu	Pro	Pro	Ala	Gln	Glu	Pro	805	810	815
Asp	Glu	Ile	Leu	Tyr	Val	Asn	Met	Asp	Glu	Gly	Gly	Gly	Tyr	Pro	Glu	820	825	830
Pro	Pro	Gly	Ala	Ala	Gly	Gly	Ala	Asp	Pro	Pro	Thr	Gln	Pro	Asp	Pro	835	840	845
Lys	Asp	Ser	Cys	Ser	Cys	Leu	Thr	Ala	Ala	Glu	Val	His	Pro	Ala	Gly	850	855	860
Arg	Tyr	Val	Leu	Cys	Pro	Ser	Thr	Thr	Pro	Ser	Pro	Ala	Gln	Pro	Ala	865	870	875
Asp	Arg	Gly	Ser	Pro	Ala	Ala	Pro	Gly	Gln	Glu	Asp	Gly	Ala			885	890	

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<213> ORGANISM: Artificial Sequence

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<223> OTHER INFORMATION: AUNP12 peptide

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Phe Ser Glu Ser Thr Asn Ser

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<212> TYPE: PRT

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<400> SEQUENCE: 301

Ser Asn Thr Ser Glu Ser Phe

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<212> TYPE: PRT

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<223> OTHER INFORMATION: AUNP12 peptide

<400> SEQUENCE: 302

Phe Arg Val Thr Gln Leu Ala Pro Lys Ala Gln Ile Lys Glu

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<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: AUNP12 peptide

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Ser Asn Thr Ser Glu Ser Phe Lys Phe Arg Val Thr Gln Leu Ala Pro

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Lys Ala Gln Ile Lys Glu

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<223> OTHER INFORMATION: BMS-936559/MDX-1105 Antibody 1 VH CDR1

<400> SEQUENCE: 400

Asp Tyr Gly Phe Ser

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<210> SEQ ID NO 401

<211> LENGTH: 17

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<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BMS-936559/MDX-1105 Antibody 1 VH CDR2

<400> SEQUENCE: 401

Trp Ile Thr Ala Tyr Asn Gly Asn Thr Asn Tyr Ala Gln Lys Leu Gln

1 5 10 15

Gly

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<210> SEQ ID NO 402
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<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 402

Asp Tyr Phe Tyr Gly Met Asp Val
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<400> SEQUENCE: 403

Arg Ala Ser Gln Ser Val Ser Ser Tyr Leu Val
1 5 10

<210> SEQ ID NO 404
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<400> SEQUENCE: 404

Asp Ala Ser Asn Arg Ala Thr
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<210> SEQ ID NO 405
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 405

Gln Gln Arg Ser Asn Trp Pro Arg Thr
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<210> SEQ ID NO 406
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 406

Thr Tyr Ala Ile Ser
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<210> SEQ ID NO 407
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<220> FEATURE:
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<400> SEQUENCE: 407

Gly Ile Ile Pro Ile Phe Gly Lys Ala His Tyr Ala Gln Lys Phe Gln
1 5 10 15

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Gly

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<400> SEQUENCE: 408

Lys Phe His Phe Val Ser Gly Ser Pro Phe Gly Met Asp Val
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<210> SEQ ID NO 409
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<212> TYPE: PRT
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<400> SEQUENCE: 409

Arg Ala Ser Gln Ser Val Ser Ser Tyr Leu Ala
1 5 10

<210> SEQ ID NO 410
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<400> SEQUENCE: 410

Asp Ala Ser Asn Arg Ala Thr
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<210> SEQ ID NO 411
<211> LENGTH: 8
<212> TYPE: PRT
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<220> FEATURE:
<223> OTHER INFORMATION: BMS-936559/MDX-1105 Antibody 2 VL CDR3

<400> SEQUENCE: 411

Gln Gln Arg Ser Asn Trp Pro Thr
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<210> SEQ ID NO 412
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<400> SEQUENCE: 412

Ser Tyr Asp Val His
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Trp Leu His Ala Asp Thr Gly Ile Thr Lys Phe Ser Gln Lys Phe Gln
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Gly

<210> SEQ ID NO 414

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Glu Arg Ile Gln Leu Trp Phe Asp Tyr
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<213> ORGANISM: Artificial Sequence

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<223> OTHER INFORMATION: BMS-936559/MDX-1105 Antibody 3 VL CDR1

<400> SEQUENCE: 415

Arg Ala Ser Gln Gly Ile Ser Ser Trp Leu Ala
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<210> SEQ ID NO 416

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<213> ORGANISM: Artificial Sequence

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<223> OTHER INFORMATION: BMS-936559/MDX-1105 Antibody 3 VL CDR2

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Ala Ala Ser Ser Leu Gln Ser
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<210> SEQ ID NO 417

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

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<223> OTHER INFORMATION: BMS-936559/MDX-1105 Antibody 3 VL CDR3

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Gln Gln Tyr Asn Ser Tyr Pro Tyr Thr
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<210> SEQ ID NO 418

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<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: durvalumab/MEDI4736 VH Sequence

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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Tyr
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Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val

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Ala	Asn	Ile	Lys	Gln	Asp	Gly	Ser	Glu	Lys	Tyr	Tyr	Val	Asp	Ser	Val
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Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Ser	Leu	Tyr
65					70				75					80	
Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
				85					90					95	
Ala	Arg	Glu	Gly	Gly	Trp	Phe	Gly	Glu	Leu	Ala	Phe	Asp	Tyr	Trp	Gly
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 <223> OTHER INFORMATION: durvalumab/MEDI4736 VL Sequence

<400> SEQUENCE: 419

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Glu	Arg	Ala	Thr	Leu	Ser	Cys	Arg	Ala	Ser	Gln	Arg	Val	Ser	Ser	Ser
			20					25					30		
Tyr	Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	Ala	Pro	Arg	Leu	Leu
		35					40					45			
Ile	Tyr	Asp	Ala	Ser	Ser	Arg	Ala	Thr	Gly	Ile	Pro	Asp	Arg	Phe	Ser
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Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Arg	Leu	Glu
65					70					75				80	
Pro	Glu	Asp	Phe	Ala	Val	Tyr	Tyr	Cys	Gln	Gln	Tyr	Gly	Ser	Leu	Pro
			85						90					95	
Trp	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys				
			100				105								

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<210> SEQ ID NO 500
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: TRX518 VL sequence

<400> SEQUENCE: 500

Glu Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Lys Ala Ser Gln Asn Val Gly Thr Asn
20 25 30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45
Tyr Ser Ala Ser Tyr Arg Tyr Ser Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Ser
65 70 75 80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Asn Thr Asp Pro Leu
85 90 95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> SEQ ID NO 501
<211> LENGTH: 118
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: TRX518 VH sequence

<400> SEQUENCE: 501

Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15
Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30
Gly Met Gly Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45
Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
50 55 60
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80
Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
85 90 95
Cys Ala Arg Thr Arg Arg Tyr Phe Pro Phe Ala Tyr Trp Gly Gln Gly
100 105 110
Thr Leu Val Thr Val Ser
115

<210> SEQ ID NO 502
<211> LENGTH: 118
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: TRX518 VH sequence

<400> SEQUENCE: 502

Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln

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1	5	10	15												
Thr	Leu	Thr	Leu	Thr	Cys	Thr	Phe	Ser	Gly	Phe	Ser	Leu	Ser	Thr	Ser
	20						25					30			
Gly	Met	Gly	Val	Gly	Trp	Ile	Arg	Gln	Pro	Pro	Gly	Lys	Ala	Leu	Glu
	35						40					45			
Trp	Leu	Ala	His	Ile	Trp	Trp	Asp	Asp	Asp	Lys	Tyr	Tyr	Gln	Pro	Ser
	50					55					60				
Leu	Lys	Ser	Arg	Leu	Thr	Ile	Ser	Lys	Asp	Thr	Ser	Lys	Asn	Gln	Val
65					70					75				80	
Val	Leu	Thr	Met	Thr	Asn	Met	Asp	Pro	Val	Asp	Thr	Ala	Thr	Tyr	Tyr
			85						90					95	
Cys	Ala	Arg	Thr	Arg	Arg	Tyr	Phe	Pro	Phe	Ala	Tyr	Trp	Gly	Gln	Gly
	100							105					110		
Thr	Leu	Val	Thr	Val	Ser										
	115														

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<210> SEQ ID NO 599

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<210> SEQ ID NO 600

<211> LENGTH: 450

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: MEDI0562 Heavy chain sequence

<400> SEQUENCE: 600

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Ser Gly
20 25 30

Tyr Trp Asn Trp Ile Arg Lys His Pro Gly Lys Gly Leu Glu Tyr Ile
35 40 45

Gly Tyr Ile Ser Tyr Asn Gly Ile Thr Tyr His Asn Pro Ser Leu Lys
50 55 60

Ser Arg Ile Thr Ile Asn Arg Asp Thr Ser Lys Asn Gln Tyr Ser Leu
65 70 75 80

Gln Leu Asn Ser Val Thr Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Tyr Lys Tyr Asp Tyr Asp Gly Gly His Ala Met Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
115 120 125

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180 185 190

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys
210 215 220

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
225 230 235 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245 250 255

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Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His
			260					265					270		
Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val
		275					280					285			
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr
	290					295					300				
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly
305					310					315					320
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile
			325						330					335	
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val
		340						345					350		
Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser
		355					360					365			
Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu
	370					375					380				
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro
385					390					395					400
Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val
			405						410					415	
Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met
			420					425					430		
His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser
		435					440					445			
Pro	Gly														
	450														

<210> SEQ ID NO 601

<211> LENGTH: 214

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: MEDI0562 Light Chain sequence

<400> SEQUENCE: 601

Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly
1				5					10					15	
Asp	Arg	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Gln	Asp	Ile	Ser	Asn	Tyr
		20					25						30		
Leu	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Leu	Leu	Ile
		35				40						45			
Tyr	Tyr	Thr	Ser	Lys	Leu	His	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly
	50					55				60					
Ser	Gly	Ser	Gly	Thr	Asp	Tyr	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Gln	Pro
65				70					75					80	
Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Gly	Ser	Ala	Leu	Pro	Trp
		85						90						95	
Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys	Arg	Thr	Val	Ala	Ala
		100						105					110		
Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	Gly
		115				120						125			
Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala
	130					135					140				

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Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn	Ser	Gln
145					150					155					160
Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	Ser
				165					170					175	
Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	Tyr
			180					185					190		
Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	Val	Thr	Lys	Ser
		195					200					205			
Phe	Asn	Arg	Gly	Glu	Cys										
		210													

<210> SEQ ID NO 602
 <211> LENGTH: 402
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: MEDI6383

<400> SEQUENCE: 602

Glu	Ser	Lys	Tyr	Gly	Pro	Pro	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Phe
1				5					10					15	
Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr
			20					25					30		
Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val
		35					40					45			
Ser	Gln	Glu	Asp	Pro	Glu	Val	Gln	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val
	50					55				60					
Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Phe	Asn	Ser
65					70					75				80	
Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu
			85					90						95	
Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Gly	Leu	Pro	Ser
			100					105					110		
Ser	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro
	115						120					125			
Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Gln	Glu	Glu	Met	Thr	Lys	Asn	Gln
	130					135					140				
Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala
145					150					155				160	
Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr
			165					170						175	
Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Arg	Leu
			180					185					190		
Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Glu	Gly	Asn	Val	Phe	Ser	Cys	Ser
		195					200					205			
Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser
	210					215					220				
Leu	Ser	Leu	Gly	Lys	Asp	Gln	Asp	Lys	Ile	Glu	Ala	Leu	Ser	Ser	Lys
225					230					235					240
Val	Gln	Gln	Leu	Glu	Arg	Ser	Ile	Gly	Leu	Lys	Asp	Leu	Ala	Met	Ala
			245						250					255	
Asp	Leu	Glu	Gln	Lys	Val	Leu	Glu	Met	Glu	Ala	Ser	Thr	Gln	Val	Ser
			260					265					270		

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His	Arg	Tyr	Pro	Arg	Ile	Gln	Ser	Ile	Lys	Val	Gln	Phe	Thr	Glu	Tyr
		275					280					285			
Lys	Lys	Glu	Lys	Gly	Phe	Ile	Leu	Thr	Ser	Gln	Lys	Glu	Asp	Glu	Ile
	290					295					300				
Met	Lys	Val	Gln	Asn	Asn	Ser	Val	Ile	Ile	Asn	Cys	Asp	Gly	Phe	Tyr
305				310						315					320
Leu	Ile	Ser	Leu	Lys	Gly	Tyr	Phe	Ser	Gln	Glu	Val	Asn	Ile	Ser	Leu
			325						330					335	
His	Tyr	Gln	Lys	Asp	Glu	Glu	Pro	Leu	Phe	Gln	Leu	Lys	Lys	Val	Arg
		340						345					350		
Ser	Val	Asn	Ser	Leu	Met	Val	Ala	Ser	Leu	Thr	Tyr	Lys	Asp	Lys	Val
		355					360					365			
Tyr	Leu	Asn	Val	Thr	Thr	Asp	Asn	Thr	Ser	Leu	Asp	Asp	Phe	His	Val
	370					375					380				
Asn	Gly	Gly	Glu	Leu	Ile	Leu	Ile	His	Gln	Asn	Pro	Gly	Glu	Phe	Cys
385				390						395					400

Val Leu

<210> SEQ ID NO 603
 <211> LENGTH: 121
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Ox40mAb24 VH sequence

<400> SEQUENCE: 603

Gln	Val	Gln	Leu	Gln	Glu	Ser	Gly	Pro	Gly	Leu	Val	Lys	Pro	Ser	Gln
1			5					10						15	
Thr	Leu	Ser	Leu	Thr	Cys	Ala	Val	Tyr	Gly	Gly	Ser	Phe	Ser	Ser	Gly
		20						25					30		
Tyr	Trp	Asn	Trp	Ile	Arg	Lys	His	Pro	Gly	Lys	Gly	Leu	Glu	Tyr	Ile
		35				40						45			
Gly	Tyr	Ile	Ser	Tyr	Asn	Gly	Ile	Thr	Tyr	His	Asn	Pro	Ser	Leu	Lys
	50				55						60				
Ser	Arg	Ile	Thr	Ile	Asn	Arg	Asp	Thr	Ser	Lys	Asn	Gln	Tyr	Ser	Leu
65				70						75					80
Gln	Leu	Asn	Ser	Val	Thr	Pro	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Ala
			85					90						95	
Arg	Tyr	Lys	Tyr	Asp	Tyr	Asp	Gly	Gly	His	Ala	Met	Asp	Tyr	Trp	Gly
		100					105						110		
Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser							
	115						120								

<210> SEQ ID NO 604
 <211> LENGTH: 107
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: OX40mAb24 VL sequence

<400> SEQUENCE: 604

Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly
1			5						10					15	
Asp	Arg	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Gln	Asp	Ile	Ser	Asn	Tyr
		20					25						30		

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Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
    35              40              45

Tyr Tyr Thr Ser Lys Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50              55              60

Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro
    65              70              75              80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Gly Ser Ala Leu Pro Trp
    85              90              95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
    100              105

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<210> SEQ ID NO 605
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: INCAGN1949 Antibody a VH CDR1

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<400> SEQUENCE: 605

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Gly Ser Ala Met His
1          5

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<210> SEQ ID NO 606
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: INCAGN1949 Antibody a VH CDR2

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<400> SEQUENCE: 606

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Arg Ile Arg Ser Lys Ala Asn Ser Tyr Ala Thr Ala Tyr Ala Ala Ser
1          5              10              15

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Val Lys Gly

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<210> SEQ ID NO 607
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: INCAGN1949 Antibody a VH CDR3

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<400> SEQUENCE: 607

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Gly Ile Tyr Asp Ser Ser Gly Tyr Asp Tyr
1          5              10

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<210> SEQ ID NO 608
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: INCAGN1949 Antibody a VL CDR1

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<400> SEQUENCE: 608

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Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp
1          5              10              15

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<210> SEQ ID NO 609
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: INCAGN1949 Antibody a VL CDR2

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<400> SEQUENCE: 609

Leu Gly Ser Asn Arg Ala Ser
1 5

<210> SEQ ID NO 610

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: INCAGN1949 Antibody a VL CDR3

<400> SEQUENCE: 610

Met Gln Ala Leu Gln Thr Pro Leu Thr
1 5

<210> SEQ ID NO 611

<211> LENGTH: 121

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: INCAGN1949 Antibody b VH sequence

<400> SEQUENCE: 611

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Gly Ser
20 25 30
Ala Met His Trp Val Arg Gln Ala Ser Gly Lys Gly Leu Glu Trp Val
35 40 45
Gly Arg Ile Arg Ser Lys Ala Asn Ser Tyr Ala Thr Ala Tyr Ala Ala
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Ala Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Thr Ser Gly Ile Tyr Asp Ser Ser Gly Tyr Asp Tyr Trp Gly
100 105 110
Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> SEQ ID NO 612

<211> LENGTH: 112

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: INCAGN1949 Antibody b VL sequence

<400> SEQUENCE: 612

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15
Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30
Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile

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<210> SEQ ID NO 623

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<210> SEQ ID NO 700

<211> LENGTH: 167

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Tremelimumab VH sequence

<400> SEQUENCE: 700

Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser
1 5 10 15

Gly Phe Thr Phe Ser Ser Tyr Gly Met His Trp Val Arg Gln Ala Pro
20 25 30

Gly Lys Gly Leu Glu Trp Val Ala Val Ile Trp Tyr Asp Gly Ser Asn
35 40 45

Lys Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp
50 55 60

Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu
65 70 75 80

Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp Pro Arg Gly Ala Thr Leu
85 90 95

Tyr Tyr Tyr Tyr Tyr Gly Met Asp Val Trp Gly Gln Gly Thr Thr Val
100 105 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
115 120 125

Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu
130 135 140

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145 150 155 160

Ala Leu Thr Ser Gly Val His
165

<210> SEQ ID NO 701

<211> LENGTH: 139

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Tremelimumab VL sequence

<400> SEQUENCE: 701

Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys
1 5 10 15

Arg Ala Ser Gln Ser Ile Asn Ser Tyr Leu Asp Trp Tyr Gln Gln Lys
20 25 30

Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Ser Leu Gln
35 40 45

Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
50 55 60

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr
65 70 75 80

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Cys	Gln	Gln	Tyr	Tyr	Ser	Thr	Pro	Phe	Thr	Phe	Gly	Pro	Gly	Thr	Lys
			85						90					95	
Val	Glu	Ile	Lys	Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro
			100					105					110		
Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu
		115					120					125			
Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala	Lys	Val					
	130					135									

1. A method for treating cancer in an individual, the method comprising administering to the individual an effective amount of ADCxAXL and a secondary agent.

2. A first composition comprising ADCxAXL for use in a method of treating cancer in an individual, wherein the treatment comprises administration of the first composition in combination with a second composition comprising a secondary agent.

3. A first composition comprising a secondary agent for use in a method of treating a disorder in an individual, wherein the treatment comprises administration of the first composition in combination with a second composition comprising ADCxAXL.

4. Use of ADCxAXL in the manufacture of a medicament for treating cancer in an individual, wherein the medicament comprises ADCxAXL, and wherein the treatment comprises administration of the medicament in combination with a composition comprising a secondary agent.

5. Use of a secondary agent in the manufacture of a medicament for treating cancer in an individual, wherein the medicament comprises a secondary agent, and wherein the treatment comprises administration of the medicament in combination with a composition comprising ADCxAXL.

6. A kit comprising:

a first medicament comprising ADCxAXL;

a second medicament comprising a secondary agent; and, optionally,

a package insert comprising instructions for administration of the first medicament to an individual in combination with the second medicament for the treatment of cancer.

7. A kit comprising a medicament comprising ADCxAXL and a package insert comprising instructions for administration of the medicament to an individual in combination with a composition comprising a secondary agent for the treatment of cancer.

8. A kit comprising a medicament comprising a secondary agent and a package insert comprising instructions for administration of the medicament to an individual in combination with a composition comprising ADCxAXL for the treatment of cancer.

9. A pharmaceutical composition comprising ADCxAXL and a secondary agent.

10. A method of treating cancer in an individual, the method comprising administering to the individual an effective amount of the composition of claim 9.

11. The composition of claim 9 for use in a method of treating cancer in an individual.

12. The use of the composition of claim 9 in the manufacture of a medicament for treating cancer in an individual.

13. A kit comprising the composition of claim 9 and a set of instructions for administration of the medicament to an individual for the treatment of cancer.

14. The composition, method, use, or kit according to any previous claim, wherein the treatment comprises administering ADCxAXL before the secondary agent, simultaneous with the secondary agent, or after the secondary agent.

15. The composition, method, use, or kit according to any previous claim, wherein the treatment further comprises administering a chemotherapeutic agent.

16. The composition, method, use, or kit according to any previous claim, wherein the individual is human.

17. The composition, method, use, or kit according to any previous claim, wherein the individual has a disorder or has been determined to have cancer.

18. The composition, method, use, or kit according to any previous claim, wherein the individual has, or has been has been determined to have, a cancer characterised by the presence of a neoplasm comprising both AXL+ve and AXL-ve cells.

19. The composition, method, use, or kit according to any previous claim, wherein the individual has, or has been has been determined to have, a cancer characterised by the presence of a neoplasm comprising, or composed of, AXL-ve neoplastic cells.

20. The composition, method, use, or kit according to any previous claim, wherein the cancer or neoplasm is all or part of a solid tumour.

21. The composition, method, use, or kit according to any previous claim, wherein the individual has, or has been has been determined to have, a cancer which expresses AXL or AXL+ tumour-associated non-tumour cells, such as AXL+ infiltrating cells.

22. The composition, method, use, or kit according to claim 21, wherein the AXL+ infiltrating cells are dendritic cells, NK cells, or macrophages.

23. The composition, method, use, or kit according to any preceding claim, wherein the individual has, or has been has been determined to have, a cancer which expresses PD-L1.

24. The composition, method, use, or kit according to any one of the preceding claims, wherein the treatment:

a) effectively treats a broader range of disorders,

b) effectively treats resistant, refractory, or relapsed disorders,

c) has an increased response rate, and/or

d) has increased durability;

as compared to treatment with either ADCxAXL or the secondary agent alone.

25. The composition, method, use, or kit according to any one of the preceding claims, wherein the cancer is selected from the group comprising: breast cancer, lung cancer, gastric cancer, head and neck cancer, colorectal cancer, renal cancer, pancreatic cancer, uterine cancer, hepatic cancer, bladder cancer, endometrial cancer, prostate cancer, non-Hodgkin's lymphoma, NHL, AML), an immune disorder, cardiovascular disorder, thrombosis, diabetes, immune checkpoint disorder, and fibrotic disorder.

26. A composition, method, use, or kit according to any one of claims **1** to **25**, wherein the secondary agent is Fludarabine.

27. A composition, method, use, or kit according to any one of claims **1** to **25**, wherein the secondary agent is Cytarabine.

28. A composition, method, use, or kit according to any one of claims **1** to **25**, wherein the secondary agent is a PD1 antagonist.

29. A composition, method, use, or kit according to claim **28**, wherein the PD1 antagonist is selected from pembrolizumab, nivolumab, MEDI0680, PDR001 (spartalizumab), Camrelizumab, AUNP12, Pidilizumab Cemiplimab (REGN-2810), AMP-224, BGB-A317 (Tisleizumab), and BGB-108.

30. A composition, method, use, or kit according to any one of claims **1** to **25**, wherein the secondary agent is a PD-L1 antagonist.

31. A composition, method, use, or kit according to claim **30**, wherein the PD-L1 antagonist is selected from atezolizumab (Tecentriq), BMS-936559/MDX-1105, durvalumab/MEDI4736, and MSB0010718C (Avelumab).

32. A composition, method, use, or kit according to any one of claims **1** to **25**, wherein the secondary agent is a GITR (Glucocorticoid-Induced TNFR-Related protein) agonist.

33. A composition, method, use, or kit according to claim **32**, wherein the GITR (Glucocorticoid-Induced TNFR-Related protein) agonist is selected from MEDI1873, TRX518, GWN323, MK-1248, MK 4166, BMS-986156 and INCAGN1876.

34. A composition, method, use, or kit according to any one of claims **1** to **25**, wherein the secondary agent is a OX40 agonist.

35. A composition, method, use, or kit according to claim **34**, wherein the OX40 agonist is selected from MEDI0562, MEDI6383, MOXR0916, RG7888, OX40mAb24, INCAGN1949, GSK3174998, and PF-04518600.

36. A composition, method, use, or kit according to any one of claims **1** to **25**, wherein the secondary agent is a CTLA-4 antagonist.

37. A composition, method, use, or kit according to claim **36**, wherein the CTLA-4 antagonist is selected from ipilimumab and Tremelimumab.

38. A composition, method, use, or kit according to any one of claims **1** to **25**, wherein the secondary agent is a hypomethylating agent.

39. A composition, method, use, or kit according to claim **38**, wherein the hypomethylating agent is azacitidine.

40. A composition, method, use, or kit according to claim **38**, wherein the hypomethylating agent is decitabine.

41. A composition, method, use, or kit according to any one of claims **1** to **25**, wherein the secondary agent is a PARP inhibitor (PARPi).

42. A composition, method, use, or kit according to claim **41**, wherein the PARPi is selected from Olaparib, CEP-9722, BMN-673/talazoparib, Rucaparib, Iniparib/SAR24-550/BSI-201, Veliparib (ABT-888), Niraparib/MK-4827, BGB-290, 3-aminobenzamide, and E7016.

43. A composition, method, use, or kit according to any one of claims **1** to **25**, wherein the secondary agent is an agent that upregulates HER2 expression.

44. A composition, method, use, or kit according to claim **41**, wherein the agent that upregulates HER2 expression is selected from gemcitabine and tamoxifen.

45. A composition, method, use, or kit according to any one of claims **1** to **25**, wherein the secondary agent is an AXL-kinase inhibitor (AXLi).

46. A composition, method, use, or kit according to claim **45**, wherein the AXLi is selected from BGB324 (bemcentinib), TP0903, Gilteritinib (ASP2215), Cabozantinib (XL184), SG17079, Merestinib, amuvatinib (MP-470), bosutinib (SKI-606), MGCD265, and foretinib (GSK1363089/XL880).

47. A composition, method, use, or kit according to any one of claims **1** to **25**, wherein the secondary agent is a BRAF inhibitor (BRAFi).

48. A composition, method, use, or kit according to claim **47**, wherein the BRAFi is selected from vemurafenib, PLX4720, dabrafenib, Sorafenib, Encorafenib, and GDC0879.

49. A composition, method, use, or kit according to any one of claims **1** to **25**, wherein the secondary agent is a MEK inhibitor (MEKi).

50. A composition, method, use, or kit according to claim **49**, wherein the AXLi is selected from Trametinib, Cobimetinib, Binimetinib, Selumetinib, PD-325901, CI-1040, PD035901, U0126, and TAK-733.

* * * * *