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(54) Title: COMBINATION THERAPY WITH AN ANTI-AXL ANTIBODY-DRUG CONJUGATE

(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.

COMBINATION THERAPY WITH AN ANTI-AXL ANTIBODY-DRUG CONJUGATE

CROSS-REFERENCE TO RELATED APPLICATIONS

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 April 2017.

FIELD

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.

BACKGROUND**Antibody Therapy**

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

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 JP, *et al* (1995) *J Bioi Chem.* 270 (2): 551-557).

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: US5468634; US6087144; US5538861; US5968508; US6211142; US6235769; WO1999/49894; WO2000/76309; WO2001/16181 and WO2001/32926.

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.

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.

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).

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, April 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.

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), March 15, 2008).

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

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.

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

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.

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.

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).

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.

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

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
10 with AXL+ve non-neoplastic cells.

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

15 "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.

Solid tumors may be neoplasms, including non-haematological cancers, comprising or composed of AXL+ve neoplastic cells. Solid tumors may be neoplasms, including non-
20 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).

For example, the solid tumour may be a tumour with high levels of infiltrating AXL+ve
25 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.

30 The ADC may be anti-AXL-ADC, such as ADCxAXL described herein.

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,
35 a PARP inhibitor (PARPi), an agent that upregulates HER2 expression, an AXL inhibitor (AXLi), a BRAF inhibitor (BRAFi), or a MEK inhibitor (MEKi).

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
40 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.

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

45

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.

5

10 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.

15 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.

20 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).

25 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),
30 systemic sclerosis, cardiac fibrosis, non-alcoholic steatohepatitis (NASH), other types of liver fibrosis, primary biliary cirrhosis, renal fibrosis, cancer, and atherosclerosis.

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

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.

40 The target neoplasm or neoplastic cells may be all or part of a solid tumour.

"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.

45

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).

The ADC may be anti-AXL-ADC, such as ADCxAXL described herein.

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).

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.

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

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.

In a further aspect, the disclosure provides the 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 secondary agent.

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.

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).

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.

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

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.

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

“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.

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The ADC may be anti-AXL-ADC, such as ADCxAXL described herein.

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).

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.

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

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.

Another aspect of the disclosure provides a kit comprising:

5 a first medicament comprising an ADC;
 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 a
disorder.

10 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.

15 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.

20 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
25 lymphomas (e.g., non-Hodgkin's lymphoma, NHL) and leukemia (particularly acute
myeloid leukemia, AML).

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,
30 cardiovascular disorders, thrombosis, diabetes, immune checkpoint disorders, or fibrotic
disorders (fibrosis) such as strabmisus, scleroderma, keloid, Nephrogenic systemic
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with AXL+ve non-neoplastic cells.

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10 The ADC may be anti-AXL-ADC, such as ADCxAXL described herein.

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

30 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.

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

40 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.

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.

45 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.

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.

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45

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).

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.

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

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

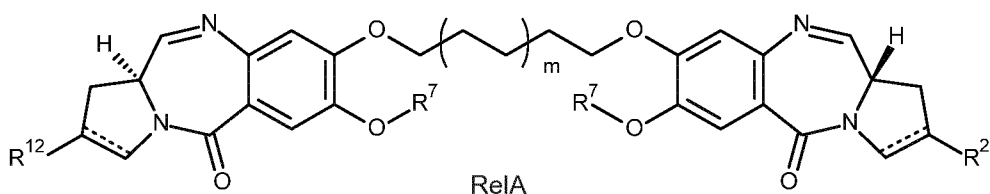
DETAILED DESCRIPTION

Antibody Drug Conjugates (ADCs)

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

The ADC of present disclosure provides a PBD dimer with a linker connected through the N10 position on one of the PBD moieties conjugated to an antibody as defined below.

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:



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 targetted 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.

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.

- 5 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

- 10 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.

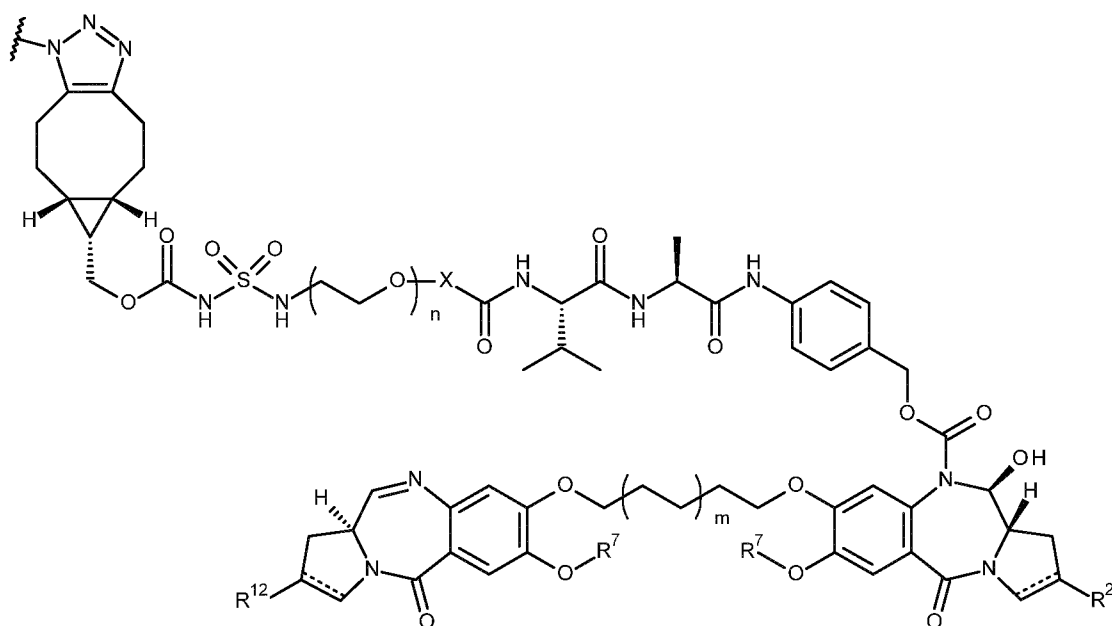
- 15 The ADC may comprise a conjugate of formula (I):



wherein:

Ab is an antibody that binds to AXL;

DL is



- 20 wherein:

X is selected from the group comprising: a single bond, $-\text{CH}_2-$ and $-\text{C}_2\text{H}_4-$;

n is from 1 to 8;

m is 0 or 1;

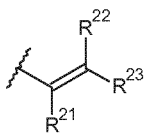
- 25 R^7 is either methyl or phenyl;

when there is a double bond between C2 and C3, R^2 is selected the group consisting of:

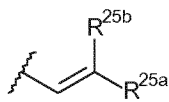
(ia) C_{5-10} aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, carboxy, ester, C_{1-7} alkyl, C_{3-7} heterocyclyl and bis-oxy- C_{1-3} alkylene;

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

(ic) C₃₋₆ saturated cycloalkyl;



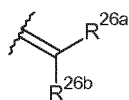
(id) , wherein each of R²¹, R²² and R²³ are independently selected from H, C₁₋₃ saturated alkyl, C₂₋₃ alkenyl, C₂₋₃ alkynyl and cyclopropyl, where the total number of carbon atoms in the R¹² group is no more than 5;



5 (ie) , 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



10 (if) , 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

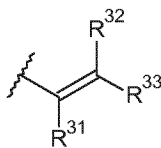


15 , where R^{26a} and R^{26b} are independently selected from H, F, C₁₋₄ saturated alkyl, C₂₋₃ alkenyl, which alkyl and alkenyl groups are optionally substituted by a group selected from C₁₋₄ alkyl amido and C₁₋₄ alkyl ester; or, when one of R^{26a} and R^{26b} is H, the other is selected from nitrile and a C₁₋₄ alkyl ester; when there is a double bond between C2' and C3', R¹² is selected the group consisting of:

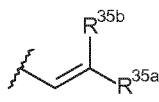
20 (ia) C₅₋₁₀ aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, carboxy, ester, C₁₋₇ alkyl, C₃₋₇ heterocyclyl and bis-oxy-C₁₋₃ alkylene;

(ib) C₁₋₅ saturated aliphatic alkyl;


(ic) C₃₋₆ saturated cycloalkyl;

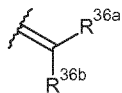


25 (id) , wherein each of R³¹, R³² and R³³ are independently selected from H, C₁₋₃ saturated alkyl, C₂₋₃ alkenyl, C₂₋₃ alkynyl and cyclopropyl, where the total number of carbon atoms in the R¹² group is no more than 5;



(ie) , 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

(if)  , where R³⁴ 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.

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).

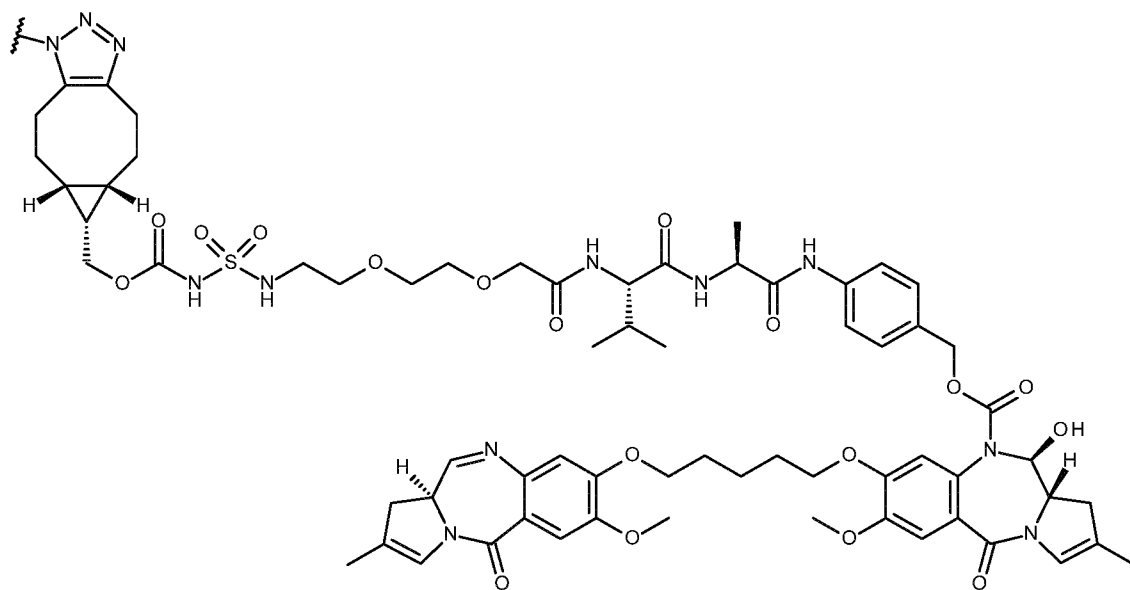
The term anti-AXL-ADC may include any embodiment described in GB1702029.8. In particular, in preferred embodiments the ADC may have the chemical structure:



wherein:

Ab is an antibody that binds to AXL;

DL is:



wherein the Ab is an anti-AXL antibody.

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.

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

- 5 (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
(b) a light chain having the sequence according to SEQ ID NO. 4.

10 DL embodiments

X

In some embodiments, X is a single bond.

In other embodiments, X is -CH₂-.

In further embodiments, X is -C₂H₄-.

15

In some embodiments, n is 1 to 4.

In some of these embodiments, n is 1.

In other of these embodiments, n is 2.

20

In further of these embodiments, n is 4.

*R*⁷

In one embodiment, *R*⁷ is methyl.

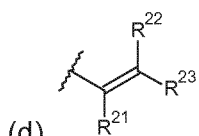
In another embodiment, *R*⁷ is phenyl.

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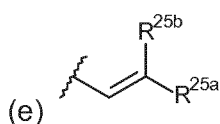
*R*²

When there is a double bond present between C2 and C3, *R*² is selected from:


- (a) C₅₋₁₀ aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, C₁₋₇ alkyl, C₃₋₇ heterocyclyl and bis-oxy-C₁₋₃ alkylene;
30 (b) C₁₋₅ saturated aliphatic alkyl;
(c) C₃₋₆ saturated cycloalkyl;



- (d) , wherein each of *R*²¹, *R*²² and *R*²³ 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 *R*² group is no more than 5;
35



- (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 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.

- 5 When R² is a C₅₋₁₀ aryl group, it may be a C₅₋₇ aryl group. A C₅₋₇ aryl group may be a phenyl group or a C₅₋₇ heteroaryl group, for example furanyl, thiophenyl and pyridyl. In some embodiments, R² is preferably phenyl. In other embodiments, R¹² is preferably thiophenyl, for example, thiophen-2-yl and thiophen-3-yl.
- 10 When R² is a C₅₋₁₀ aryl group, it may be a C₈₋₁₀ 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
- 15 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.

20 When R² is a C₅₋₁₀ 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.

25 Where R² is C₅₋₇ 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 C₅₋₇ aryl group is phenyl, the substituent is preferably in the meta- or para- positions, and more preferably is in the para- position.

30 Where R² is a C₈₋₁₀ 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).

R² substituents, when R² is a C₅₋₁₀ aryl group

35 If a substituent on R² when R² is a C₅₋₁₀ aryl group is halo, it is preferably F or Cl, more preferably Cl.

40 If a substituent on R² when R² is a C₅₋₁₀ aryl group is ether, it may in some embodiments be an alkoxy group, for example, a C₁₋₇ alkoxy group (e.g. methoxy, ethoxy) or it may in some embodiments be a C₅₋₇ aryloxy group (e.g. phenoxy, pyridyloxy, furanyloxy). The alkoxy group may itself be further substituted, for example by an amino group (e.g. dimethylamino).

If a substituent on R² when R² is a C₅₋₁₀ aryl group is C₁₋₇ alkyl, it may preferably be a C₁₋₄ alkyl group (e.g. methyl, ethyl, propyl, butyl).

If a substituent on R² when R² is a C₅₋₁₀ aryl group is C₃₋₇ heterocyclyl, it may in some embodiments be C₆ 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 C₁₋₄ alkyl groups. If the C₆ nitrogen containing heterocyclyl group is piperazinyl, the said further substituent may be on the second nitrogen ring atom.

If a substituent on R² when R² is a C₅₋₁₀ aryl group is bis-oxy-C₁₋₃ alkylene, this is preferably bis-oxy-methylene or bis-oxy-ethylene.

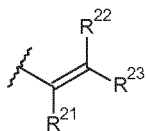
If a substituent on R² when R² is a C₅₋₁₀ aryl group is ester, this is preferably methyl ester or ethyl ester.

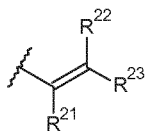
Particularly preferred substituents when R² is a C₅₋₁₀ aryl group include methoxy, ethoxy, fluoro, chloro, cyano, bis-oxy-methylene, methyl-piperazinyl, morpholino and methyl-thiophenyl. Other particularly preferred substituent for R² are dimethylaminopropoxy and carboxy.

Particularly preferred substituted R² groups when R² is a C₅₋₁₀ 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 R² group is 4-nitrophenyl. R² groups of particular interest include 4-(4-methylpiperazin-1-yl)phenyl and 3,4-bisoxymethylene-phenyl.

When R² is C₁₋₅ 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.

When R² is C₃₋₆ saturated cycloalkyl, it may be cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl. In some embodiments, it may be cyclopropyl.



When R² is , 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 R² group is no more than 5. In some embodiments, the total number of carbon atoms in the R² group is no more than 4 or no more than 3.

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.

5 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.

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.

10 In some embodiments, R^{21} is H.

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

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

15

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

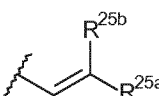
In some embodiments, R^{21} and R^{23} are H.

20


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

A R^2 group of particular interest is: .

25

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.

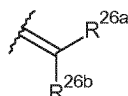
30

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.

35

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.

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

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 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.

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

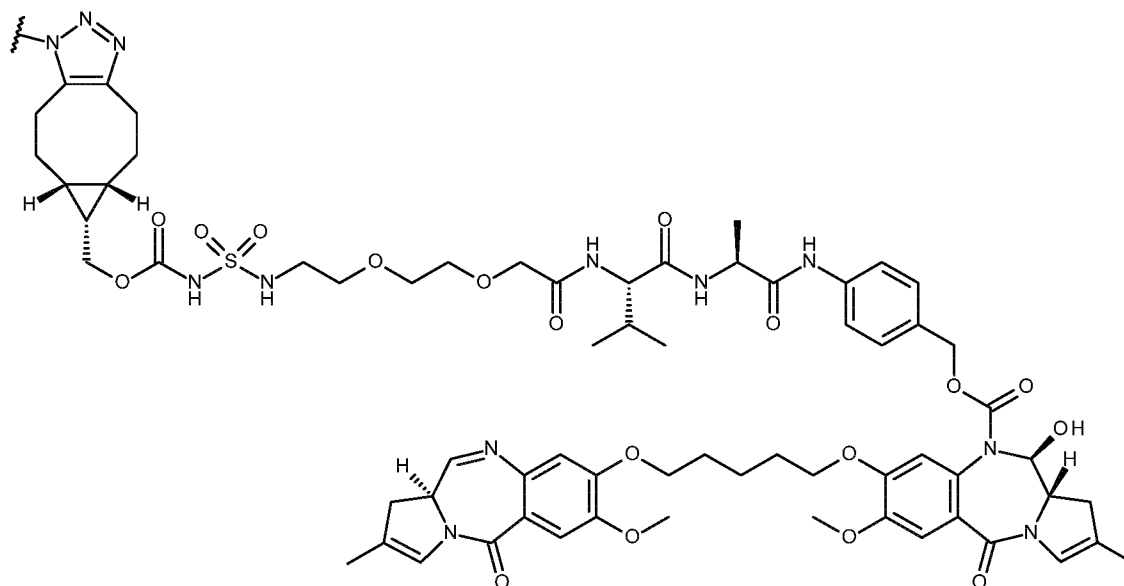
In other embodiments, it is preferred that R^{26a} and R^{26b} are both methyl.

In further embodiments, it is preferred that one of R^{26a} and R^{26b} is H, and the other is selected from C₁₋₄ saturated alkyl, C₂₋₃ 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.

R¹²

The above preferences for R² apply equally to R¹².

In one preferred embodiment of the invention, DL is



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.

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



wherein:

Ab is an antibody comprising:

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

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

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

[GlcNAc] is a N-acytel glucsamine residue; and
DL is the drug-linker described immediately above.

The antibody component of the anti-AXL ADC

5 In one aspect the antibody is an antibody that binds to AXL.

1H12

10 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 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
15 comprises a VH domain having the sequence according to SEQ ID NO. 1.

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
20 sequence according to SEQ ID NO. 2.

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.
25

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

35 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.

40 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.

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.

5 In some embodiments the antibody is a fully human monoclonal IgG1 antibody, preferably IgG1, κ .

10 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.

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

15 5F11

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 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.

20 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.

30 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 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.

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

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.

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.

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.

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

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

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

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

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.

"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.

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

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).

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

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 V_H is then combined with a V_L library to generate a completely human V_HxV_L combination. The method is described in Nature Biotechnology (N.Y.) 12, (1994) 899-903.

Composite antibodies

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 V_H and V_L 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

5 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
10 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
15 amphipathicity, Rothbard motifs, and cleavage sites for cathepsin B and other processing enzymes.

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
20 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.

25 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

This method involves:

(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;

35 (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;

40 (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);

(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;

(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);

(f) constructing a three-dimensional model of the variable region of the non-human antibody resulting from the substituting specified in step (e);

(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

(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

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

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

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

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

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

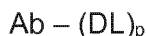
- (3) enzymatic transfer to the core GlcNAc of a N-acetylgalactose residue harboring an azide group for conjugation to the drug linker.

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

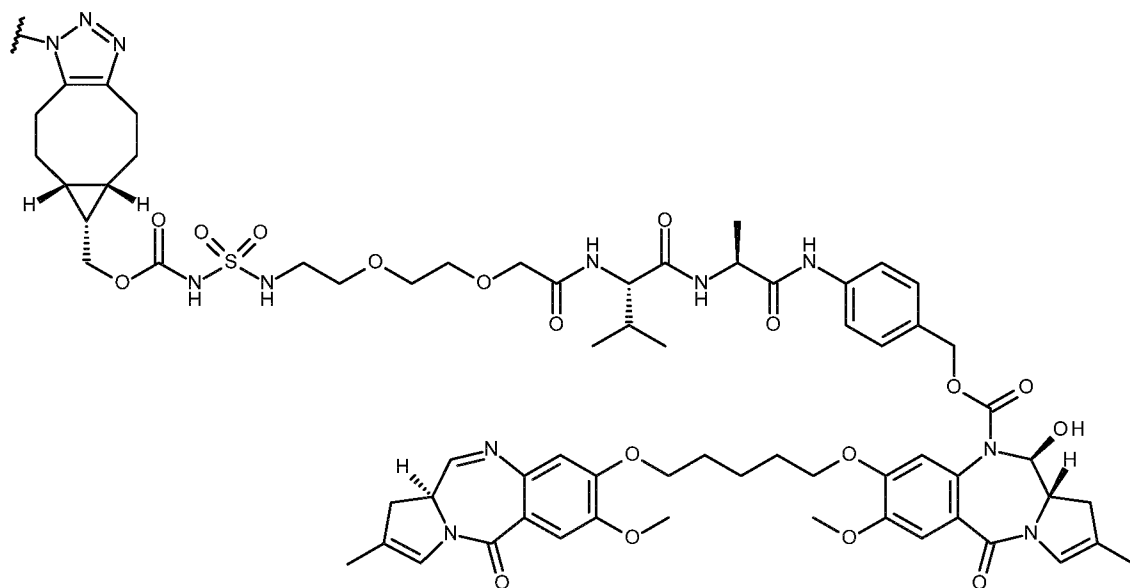
ADCxAXL is an antibody drug conjugate composed of a humanized antibody against human AXL attached to a pyrrolobenzodiazepine (PBD) warhead via a cleavable linker. The mechanism of action of ADCxAXL depends on AXL binding. The AXL specific antibody targets the antibody drug conjugate (ADC) to cells expressing 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).

It has the chemical structure:



wherein:

DL is:



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

- (a) a heavy chain having the sequence according to SEQ ID NO. 3;
- (b) a light chain having the sequence according to SEQ ID NO. 4.

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 ...SPG**K**), with the terminal K being optionally removed post-translationally to improve the homogeneity of the final therapeutic ADC product.

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

The "first target protein" (FTP) as used herein may be AXL.

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, 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 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} .

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, 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 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} .

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: March 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

The phrase "optionally substituted" as used herein, pertains to a parent group which may be unsubstituted or which may be substituted.

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.

Examples of substituents are described in more detail below.

5

C₁₋₁₂ alkyl: The term "C₁₋₁₂ 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 "C₁₋₄ 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.

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Examples of saturated alkyl groups include, but are not limited to, methyl (C₁), ethyl (C₂), propyl (C₃), butyl (C₄), pentyl (C₅), hexyl (C₆) and heptyl (C₇).

20

Examples of saturated linear alkyl groups include, but are not limited to, methyl (C₁), ethyl (C₂), n-propyl (C₃), n-butyl (C₄), n-pentyl (amyl) (C₅), n-hexyl (C₆) and n-heptyl (C₇).

Examples of saturated branched alkyl groups include iso-propyl (C₃), iso-butyl (C₄), sec-butyl (C₄), tert-butyl (C₄), iso-pentyl (C₅), and neo-pentyl (C₅).

25

C₂₋₁₂ Alkenyl: The term "C₂₋₁₂ alkenyl" as used herein, pertains to an alkyl group having one or more carbon-carbon double bonds.

30

Examples of unsaturated alkenyl groups include, but are not limited to, ethenyl (vinyl, -CH=CH₂), 1-propenyl (-CH=CH-CH₃), 2-propenyl (allyl, -CH-CH=CH₂), isopropenyl (1-methylvinyl, -C(CH₃)=CH₂), butenyl (C₄), pentenyl (C₅), and hexenyl (C₆).

C₂₋₁₂ alkynyl: The term "C₂₋₁₂ alkynyl" as used herein, pertains to an alkyl group having one or more carbon-carbon triple bonds.

35

Examples of unsaturated alkynyl groups include, but are not limited to, ethynyl (-C≡CH) and 2-propynyl (propargyl, -CH₂-C≡CH).

40

C₃₋₁₂ cycloalkyl: The term "C₃₋₁₂ 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.

Examples of cycloalkyl groups include, but are not limited to, those derived from:
saturated monocyclic hydrocarbon compounds:

45

cyclopropane (C₃), cyclobutane (C₄), cyclopentane (C₅), cyclohexane (C₆), cycloheptane (C₇), methylcyclopropane (C₄), dimethylcyclopropane (C₅), methylcyclobutane (C₅),

dimethylcyclobutane (C₆), methylcyclopentane (C₆), dimethylcyclopentane (C₇) and methylcyclohexane (C₇);

unsaturated monocyclic hydrocarbon compounds:

cyclopropene (C₃), cyclobutene (C₄), cyclopentene (C₅), cyclohexene (C₆), methylcyclopropene (C₄), dimethylcyclopropene (C₅), methylcyclobutene (C₅), dimethylcyclobutene (C₆), methylcyclopentene (C₆), dimethylcyclopentene (C₇) and methylcyclohexene (C₇); and

saturated polycyclic hydrocarbon compounds:

norcarane (C₇), norpinane (C₇), norbornane (C₇).

C₃₋₂₀ heterocyclyl: The term “C₃₋₂₀ 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.

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

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

N₁: aziridine (C₃), azetidine (C₄), pyrrolidine (tetrahydropyrrole) (C₅), pyrroline (e.g., 3-pyrroline, 2,5-dihydropyrrole) (C₅), 2H-pyrrole or 3H-pyrrole (isopyrrole, isoazole) (C₅), piperidine (C₆), dihydropyridine (C₆), tetrahydropyridine (C₆), azepine (C₇);

O₁: oxirane (C₃), oxetane (C₄), oxolane (tetrahydrofuran) (C₅), oxole (dihydrofuran) (C₅), oxane (tetrahydropyran) (C₆), dihydropyran (C₆), pyran (C₆), oxepin (C₇);

S₁: thiirane (C₃), thietane (C₄), thiolane (tetrahydrothiophene) (C₅), thiane (tetrahydrothiopyran) (C₆), thiepane (C₇);

O₂: dioxolane (C₅), dioxane (C₆), and dioxepane (C₇);

O₃: trioxane (C₆);

N₂: imidazolidine (C₅), pyrazolidine (diazolidine) (C₅), imidazoline (C₅), pyrazoline (dihydropyrazole) (C₅), piperazine (C₆);

N₁O₁: tetrahydrooxazole (C₅), dihydrooxazole (C₅), tetrahydroisoxazole (C₅), dihydroisoxazole (C₅), morpholine (C₆), tetrahydrooxazine (C₆), dihydrooxazine (C₆), oxazine (C₆);

N₁S₁: thiazoline (C₅), thiazolidine (C₅), thiomorpholine (C₆);

N₂O₁: oxadiazine (C₆);

O₁S₁: oxathiole (C₅) and oxathiane (thioxane) (C₆); and,

N₁O₁S₁: oxathiazine (C₆).

Examples of substituted monocyclic heterocyclyl groups include those derived from saccharides, in cyclic form, for example, furanoses (C₅), such as arabinofuranose, lyxofuranose, ribofuranose, and xylofuranse, and pyranoses (C₆), such as allopyranose,

altropyranose, glucopyranose, mannopyranose, gulopyranose, idopyranose, galactopyranose, and talopyranose.

C₅₋₂₀ aryl: The term "C₅₋₂₀ 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 "C₅₋₇ 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 "C₅₋₁₀ 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.

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.

The ring atoms may be all carbon atoms, as in "carboaryl groups".

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).

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).

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).

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

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);

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);

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

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

C₁₄ (with 3 fused rings) derived from acridine (N₁), xanthene (O₁), thioxanthene (S₁), oxanthrene (O₂), phenoxathiin (O₁S₁), phenazine (N₂), phenoxazine (N₁O₁), phenothiazine (N₁S₁), thianthrene (S₂), phenanthridine (N₁), phenanthroline (N₂), phenazine (N₂).

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.

Halo: -F, -Cl, -Br, and -I.

Hydroxy: -OH.

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 heterocyclyloxy group), or a C₅₋₂₀ aryl group (also referred to as a C₅₋₂₀ aryloxy group), preferably a C₁₋₇ alkyl group.

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).

Carboxy (carboxylic acid): -C(=O)OH.

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.

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 ($-^+NR^1R^2R^3$). Examples of amino groups include, but are not limited to, $-NH_2$, $-NHCH_3$, $-NHC(CH_3)_2$, $-N(CH_3)_2$, $-N(CH_2CH_3)_2$, and $-NHPh$. Examples of cyclic amino groups include, but are not limited to, aziridino, azetidino, pyrrolidino, piperidino, piperazino, morpholino, and thiomorpholino.

Amido (carbamoyl, carbamyl, aminocarbonyl, carboxamide): $-C(=O)NR^1R^2$, wherein R^1 and R^2 are independently amino substituents, as defined for amino groups. Examples of amido groups include, but are not limited to, $-C(=O)NH_2$, $-C(=O)NHCH_3$, $-C(=O)N(CH_3)_2$, $-C(=O)NHCH_2CH_3$, and $-C(=O)N(CH_2CH_3)_2$, as well as amido groups in which R^1 and R^2 , together with the nitrogen atom to which they are attached, form a heterocyclic structure as in, for example, piperidinocarbonyl, morpholinocarbonyl, thiomorpholinocarbonyl, and piperazinocarbonyl.

Nitro: $-NO_2$.

Azido: $-N_3$.

Cyano (nitrile, carbonitrile): $-CN$.

Drug loading

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

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.

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.

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.

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.

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.

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.

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

Includes Other Forms

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 (-COOH) also includes the anionic (carboxylate) form (-COO⁻), a salt or solvate thereof, as well as conventional protected forms. Similarly, a reference to an amino group includes the protonated form (-N⁺HR¹R²), 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 (-O⁻), a salt or solvate thereof, as well as conventional protected forms.

Salts

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).

For example, if the compound is anionic, or has a functional group which may be anionic (e.g. -COOH may be -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 Ca²⁺ and Mg²⁺, and other cations such as Al³⁺. Examples of suitable organic cations include, but are not limited to, ammonium ion (i.e. NH₄⁺) and substituted ammonium ions (e.g. NH₃R⁺, NH₂R₂⁺, NHR₃⁺, NR₄⁺). 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 $N(CH_3)_4^+$.

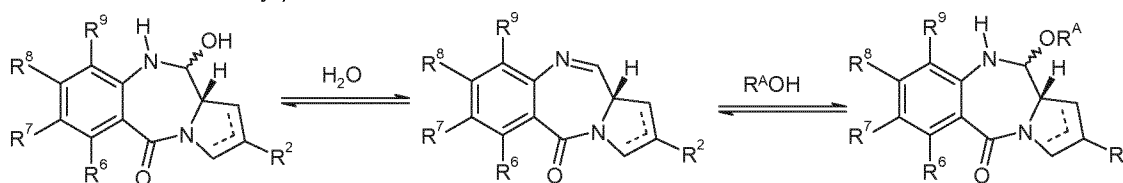
If the compound is cationic, or has a functional group which may be cationic (e.g. $-NH_2$ may be $-NH_3^+$), then a salt may be formed with a suitable anion. Examples of suitable inorganic anions include, but are not limited to, those derived from the following inorganic acids: hydrochloric, hydrobromic, hydroiodic, sulfuric, sulfurous, nitric, nitrous, phosphoric, and phosphorous.

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, ethanedithionyl, ethanesulfonic, fumaric, glucelonic, 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

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.

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 ($R^A OH$, where R^A is C_{1-4} alkyl):



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.

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

Isomers

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").

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.

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.

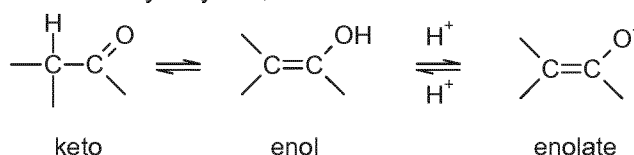
"Diastereomer" refers to a stereoisomer with two or more centers of chirality and whose molecules are not mirror images of one another. Diastereomers have different physical properties, e.g. melting points, boiling points, spectral properties, and reactivities. Mixtures of diastereomers may separate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

"Enantiomers" refer to two stereoisomers of a compound which are non-superimposable mirror images of one another.

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.

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, -OCH₃, is not to be construed as a reference to its structural isomer, a hydroxymethyl group, -CH₂OH. 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. C₁₋₇ alkyl includes n-propyl and iso-propyl; butyl includes n-, iso-, sec-, and tert-butyl; methoxyphenyl includes ortho-, meta-, and para-methoxyphenyl).

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.



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.

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 ¹²C, ¹³C, and ¹⁴C; O may be in any isotopic form, including ¹⁶O and ¹⁸O; and the like.

Examples of isotopes that can be incorporated into compounds of the invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, fluorine, and chlorine, such as, but not limited to ²H (deuterium, D), ³H (tritium), ¹¹C, ¹³C, ¹⁴C, ¹⁵N, ¹⁸F, ³¹P, ³²P, ³⁵S, ³⁶Cl, and ¹²⁵I. Various isotopically labeled compounds of the present invention, for example those into which radioactive isotopes such as ³H, ¹³C, and ¹⁴C are incorporated. Such isotopically labelled 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 labelled 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 ¹⁸F 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.

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

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.

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.

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

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 KS Peggs et al.2009, Clinical and Experimental Immunology, 157: 9–19 [doi:10.1111/j.1365-2249.2009.03912.x]; DM Pardoll 2012 [doi:10.1038/nrc3239]).

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.

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.

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.

The secondary agent may be:

- (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;
- (b) a PD-L1 antagonist, such as atezolizumab (Tecentriq), BMS-936559/MDX-1105, durvalumab/MEDI4736, or MSB0010718C (Avelumab);
- (c) a GITR (Glucocorticoid-Induced INFR-Related protein) agonist, such as MEDI1873, TRX518, GWN323, MK-1248, MK-4166, BMS-986156 or INCAGN1876;
- (d) an OX40 agonist, such as MEDI0562, MEDI6383, MOXR0916, RG7888, OX40mAb24, INCAGN1949, GSK3174998, or PF-04518600;
- (e) a CTLA-4 antagonist, such as ipilimumab (brand name Yervoy) or Tremelimumab (Originally developed by Pfizer, now Medimmune);
- (f) Fludarabine or Cytarabine;
- (g) a hypomethylating agent, such as cytidine analogs – for example, 5-azacytidine (azacitidine) and 5-aza-2'-deoxycytidine (decitabine); or
- (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;
- (i) an agent that upregulates HER2 expression, such as gemcitabine and tamoxifen;
- (j) an AXL-kinase inhibitor (AXLi) such as BGB324 (bemcentinib), TP0903, Gilteritinib (ASP2215), Cabozantinib (XL184), SGI7079, Merestinib, amuvatinib (MP-470), bosutinib (SKI-606), MGCD265, and foretinib (GSK1363089/XL880);

- (k) a BRAF inhibitor (BRAFi), such as vemurafenib, PLX4720, dabrafenib, Sorafenib, Encorafenib, and GDC0879; or
- (l) a MEK inhibitor (MEKi), such as Trametinib, Cobimetinib, Binimetinib, Selumetinib, PD-325901, CI-1040, PD035901, U0126 and TAK-733.

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Each of these classes of secondary agent is described in more detail below.

PD1 antagonists

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Programmed death receptor 1 (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.

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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: 3011-6). 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).

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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; Wintterle, et al.,

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(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).

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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).

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

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.

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.

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.

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.

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

- a) a PD1 antagonist which inhibits the binding of PD1 to its ligand binding partners.
- b) a PD1 antagonist which inhibits the binding of PD1 to PD- L1.
- c) a PD1 antagonist which inhibits the binding of PD-1 to PDL2.
- d) a PD1 antagonist which inhibits the binding of PD-1 to both PDL1 and PDL2.
- e) a PD1 antagonist of parts (a) to (d) which is an antibody.

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

- a) pembrolizumab (brand name Keytruda)
 - i. CAS Number → 1374853-91-4
(see <http://www.cas.org/content/chemical-substances/faqs>)
 - ii. NCBI Pubchem reference → 254741536
(see <https://pubchem.ncbi.nlm.nih.gov/>)
 - iii. DrugBank reference → DB09037
(see <https://www.drugbank.ca/>)
 - iv. Unique Ingredient Identifier (UNII) → DPT003T46P
(see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

- b) nivolumab (brand name Opdivo)
 - i. CAS Number → 946414-94-4
(see <http://www.cas.org/content/chemical-substances/faqs>)
 - ii. DrugBank reference → DB09035
(see <https://www.drugbank.ca/>)

- c) MEDI0680 (formerly AMP-514)
 - As described in WO2014/055648, WO2015/042246, WO2016/127052, WO2017/004016, WO2012/145493, US8609089, WO2016/007235, WO2016/011160; Int. J. Mol. Sci. 2016 Jul; 17(7): 1151, doi: 10.3390/ijms17071151; and Drug Discov Today, 2015 Sep;20(9):1127-34. doi: 10.1016/j.drudis.2015.07.003.
 - See also clinical trials NCT02271945 and NCT02013804 at <https://clinicaltrials.gov/ct2/home>

- d) PDR001 (spartalizumab)
 - i. CAS Number → 1935694-88-4

- (see <http://www.cas.org/content/chemical-substances/faqs>)
- ii. Unique Ingredient Identifier (UNII) → QOG25L6Z8Z
(see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

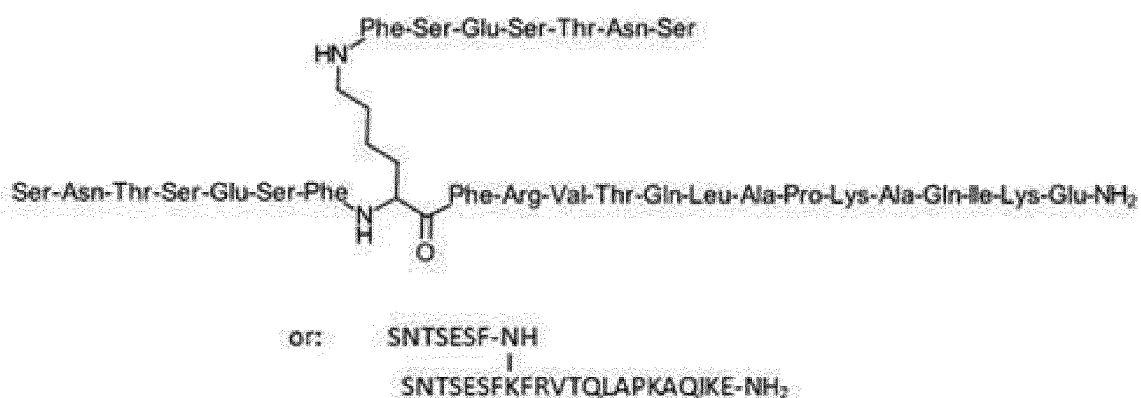
- As described in WO2016/007235 and WO2016/011160
- NCI thesaurus code → C121625
(see <https://ncit.nci.nih.gov/ncitbrowser/>)

e) Camrelizumab [INCSHR-1210] (Incyte)

- i. CAS Number → 1798286-48-2
(see <http://www.cas.org/content/chemical-substances/faqs>)
- ii. Unique Ingredient Identifier (UNII) → 73096E137E
(see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

f) AUNP12 (peptide) (Aurigene/PierreFabre)

- 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.
- ii. CAS Number → 1353563-85-5
(see <http://www.cas.org/content/chemical-substances/faqs>)



g) Pidilizumab (CT-01 1)

- i. CAS Number → 1036730-42-3
(see <http://www.cas.org/content/chemical-substances/faqs>)
- ii. Unique Ingredient Identifier (UNII) → B932PAQ1BQ
(see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

h) Cemiplimab (formerly REGN-2810, SAR-439684)

- i. CAS Number → 1801342-60-8

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

- ii. Unique Ingredient Identifier (UNII) → 6QVL057INT

(see

<http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

- As described in WO2016/007235

- NCI thesaurus code → C121540

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

- i) BGB-A317 (Tislelizumab)

- i. As described in US 9,834,606 B2

- ii. See clinical trial NCT03209973 (<https://clinicaltrials.gov/>)

- iii. NCI thesaurus code C121775

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

- j) BGB-108

- See WO2016/000619 and US8735553

- k) AMP-224

see clinical trial NCT02298946, <https://clinicaltrials.gov/ct2/home>

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

"PD-L1 antagonist" means any chemical compound or biological molecule that stimulates an immune reaction through inhibition of PD-L1 signalling.

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.

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.

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).

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.

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.

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

- (a) are PD-L1 binding antagonists;
- (b) inhibit the binding of PD-L1 to PD1;
- (c) inhibit the binding of PD-L1 to B7-1;
- (d) inhibit the binding of PD-L1 to both PD1 and B7-1;
- (e) are anti-PD-L1 antibodies.

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

- a) atezolizumab (MPDL3280A, brand name Tecentriq)
 - i. CAS Number → 1380723-44-3
(see <http://www.cas.org/content/chemical-substances/faqs>)
 - ii. DrugBank reference → DB11595

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

- iii. Unique Ingredient Identifier (UNII) → 52CMI0WC3Y

(see

<http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

b) BMS-936559 / MDX-1105

- I. CAS Number → 1422185-22-5

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

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

- III. See WO2007/005874 for antibody sequences, in particular the:

i. Antibody having:

- a. VH CDR1 = DYGFS
- b. VH CDR2 = WITAYNGNTNYAQKLQG
- c. VH CDR3 = DYFYGMDV
- d. VL CDR1 = RASQSVSSYLV
- e. VL CDR2 = DASNRAT
- f. VL CDR3 = QQRSNWPRT

ii. Antibody having:

- a. VH CDR1 = TYAIS
- b. VH CDR2 = GIPIFGKAHYAQKFQG
- c. VH CDR3 = KFHFVSGSPFGMDV
- d. VL CDR1 = RASQSVSSYLA
- e. VL CDR2 = DASNRAT
- f. VL CDR3 = QQRSNWPT

iii. Antibody having:

- a. VH CDR1 = SYDVH
- b. VH CDR2 = WLHADTGITKFSQKFQG
- c. VH CDR3 = ERIQLWFDY
- d. VL CDR1 = RASQGISSWLA
- e. VL CDR2 = AASSLQS
- f. VL CDR3 = QQYNSYPYT

c) durvalumab/MEDI4736

- i. CAS Number → 1428935-60-7

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

- ii. Unique Ingredient Identifier (UNII) → 28X28X9OKV

(see

<http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

- iii. VH sequence

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWMSWVRQAPGKGLEW
 VANIKQDGSEKYYVDSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYC
 AREGGWFGELAFDYWGQGTLVTVSS

- iv. VL sequence

EIVLTQSPGTL~~SL~~SPGERATL~~SC~~RASQ~~RV~~SSSYLA~~WY~~QQKPGQAP~~RLLI~~Y
 DASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC~~QQY~~GSLPWTF
 GGGTKVEIK

- 5 d) Avelumab / MSB0010718C
- i. CAS Number → 1537032-82-8
 (see <http://www.cas.org/content/chemical-substances/faqs>)
- 10 ii. Unique Ingredient Identifier (UNII) → KXG2PJ551I
 (see
<http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

15 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

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).

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 21:17-129). 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).

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.

"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.

- 5 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.
- 20 Combining an ADC, which targets a first target protein (FTP) positive lymphomas and leukemias with GITR agonists is advantageous, because on the one hand the ADC will directly kill the FTP positive tumor cells, while on the other hand the GITR 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 GITR agonist.
- 30 GITR (Glucocorticoid-Induced INFR-Related protein) is expressed transiently on activated T-cells and expressed constitutively at high levels on T-reg with further induction following activation. GITR ligation via its ligand GITRL 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.
- 35 Therefore it will be beneficial to target a FTP(+) tumor with the ADC, causing the antigenic cell death, while the GITR agonist induces a stronger, durable immune response.

40 Specific GITR agonists suitable for use as secondary agents in the present disclosure include:

- a) MEDI1873, a GITR ligand fusion protein developed by MedImmune
 - See WO2016/196792, US20160304607
 - NCI thesaurus code → C124651
(see <https://ncit.nci.nih.gov/ncitbrowser>)
 - See also clinical trial NCT023126110 at <https://clinicaltrials.gov/ct2/home>

- See Tigue NJ, 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.

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- 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

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

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

15

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

- VL comprising the sequence (CDR underline):
EIVMTQSPATLSVSPGERATLSCKASQNVGTNVAWYQQKPG
QAPRLLIYSASYRYS~~G~~IPARFSGSGSGTEFTLTISLQSEDFA
VYYCQQYNTDPLTFGGGTKVEIK

20

- VH comprising the sequence (CDR underline):
QVTLRSGPALVKPTQTLTLCTFSGFSLSTSGMGVGWIRQP
PGKALEWLAHIWWDDDKYY~~Q~~PSLKSRLTISKDTSKNQVVLTM
TNMDPVDATYYCARTRYFPFAYWGQGTLLTVS

25

QVTLRSGPALVKPTQTLTLCTFSGFSLSTSGMGVGWIRQP
PGKALEWLAHIWWDDDKYY~~Q~~PSLKSRLTISKDTSKNQVVLTM
TNMDPVDATYYCARTRYFPFAYWGQGTLLTVS

- o See clinical trials NCT01239134 and NCT02628574 at <https://clinicaltrials.gov/ct2/home>

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- o NCI thesaurus code → C95023
(see <https://ncit.nci.nih.gov/ncitbrowser>)

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

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- See WO2016/196792
- NCI thesaurus code → C128028
(see <https://ncit.nci.nih.gov/ncitbrowser>)
- See clinical trial NCT02740270 at <https://clinicaltrials.gov/ct2/home>

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

- See clinical trial NCT02553499 at <https://clinicaltrials.gov/ct2/home>
- MK-1248 has the same CDR as MK4166 (see Sukumar et al., Cancer Res. 2017)

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

- See clinical trial NCT02132754 at <https://clinicaltrials.gov/ct2/home>
- See Sukumar, et al., (2017), Cancer Research. 77. canres.1439.2016. 10.1158/0008-5472.CAN-16-1439.
- NCI thesaurus code C116065
(see <https://ncit.nci.nih.gov/ncitbrowser/>)

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

- See clinical trial NCT02598960 at <https://clinicaltrials.gov/ct2/home>
- NCI thesaurus code C132267
(see <https://ncit.nci.nih.gov/ncitbrowser/>)

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

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

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.

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.

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.

"OX40 agonist" means any chemical compound or biological molecule that stimulates an immune reaction through iactivation of OX40 signalling.

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.

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.

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

a) MEDI0562 (aka Tavolixizumab, Tavolimab)

a) CAS Number → 1635395-25-3

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

b) Unique Ingredient Identifier (UNII) → 4LU9B48U4D

(see

<http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

- See clinical trial NCT02318394 at <https://clinicaltrials.gov/ct2/home>

- As described in WO2015/095423, WO2015/153514, WO2016/073380 & WO2016/081384

- NCI thesaurus code → C120041

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

- Heavy Chain sequence:

QVQLQESGPGLVKPSQTLSTCAVYGGSFSSGYWNWIRKHPGKGLEIYIGYI
SYNGITYHNPSLKSRTINRDTSKNQYSLQLNSVTPEDTAVYYCARYKYDYDG
GHAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP
EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVN
HKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR
TPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKL
TVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG

- Light chain sequence:

DIQMTQSPSSLSASVGDRVTITCRASQDISNYLNWYQQKPGKAPKLLIYYTSK
LHSGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGSALPWTFGQGTKV
EIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQS
GNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTK
SFN RGE C

b) MEDI6383 (Efizonerimod alfa)

a) CAS Number → 1635395-27-5

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

b) Unique Ingredient Identifier (UNII) → 1MH7C2X8KE

(see

<http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

- See clinical trial NCT02221960 at <https://clinicaltrials.gov/ct2/home>

- As described in WO2015/095423, WO2016/081384, and WO2016/189124

- NCI thesaurus code → C118282

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

- Amino acid sequence (Seq ID no.17 from WO2016/189124):

ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQED
PEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKGLPSSIEKISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGF
YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVF
SCSVMHEALHNNHYTQKSLSLSLGKDQDKIEALSSKVQQLERSIGLKDLMAD
LEQKVLEMEASTQVSHRYPRIQSIKVFTEYKKEKGFILTSQKEDEIMKVQNN
SVIINCDFYLI SLKGYFSQEVNLSLHYQKDEEPLFQLKKVRSVNSLMVASLTY
KDKVYLVNTDNTSLDDFHVNGGELILIHQNPGEFCVL

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

a) CAS Number → 1638935-72-4

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

b) Unique Ingredient Identifier (UNII) → C78148TF1D

(see

<http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

c) NCI thesaurus code → C121376

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

d) OX40mAb24 (9B12)

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)).

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

QVQLQESGPGLVKPSQTLSTCAVYGGSFSSGYWNWIRKHPGKGLEIYI
GYISYNGITYHNPSLKSRLTINRDTSKNQYSLQLNSVTPEDTAVYYCARYK
YDYDGGHAMDYWGQGTLLTVSS

VL sequence

DIQMTQSPSSLSASVGDRVITICRASQDISNYLNWYQQKPGKAPKLLIYY
 TSKLHSGVPSRFGSGSGTDYTLTISSLQPEDFATYYCQQGSALPWTFG
 QGTKVEIK

5 e) INCAGN1949

- a) See Gonzalez et al. 2016, DOI: 10.1158/1538-7445.AM2016-3204
 b) See clinical trial NCT02923349 at <https://clinicaltrials.gov/ct2/home>
 c) Antibody sequences are disclosed in WO2016/179517 A1:

i. In particular, an antibody comprising the sequences:

VH CDR1 → GSAMH

VH CDR2 → RIRSKANSYATAYAASVKG

VH CDR3 → GIYDSSGYDY

VL CDR1 → RSSQSLHNSNGYNYLD

VL CDR2 → LGSNRAS

VL CDR3 → MQALQTPLT

ii. Such as, an antibody comprising the sequences:

VH →

EVQLVESGGGLVQPGGSLKLSCAASGFTFSGSAMHWVR

QASGKGLEWVGRIRSKANSYATAYAASVKG RFTISRDDS

KNTAYLQMNSLKTEDTAVYYCTSGIYDSSGYDYWGQGTL

VTVSS

VL →

DIVMTQSPLSLPVTPGEPASISCRSSQSLHNSNGYNYLDW

YLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKI

SRVEAEDVGVYYCMQALQTPLTFGGGTKVEIK

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

- See clinical trial NCT02528357 at <https://clinicaltrials.gov/ct2/home>

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

- See patent [WO 2017/130076 A1](#)

- See clinical trial NCT02315066 at <https://clinicaltrials.gov/ct2/home>-

NCI thesaurus code → C121927

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

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

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.

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

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.

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.

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 co-stimulatory 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.

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

a) ipilimumab

- i. CAS Number → 477202-00-9

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

- ii. Unique Ingredient Identifier (UNII) → 6T8C155666

(see

<http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

b) Tremelimumab

- i. CAS Number → 745013-59-6

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

- ii. Unique Ingredient Identifier (UNII) → QEN1X95CIX

(see

<http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

- iii. VH sequence

GVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWIYDGS
NKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARDPRGATL
YYYYYGMDVWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALG
CLVKDYFPEPVTVSWNSGALTSGVH [SEQ ID NO. 1]

- iv. VL sequence

PSSLSASVGDRTITCRASQSINSYLDWYQQKPGKAPKLLIYAASSLQSG
VPSRFSGSGSGTDFTLTISLQPEDFATYYCQQYYSTPFTFGPGTKVEIK
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV [SEQ ID NO. 2]

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

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

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.

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.

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 concentration 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.

CAS Number → 21679-14-1

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

ii. NCBI Pubchem reference → 657237

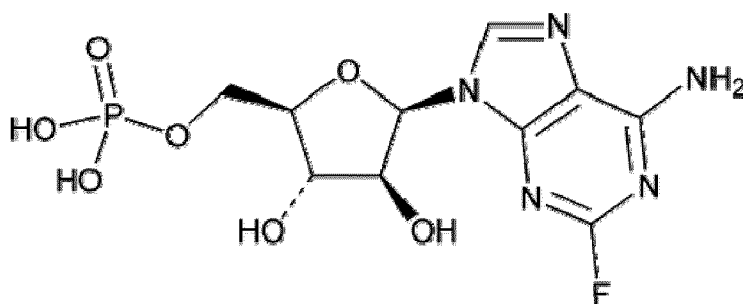
(see <https://pubchem.ncbi.nlm.nih.gov/>)

iii. IUPHAR/BPS reference → 4802

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

iv. Unique Ingredient Identifier (UNII) → 1X9VK9O1SC

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



Formula I, Fludarabine: [(2R,3R,4S,5R)-5-(6-amino-2-fluoro-purin-9-yl)- 3,4-dihydroxy-oxolan-2-yl]methoxyphosphonic acid

Cytarabine

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.

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.

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 concentration 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).

CAS Number → 147-94-4

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

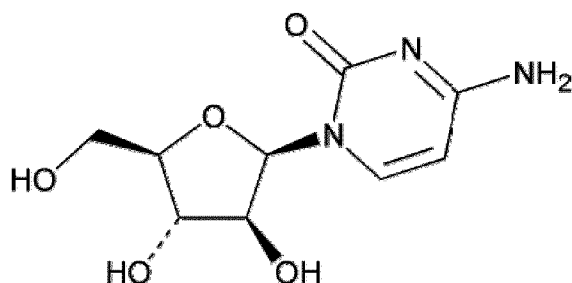
ii. NCBI Pubchem reference → 6253

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

iii. IUPHAR/BPS reference → 4827

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

iv. Unique Ingredient Identifier (UNII) → 04079A1RDZ
(see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)



Formula II, Cytarabine: 4-amino-1-[(2R,3S,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl] pyrimidin-2-one

Hypomethylating agent

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.

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.

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.

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

a) 5-azacytidine (azacitidine)

- i. CAS Number → 320-67-2

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

- ii. NCBI Pubchem reference → 9444

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

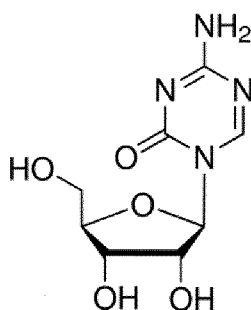
- iii. IUPHAR/BPS reference → 6796

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

- iv. Unique Ingredient Identifier (UNII) → M801H13NRU

(see

<http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)



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

b) 5-aza-2'-deoxycytidine (decitabine)

- i. CAS Number → 2353-33-5

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

- ii. NCBI Pubchem reference → 451668

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

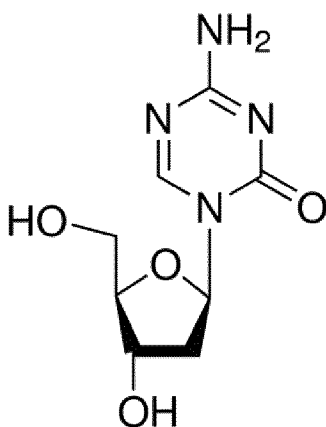
- iii. IUPHAR/BPS reference → 6805

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

- iv. Unique Ingredient Identifier (UNII) → 776B62CQ27

(see

<http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)



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

PARP inhibitors

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.

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.

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).

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.

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.

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.

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.

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.

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

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%.

Specific PARPi suitable for use in the present disclosure include:

a) Olaparib

- i. CAS Number → 763113-22-0

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

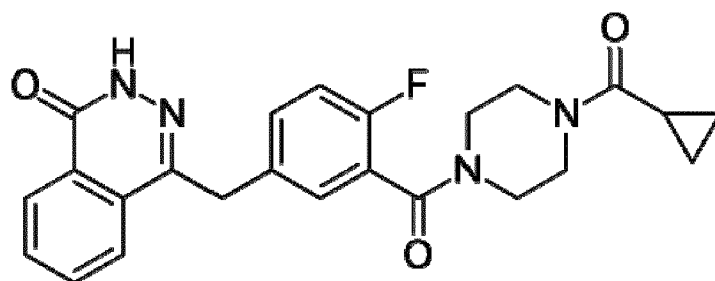
- ii. NCBI Pubchem reference → 23725625

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

- iii. Unique Ingredient Identifier (UNII) → WOH1JD9AR8

(see

<http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

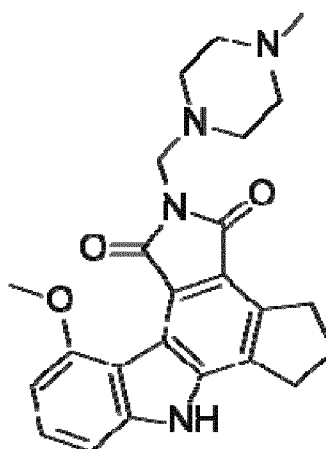


Formula V, olaparib: 4-[(3-[(4-cyclopropylcarbonyl)piperazin-1-yl]carbonyl)-4-fluorophenyl]methyl(2H)phthalazin-1-one

b) CEP-9722

- i. CAS Number → 916574-83-9

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



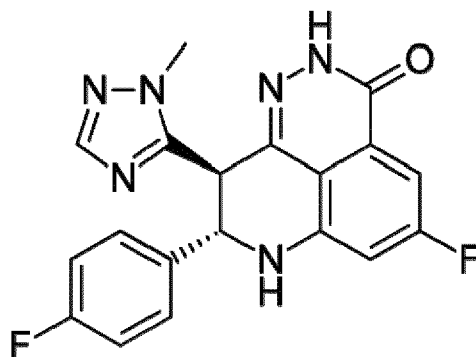
Formula VI, CEP-9722: 11-methoxy-2-((4-methylpiperazin-1-yl)methyl)-4,5,6,7-tetrahydro-1H-cyclopenta[a]pyrrolo[3,4-c]carbazole-1,3(2H)-dione

c) BMN-673/talazoparib

- i. CAS Number → 1207456-01-6

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

- 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

d) Rucaparib

- i. CAS Number → 283173-50-2

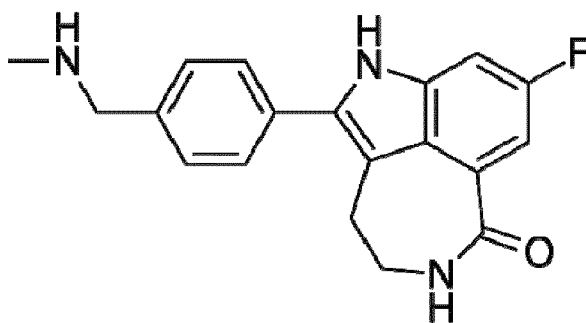
(see <http://www.cas.org/content/chemical-substances/faqs>)

- ii. NCBI Pubchem reference → 9931954

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

- iii. Unique Ingredient Identifier (UNII) → 8237F3U7EH

(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

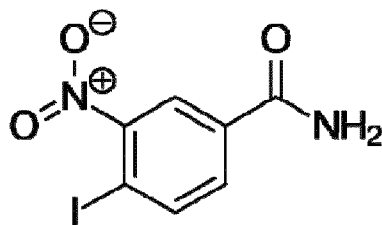
e) Iniparib/SAR24-550/BSI-201

- i. CAS Number → 160003-66-7

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

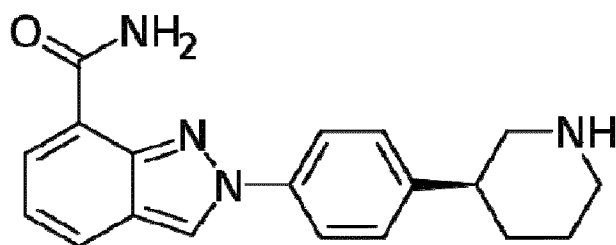
- ii. NCBI Pubchem reference → 9796068

- (see <https://pubchem.ncbi.nlm.nih.gov/>)
- iii. Unique Ingredient Identifier (UNII) → 2ZWI7KHK8F
(see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)



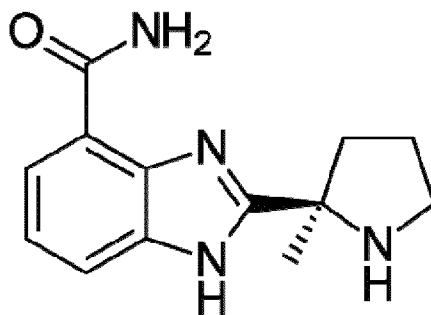
Formula IX, Iniparib: 4-Iodo-3-nitrobenzamide

- f) Veliparib (ABT-888)
- i. CAS Number → 912444-00-9
(see <http://www.cas.org/content/chemical-substances/faqs>)
- ii. NCBI Pubchem reference → 11960529
(see <https://pubchem.ncbi.nlm.nih.gov/>)
- iii. Unique Ingredient Identifier (UNII) → 01O4K0631N
(see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)



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

- g) Niraparib/MK-4827
- i. CAS Number → 1038915-60-4
(see <http://www.cas.org/content/chemical-substances/faqs>)
- ii. NCBI Pubchem reference → 24958200
(see <https://pubchem.ncbi.nlm.nih.gov/>)
- iii. Unique Ingredient Identifier (UNII) → HMC2H89N35
(see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)



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

h) BGB-290

i. CAS Number → 1820833-75-7

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

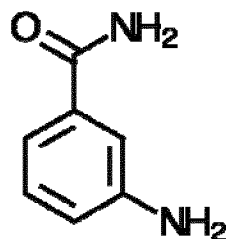
i) 3-aminobenzamide

i. CAS Number → 3544-24-9

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

ii. NCBI Pubchem reference → 1645

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

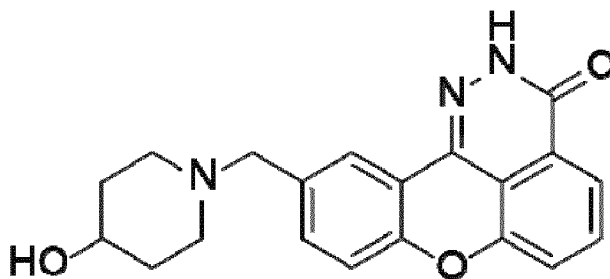


Formula XII: 3-Aminobenzamide

j) E7016

i. CAS Number → 902128-92-1

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



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

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

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.

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.

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

a) gemcitabine

- i. CAS Number → 95058-81-4

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

- ii. NCBI Pubchem reference → 60750

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

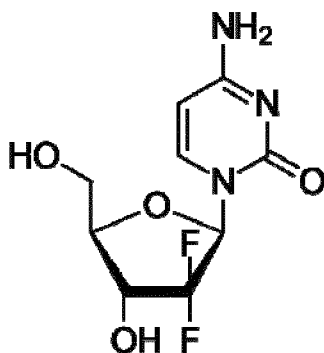
- iii. DrugBank reference → DB00441

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

- iv. Unique Ingredient Identifier (UNII) → B76N6SBZ8R

(see

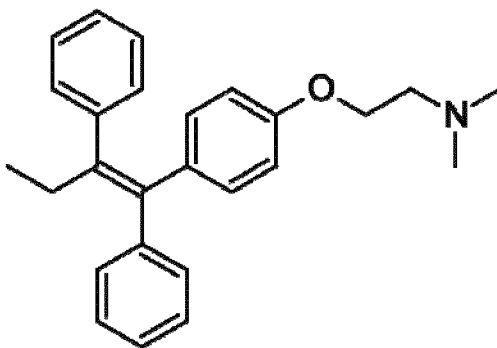
<http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)



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

b) tamoxifen

- i. CAS Number → 10540-29-1
(see <http://www.cas.org/content/chemical-substances/faqs>)
- ii. NCBI Pubchem reference → 2733526
(see <https://pubchem.ncbi.nlm.nih.gov/>)
- iii. DrugBank reference → DB00675
(see <https://www.drugbank.ca/>)
- iv. Unique Ingredient Identifier (UNII) → 094ZI81Y45
(see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)



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

Gemcitabine is the preferred agent that upregulated HER2.

AXLi

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

"AXL inhibitor" means any chemical compound or biological molecule that reduces AXL signalling.

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.

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.

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.

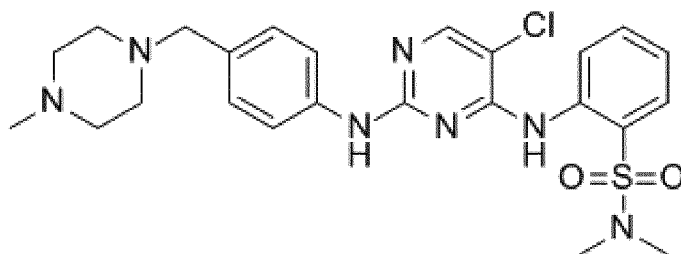
To show that co-treatment of AXL-positive cancer cell lines with ADC and the AXLi (including but not limited to BGB324 and TP0903) has an additive or synergistic anti-tumor effect, a panel of cell lines including, but not limited to MDA-MB-157 and SKLU1 will be co-treated with a range of concentration 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.

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

c) TP0903

i. CAS Number → 1341200-45-0

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



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

d) BGB324

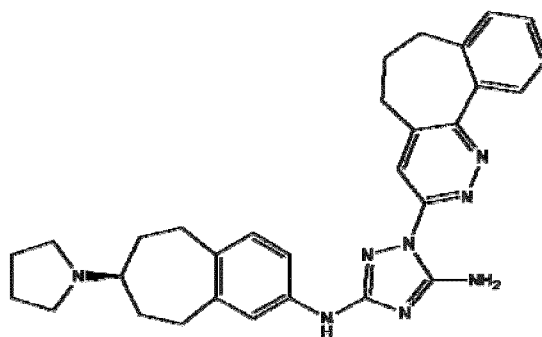
i. CAS Number → 1037624-75-1

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

ii. Unique Ingredient Identifier (UNII) → 0ICW2LX8AS

(see

<http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

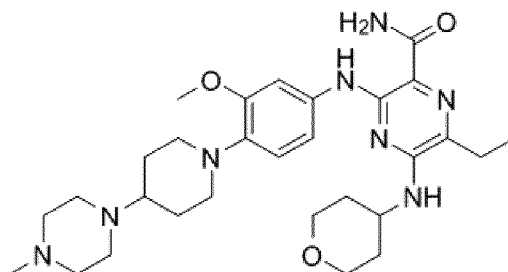


Formula XVII: 1-(6,7-dihydro-5H-benzo[2,3]cyclohepta[2,4-d]pyridazin-3-yl)-3-N-[(7S)-7-pyrrolidin-1-yl-6,7,8,9-tetrahydro-5H-benzo[7]annulen-3-yl]-1,2,4-triazole-3,5-diamine [BGB324]

e) Gilteritinib (ASP2215)

i. CAS Number → 1254053-43-4

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



Formula XVIII: 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]

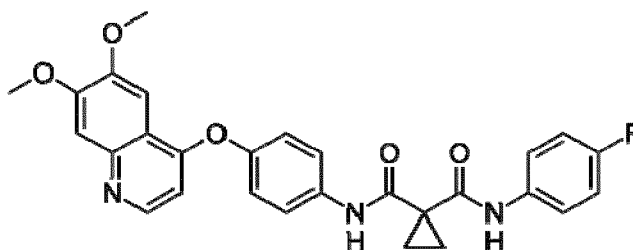
f) Cabozantinib

- i. CAS Number → 849217-68-1

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

- ii. Unique Ingredient Identifier (UNII) → 1C39JW444G

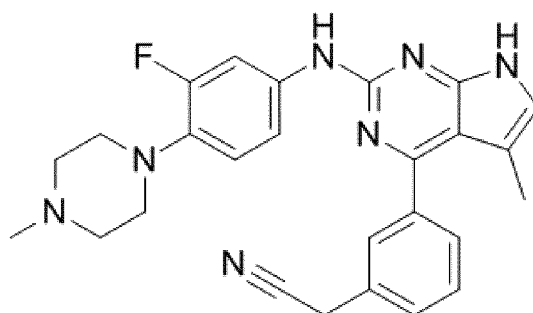
(see

<http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

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

g) SGI7079

- i. CAS Number → 1239875-86-5

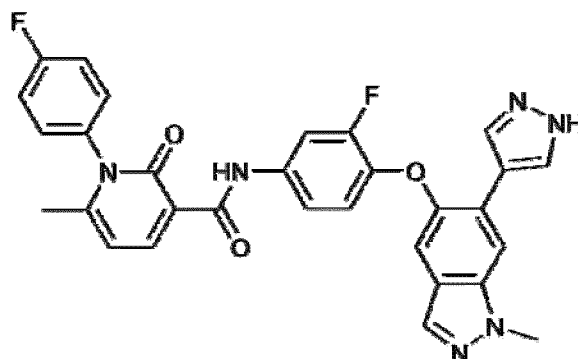
(see <http://www.cas.org/content/chemical-substances/faqs>)

Formula XX: 2-(3-(2-((3-fluoro-4-(4-methylpiperazin-1-yl)phenyl)amino)-5-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)acetonitrile [SGI7079]

h) Merestinib

- i. CAS Number → 1206799-15-6

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



Formula XXI: N-(3-Fluoro-4-{{1-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]

i) amuvatinib (MP-470)

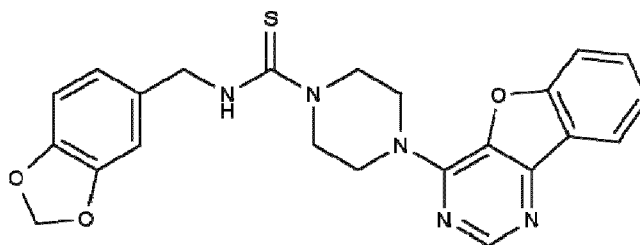
i. CAS Number → 850879-09-3

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

ii. Unique Ingredient Identifier (UNII) → SO9S6QZB4R

(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]

j) bosutinib (SKI-606)

i. CAS Number → 380843-75-4

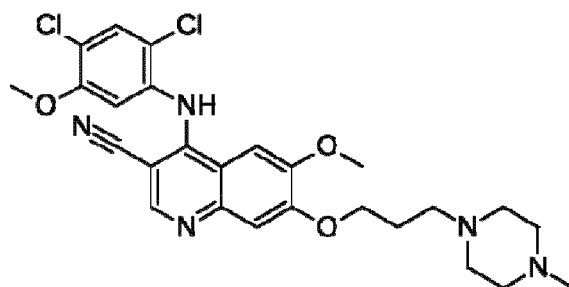
(see <http://www.cas.org/content/chemical-substances/faqs>)

ii. Unique Ingredient Identifier (UNII) → 5018V4AEZ0

(see

<http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

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

k) MGCD265

i. CAS Number → 875337-44-3

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

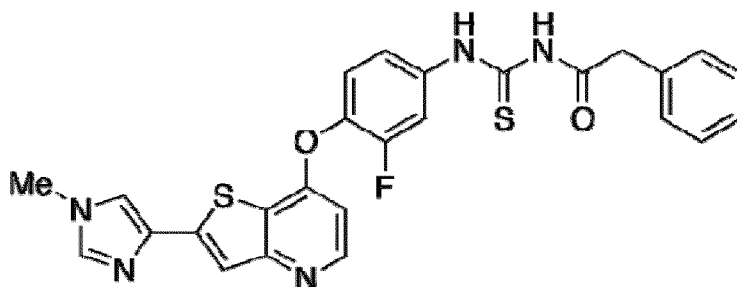
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ii. Unique Ingredient Identifier (UNII) → 93M6577H9D

(see

<http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

20



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Formula XXIV: N-(3-fluoro-4-(2-(1-methyl-1H-imidazol-4-yl)thieno(3,2-b)pyridin-7-yloxy)phenylcarbamothioyl)-2-phenylacetamide [MGCD265]

30

l) foretinib (GSK1363089/XL880)

i. CAS Number → 849217-64-7

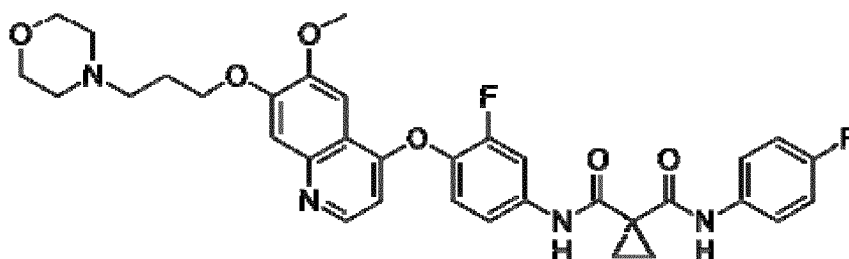
(see <http://www.cas.org/content/chemical-substances/faqs>)

ii. Unique Ingredient Identifier (UNII) → 81FH7VK1C4

(see

<http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

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

BRAFi

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

- 5 "BRAF inhibitor" means any chemical compound or biological molecule that reduces BRAF activity.

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.

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.

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.

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.

Examples of such potential combinations are BRAF inhibitors such as vemurafenib and dabrafenib. These BRAF inhibitors inhibits the B-RAF protein directly.

Combining ADC, which targets AXL positive tumors, with BRAFi 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, BRAFi will interfere with cell proliferation through inhibition of BRAF.

To show that ADC works synergistically with BRAFi, 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 BRAFi. 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 (Table 1) using the CalcuSyn analysis program.

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

a) vemurafenib

- i. CAS Number → 918504-65-1

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

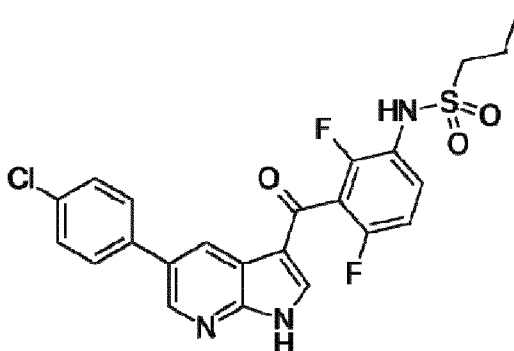
- ii. DrugBank reference → DB08881

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

- iii. Unique Ingredient Identifier (UNII) → 207SMY3FQT

(see

<http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)



Formula XXVI: N-(3-([5-(4-Chlorophenyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]carbonyl)-2,4-difluorophenyl)propane-1-sulfonamide [vemurafenib]

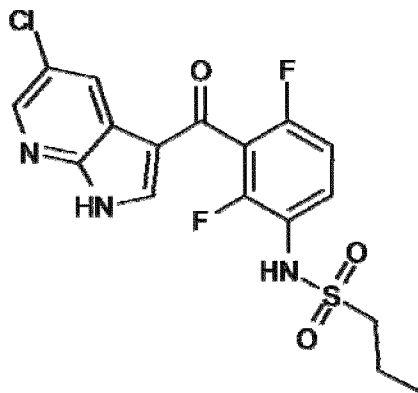
b) PLX4720

- i. CAS Number → 918505-84-7

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

- ii. Unique Ingredient Identifier (UNII) → EQY31RO8HA

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

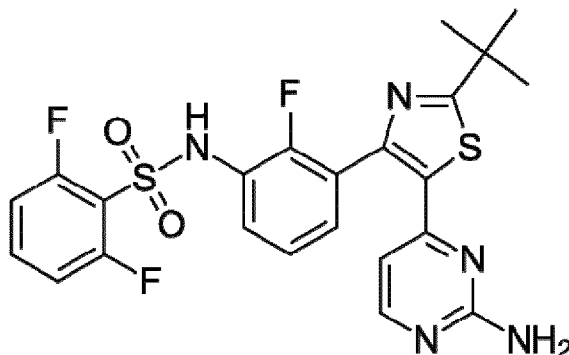


Formula XXVII: N-(3-(5-chloro-1H-pyrrolo[2,3-b]pyridine-3-carbonyl)-2,4-difluorophenyl)propane-1-sulfonamide [PLX4720]

c) dabrafenib

i. CAS Number → 1195765-45-7

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



Formula XXVIII: N-{3-[5-(2-aminopyrimidin-4-yl)-2-tert-butyl-1,3-thiazol-4-yl]-2-fluorophenyl}-2,6-difluorobenzenesulfonamide [dabrafenib]

d) Sorafenib

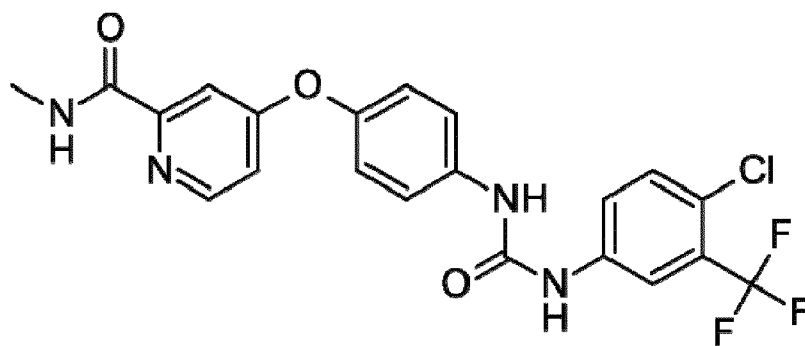
i. CAS Number → 284461-73-0

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

ii. Unique Ingredient Identifier (UNII) → 9ZOQ3TZI87

(see

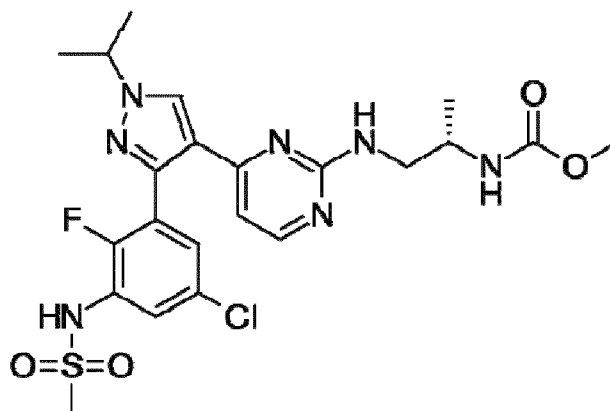
<http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)



Formula XXIX: 4-[4-[[4-chloro-3-(trifluoromethyl)phenyl]carbamoylamino]phenoxy]-N-methyl-pyridine-2-carboxamide [sorafenib]

e) Encorafenib

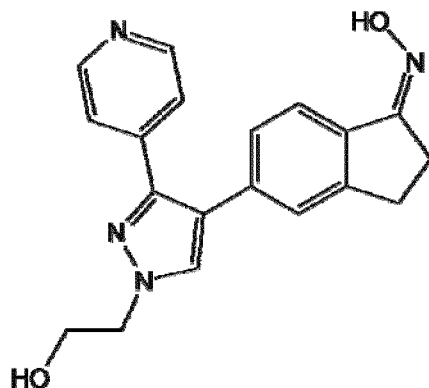
- i. CAS Number → 1269440-17-6
(see <http://www.cas.org/content/chemical-substances/faqs>)
- ii. Unique Ingredient Identifier (UNII) → 8L7891MRB6
(see <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)



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]

f) GDC0879

- i. CAS Number → 905281-76-7
(see <http://www.cas.org/content/chemical-substances/faqs>)



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

MEKi

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

"MEK inhibitor" means any chemical compound or biological molecule that reduces MEK1 and/or MEK2 activity.

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.

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.

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.

5 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.

10 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.

15 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.

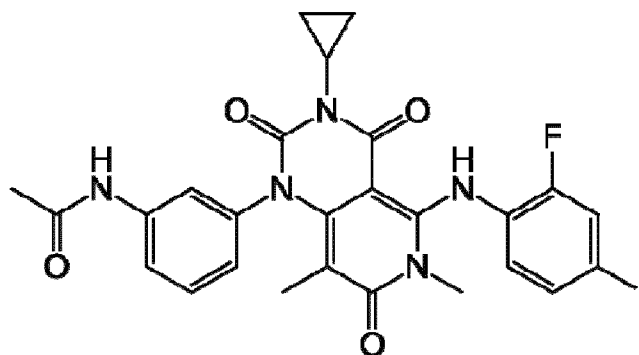
20 To show that ADC works synergistically with MEKi, a panel of AXL (+) cell lines including, but not limited to MDA-MB231, H1299 and SNU12C cells, will be co-treated with a range of concentration of both ADC and MEKi. As negative controls, the same panel of cell lines will be co-treated with a range of concentrations of MEKi or with a range of concentration
25 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
30 index using the CalcuSyn analysis program.

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

a) Trametinib

- 35 i. CAS Number → 871700-17-3
(see <http://www.cas.org/content/chemical-substances/faqs>)
ii. Unique Ingredient Identifier (UNII) → 33E86K87QN
(see
40 <http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

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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

b) Cobimetinib

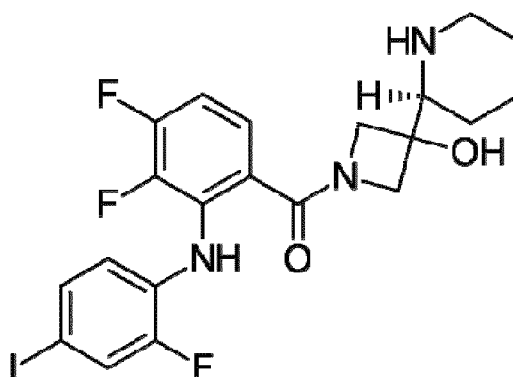
- i. CAS Number → 934660-93-2

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

- ii. Unique Ingredient Identifier (UNII) → ER29L26N1X

(see

<http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)



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

c) Binimetinib

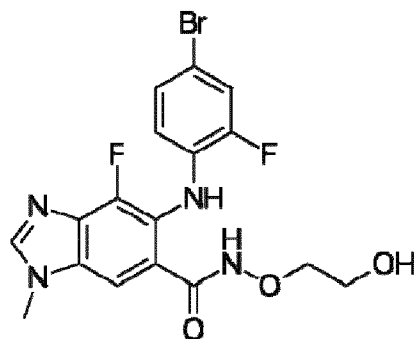
- i. CAS Number → 606143-89-9

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

- ii. Unique Ingredient Identifier (UNII) → 181R97MR71

(see

<http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)



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

d) Selumetinib

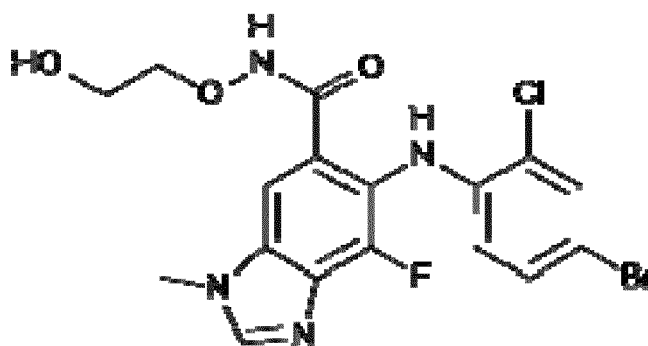
- i. CAS Number → 606143-52-6

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

- ii. Unique Ingredient Identifier (UNII) → 6UH91I579U

(see

<http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)



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

e) PD-325901

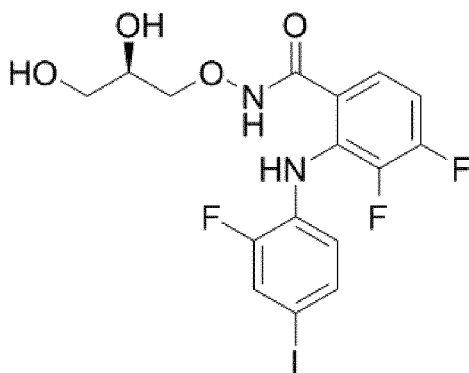
- i. CAS Number → 391210-10-9

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

- ii. Unique Ingredient Identifier (UNII) → 86K0J5AK6M

(see

<http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)



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

f) CI-1040

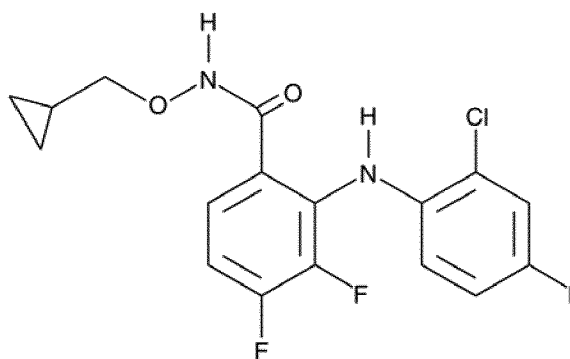
i. CAS Number → 212631-79-3

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

ii. Unique Ingredient Identifier (UNII) → R3K9Y00J04

(see

<http://www.fda.gov/ForIndustry/DataStandards/SubstanceRegistrationSystem-UniqueIngredientIdentifierUNII/default.htm>)

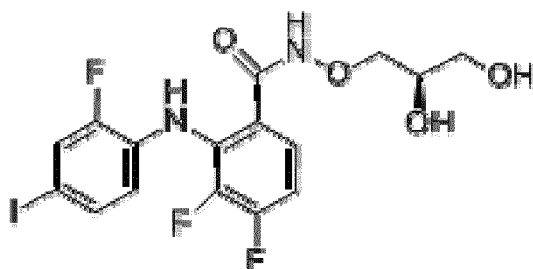


Formula XXXVII: 2-[(2-chloro-4-iodophenyl)amino]-N-(cyclopropylmethoxy)-3,4-difluorobenzamide

g) PD035901

i. CAS Number → 391210-10-9

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



Formula XXXVIII: PD035901

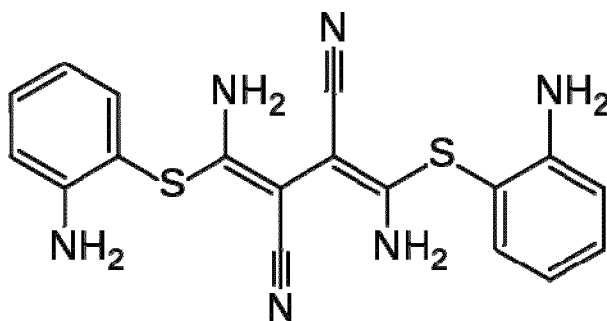
h) U0126

i. CAS Number → 218601-62-8

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

ii. Unique Ingredient Identifier (UNII) → 8027P94HLL

(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

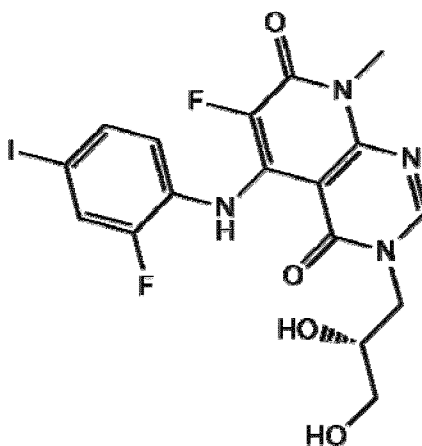
i) TAK-733

iii. CAS Number → 1035555-63-5

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

iv. Unique Ingredient Identifier (UNII) → 5J61HSP0QJ

(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

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
 5 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.

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
 10 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.

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
 15 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

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
 25 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:

- 1) effective treatment of a broader range of cancers;
- 2) effective treatment of resistant or refractory forms of disorders such as
 30 cancer, and individuals with disorders such as cancer who have relapsed after a period of remission;
- 3) increased response rate to treatment; and / or
- 4) Increased durability of treatment.

Effective treatment of a broader range of cancers as used herein means that following
 35 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.

Effective treatment of a resistant, refractory, or relapsed forms as used herein means that
 40 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
 45 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.

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 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 treated individuals.

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.

'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

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

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.

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.

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 strabmisus, 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.

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.

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.

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*.

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. histocytoma, glioma, astrocyoma, osteoma), cancers (e.g. lung cancer, small cell lung cancer, gastrointestinal cancer, bowel cancer, colon cancer, breast carinoma, 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.

5 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.

10 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).

15 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
20 cancer. Furthermore, Axl is known to play a role in many cancers of epithelial origin.

Fibrotic disorders of interest include strabmisus, scleroderma, keloid, Nephrogenic systemic fibrosis, pulmonary fibrosis, idiopathic pulmonary fibrosis (IPF), cystic fibrosis (CF), systemic sclerosis, cardiac fibrosis, non-alcoholic steatohepatitis (NASH), other
25 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
30 (see Rockey, D.C., Bell, P.D. and Hill, J.A. (2015), N. Engl. Med., Vol. 372, pp. 1138-1149).

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

35 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.

40 The target neoplasm or neoplastic cells may be all or part of a solid tumour.

“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.

45

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).

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, blastocoelic, inflammatory, angiogenic and immunologic, including autoimmune disorders and graft-versus-host disease (GVHD).

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.

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 polyarteriitis), 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.

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.

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.

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.

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

"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.

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

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

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.

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.

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.

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

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.

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.

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.

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.

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.

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.

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.

The first target protein is preferably AXL.

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

Samples

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.

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.

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.

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.

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

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.

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.

5 The first target polypeptide is preferably AXL.

Individual status

10 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.

15 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.

20 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).

30 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.

40 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.

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

- 5 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

- 10 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.

- 15 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.

- 20 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.

- 25 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.

- 30 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.

In some cases, the control is a cell culture sample.

- 35 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.

- 40 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.

- 45 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.

The first target protein is preferably AXL.

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

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.

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.

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.

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.

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, or PCT/EP2018/053163.

5 The secondary agent may be:

- (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;
- (b) a PD-L1 antagonist, such as atezolizumab (Tecentriq), BMS-936559/MDX-1105, durvalumab/MEDI4736, or MSB0010718C (Avelumab);
- (c) a GITR (Glucocorticoid-Induced INFR-Related protein) agonist, such as MEDI1873, TRX518, GWN323, MK-1248, MK-4166, BMS-986156 or INCAGN1876;
- (d) an OX40 agonist, such as MEDI0562, MEDI6383, MOXR0916, RG7888, OX40mAb24, INCAGN1949, GSK3174998, or PF-04518600;
- (e) a CTLA-4 antagonist, such as ipilimumab (brand name Yervoy) or Tremelimumab (Originally developed by Pfizer, now Medimmune);
- (f) Fludarabine or Cytarabine;
- (g) a hypomethylating agent, such as cytidine analogs – for example, 5-azacytidine (azacitidine) and 5-aza-2'-deoxycytidine (decitabine); or
- (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;
- (i) an agent that upregulates HER2 expression, such as gemcitabine and tamoxifen;
- (j) an AXL-kinase inhibitor (AXLi) such as BGB324 (bemcentinib), TP0903, Gilteritinib (ASP2215), Cabozantinib (XL184), SGI7079, Merestinib, amuvatinib (MP-470), bosutinib (SKI-606), MGCD265, and foretinib (GSK1363089/XL880);
- (k) a BRAF inhibitor (BRAFi), such as vemurafenib, PLX4720, dabrafenib, Sorafenib, Encorafenib, and GDC0879; or
- (l) a MEK inhibitor (MEKi), such as Trametinib, Cobimetinib, Binimetinib, Selumetinib, PD-325901, CI-1040, PD035901, U0126, and TAK-733.

35 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.

40 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

45 kinase inhibitors. Chemotherapeutic agents include compounds used in “targeted therapy” and conventional chemotherapy.

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), 5
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-
10 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.

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
20 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), lonafarnib (SARASAR™, SCH 66336, Schering Plough), sorafenib (NEXAVAR®, BAY43-
25 9006, Bayer Labs), gefitinib (IRESSA®, AstraZeneca), irinotecan (CAMPTOSAR®, CPT-11, Pfizer), tipifarnib (ZARNESTRA™, Johnson & Johnson), ABRAXANE™ (Cremophor-free), albumin-engineered nanoparticle formulations of paclitaxel (American Pharmaceutical Partners, Schaumburg, IL), vandetanib (rINN, ZD6474, ZACTIMA®, AstraZeneca), chlorambucil, AG1478, AG1571 (SU 5271; Sugen), temsirolimus (TORISEL®, Wyeth), pazopanib (GlaxoSmithKline), canfosfamide (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, triethylenephosphoramidate, triethylenethiophosphoramidate and trimethylomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analog topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogs); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the
40 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 omegal1 (*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 enediynes (antibiotic chromophores), aclacinomysins, actinomycin, anthramycin, azaserine, bleomycins, cactinomycin, carabycin, 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, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitio stanol, mepitio stanane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglutone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; 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, OR); 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; thiotepa; 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; difluoromethylornithine (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).

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® (toremifine 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-alpha, 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.

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 (PERJETA™, OMNITARG™, 2C4, Genentech), trastuzumab (HERCEPTIN®, Genentech), tositumomab (Bexxar, Corixa), MDX-060 (Medarex) and the antibody drug conjugate, gemtuzumab ozogamicin (MYLOTARG®, Wyeth).

Humanized monoclonal antibodies with therapeutic potential as chemotherapeutic agents in combination with the conjugates of the disclosure include: alemtuzumab, apolizumab, aselizumab, atlizumab, bapineuzumab, bevacizumab, bivatumab 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, pectusituzumab, pectuzumab, pertuzumab, pexelizumab, ralivizumab, ranibizumab, reslivizumab, reslizumab, resyvizumab, rovelizumab, ruplizumab, sibrotuzumab, sipilizumab, sontuzumab, tacatuzumab tetraxetan, tadocizumab, talizumab, tefibazumab, tocilizumab, toralizumab, trastuzumab, tucotuzumab celmoleukin, tucusituzumab, umavizumab, urtoxazumab, and visilizumab.

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.

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 a gelatin.

5 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.
10 Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

Dosage

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.
25

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 localisation 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.
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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
40

conjunction with a chemotherapeutic agent. A high expression level of the second target protein may indicate a more aggressive therapy.

5 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.

10 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
15 cells infiltrating a solid tumour comprising or composed of neoplastic cells which do not express the first target protein.

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
20 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.

25 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 µg 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
30 so the actual weight to be used is increased proportionately.

In one embodiment, each active compound is administered to a human subject according to the following dosage regime: about 100 mg, 3 times daily.

35 In one embodiment, each active compound is administered to a human subject according to the following dosage regime: about 150 mg, 2 times daily.

In one embodiment, each active compound is administered to a human subject according to the following dosage regime: about 200 mg, 2 times daily.
40

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.

45 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.

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.

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.

The secondary agent may a PD1 antagonist. Suitable PD1 antagonists include pembrolizumab, nivolumab, MEDI0680, PDR001, Camrelizumab, AUNP12, Pidilizumab REGN-2810, and BGB-108.

Antibodies

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.

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 antibodies. 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.

"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.

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, US 4816567). 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).

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 (US 4816567; 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.

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.

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.

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

Embodiments and experiments illustrating the principles of the disclosure will now be discussed with reference to the accompanying figures in which:

Figure 1. Sequences

Figure 2. Binding of a conjugate according to the invention to AXL

Figure 3. *in vivo* efficacy of a conjugate according to the invention

Figure 4. *in vitro* synergy between ADCxAXL and Cytarabine in SN12C cells

Figure 5. *in vitro* synergy between ADCxAXL and Fludarabine in SN12C cells

Figure 6. *in vitro* synergy between ADCxAXL and Decitabine in SN12C cells

Figure 7. *in vitro* synergy between ADCxAXL and Olaparib in SN12C cells

Figure 8. *in vitro* synergy between ADCxAXL and Gemcitabine in SN12C cells

The disclosure includes the combination of the aspects and preferred features described except where such a combination is clearly impermissible or expressly avoided.

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

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.

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.

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

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

The following paragraphs describe some specific embodiments of the present disclosure:

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 paragraph 9.

11. The composition of paragraph 9 for use in a method of treating cancer in an individual.

12. The use of the composition of paragraph 9 in the manufacture of a medicament for treating cancer in an individual.

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.

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.

15. The composition, method, use, or kit according to any previous paragraph, wherein the treatment further comprises administering a chemotherapeutic agent.

16. The composition, method, use, or kit according to any previous paragraph, wherein the individual is human.

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.

18. The composition, method, use, or kit according 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.

19. The composition, method, use, or kit according 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.

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.

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.

22. The composition, method, use, or kit according to paragraph 21, wherein the AXL+ infiltrating cells are dendritic cells, NK cells, or macrophages.

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.

24. The composition, method, use, or kit according to any one of the preceding paragraphs, 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 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.

26. A composition, method, use, or kit according to any one of paragraphs 1 to 25, wherein the secondary agent is a PD1 antagonist.

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.

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.

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).

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.

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.

32. A composition, method, use, or kit according to any one of paragraphs 1 to 25, wherein the secondary agent is a OX40 agonist.

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.

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.

35. A composition, method, use, or kit according to paragraph 34, wherein the CTLA-4 antagonist is selected from ipilimumab and Tremelimumab.

36. A composition, method, use, or kit according to any one of paragraphs 1 to 25, wherein the secondary agent is Fludarabine.

37. A composition, method, use, or kit according to any one of paragraphs 1 to 25, wherein the secondary agent is Cytarabine.

38. A composition, method, use, or kit according to any one of paragraphs 1 to 25, wherein the secondary agent is a hypomethylating agent.

39. A composition, method, use, or kit according to paragraph 38, wherein the hypomethylating agent is azacitidine.

40. A composition, method, use, or kit according to paragraph 38, wherein the hypomethylating agent is decitabine.

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).

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), Niraparib/MK-4827, BGB-290, 3-aminobenzamide, and E7016.

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.

44. A composition, method, use, or kit according to paragraph 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 paragraphs 1 to 25, wherein the secondary agent is an AXL-kinase inhibitor (AXLi).

46. A composition, method, use, or kit according to paragraph 45, wherein the AXLi is selected from BGB324 (bemcentinib), TP0903, Gilteritinib (ASP2215), Cabozantinib (XL184), SGI7079, Merestinib, amuvatinib (MP-470), bosutinib (SKI-606), MGCD265, and foretinib (GSK1363089/XL880).

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).

48. A composition, method, use, or kit according to paragraph 47, wherein the BRAFi is selected from vemurafenib, PLX4720, dabrafenib, Sorafenib, Encorafenib, and GDC0879.

5 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).

10 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.

15

STATEMENTS OF INVENTION

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.

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.

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.

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.

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.

6. A kit comprising:
a first medicament comprising an ADC;
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 a disorder.

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.

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.

9. A pharmaceutical composition comprising an ADC and a secondary agent.

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.

11. The composition of paragraph 9 for use in a method of treating a disorder in an individual.

12. The use of the composition of paragraph 9 in the manufacture of a medicament for treating a disorder in an individual.

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.

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.

15. The composition, method, use, or kit according to any previous paragraph, wherein the treatment further comprises administering a chemotherapeutic agent.

16. The composition, method, use, or kit according to any previous paragraph, wherein the individual is human.

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.

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.

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).

20. The composition, method, use, or kit according to any one of the preceding paragraphs, 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 the ADC or the secondary agent alone.

21. A composition, method, use, or kit according to any previous paragraph, wherein the ADC is an anti-AXL ADC.

22. A composition, method, use, or kit according to paragraph 21, wherein the anti-AXL ADC is ADCxAXL.

23. A composition, method, use, or kit according to any previous paragraph, wherein the FTP is AXL.

24. A composition, method, use, or kit according to any previous paragraph, wherein the disorder is a proliferative disease.

25. The composition, method, use, or kit of paragraph 24, wherein the disorder is cancer.

26. The composition, method, use, or kit according 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.

27. The composition, method, use, or kit according 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.

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.

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.

30 A composition, method, use, or kit according to any previous paragraph, wherein the STP is PD-L1.

31. A composition, method, use, or kit according to any one of paragraphs 1 to 30, wherein the secondary agent is a PD1 antagonist.

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 (Tisleizumab), and BGB-108.

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.

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).

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 INFR-Related protein) agonist.

36. A composition, method, use, or kit according to paragraph 35, wherein the G₁T_R (Glucocorticoid-Induced TNFR-Related protein) agonist is selected from MEDI1873, TRX518, GWN323, MK-1248, MK 4166, BMS-986156 and INCAGN1876.

37. A composition, method, use, or kit according to any one of paragraphs 1 to 30, wherein the secondary agent is a OX40 agonist.

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.

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.

40. A composition, method, use, or kit according to paragraph 39, wherein the CTLA-4 antagonist is selected from ipilimumab and Tremelimumab.

41. A composition, method, use, or kit according to any one of paragraphs 1 to 30, wherein the secondary agent is Fludarabine.

42. A composition, method, use, or kit according to any one of paragraphs 1 to 30, wherein the secondary agent is Cytarabine.

43. A composition, method, use, or kit according to any one of paragraphs 1 to 30, wherein the secondary agent is a hypomethylating agent.

44. A composition, method, use, or kit according to paragraph 43, wherein the hypomethylating agent is azacitidine.

45. A composition, method, use, or kit according to paragraph 43, wherein the hypomethylating agent is decitabine.

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).

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), Niraparib/MK-4827, BGB-290, 3-aminobenzamide, and E7016.

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.

49. A composition, method, use, or kit according to paragraph 48, wherein the agent that upregulates HER2 expression is selected from gemcitabine and tamoxifen.

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).

51. A composition, method, use, or kit according to paragraph 50, wherein the AXLi is selected from BGB324 (bemcentinib), TP0903, Gilteritinib (ASP2215), Cabozantinib (XL184), SGI7079, Merestinib, amuvatinib (MP-470), bosutinib (SKI-606), MGCD265, and foretinib (GSK1363089/XL880).

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).

53. A composition, method, use, or kit according to paragraph 52, wherein the BRAFi is selected from vemurafenib, PLX4720, dabrafenib, Sorafenib, Encorafenib, and GDC0879.

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).

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

In the following examples:

- the FTP is preferably AXL.
- Cell lines expressing AXL suitable for use in the examples include MDA-MB231, NCI-H1299 and SN12C.
- Disease A – Colorectal
- Disease B – Gastric cancer
- Disease C – Pancreatic cancer

Example 1

To show that a PBD-ADC can induce ICD and therefore can be a suitable combination agent with immune-oncology (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).

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

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).

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

The purpose of this study is to preliminarily assess the safety, tolerability, pharmacological and clinical activity of this combination

The following cancer types have been chosen for study: Disease A, Disease B, and Disease C

Evidence for efficacy as single agents exists for both drugs:

- ADC (see, for example, GB1702029.8, GB1719906.8, and PCT/EP2018/053163.)
- Secondary agent (see KS Peggs et al.2009, Clinical and Experimental Immunology, 157: 9–19 [doi:10.1111/j.1365-2249.2009.03912.x])

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.

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.

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

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

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

Objective	Endpoint
<p><i>Primary Objective</i></p> <p>To characterize the safety and tolerability of ADC in combination with the secondary agent, and to identify the recommended dose and schedules for future studies</p>	<p>Frequency and severity of treatment-emergent AEs and SAEs</p> <p>Changes between baseline and post-baseline laboratory parameters and vital signs</p> <p>Incidence of dose limiting toxicities (DLTs) during the first cycle of treatment (dose escalation only)</p> <p>Frequency of dose interruptions and dose reductions</p>

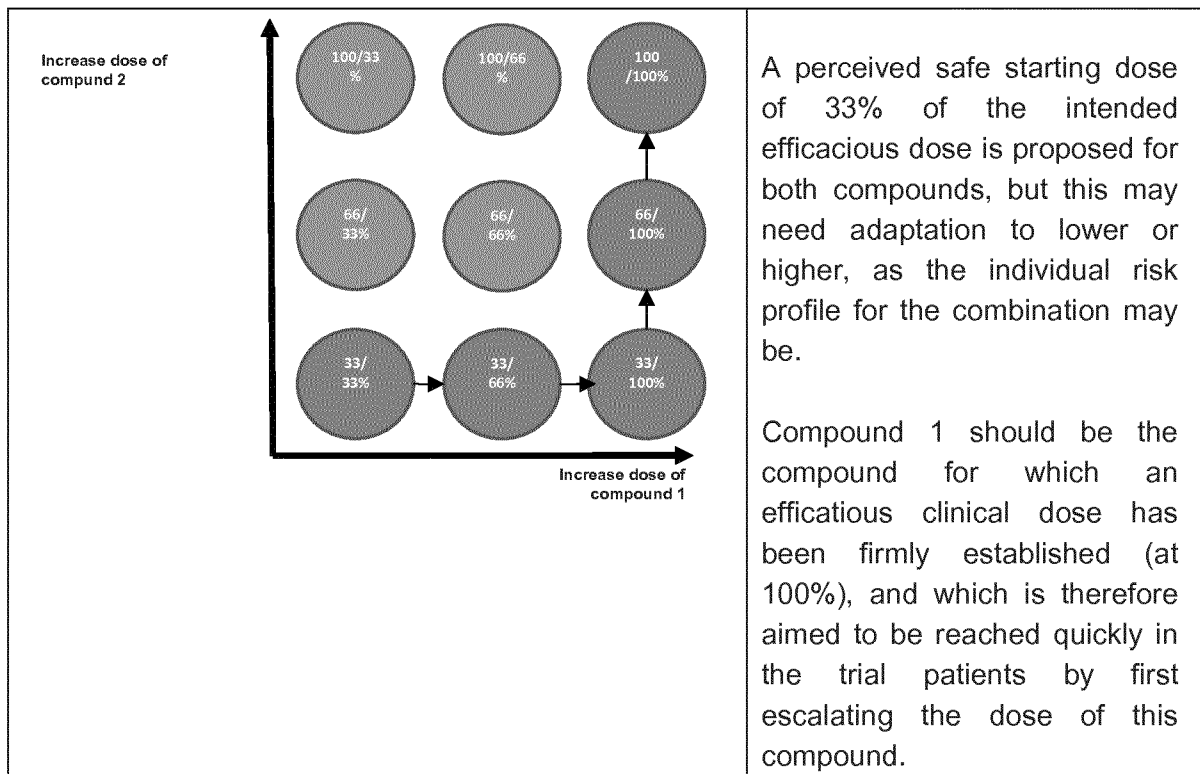
<p><i>Secondary Objectives</i></p> <p>To evaluate the clinical activity of the combination of ADC with the secondary agent</p> <p>To characterize the pharmacokinetic (PK) profile of each of the two compounds ADC and the secondary agent</p> <p>Evidence for immunogenicity and ADAs to ADC</p>	<p>ORR, DOR, PFS, OS</p> <p>AUC and Cmax for each compound</p> <p>Anti-Drug-Antibodies (ADAs) before, during and after treatment with ADC</p>
<p><i>Exploratory Objectives</i></p> <p>To examine potential correlation of PK profiles with safety/tolerability and efficacy</p> <p>To characterize changes in the immune infiltrate in tumors</p> <p>To characterize changes in circulating levels of cytokines in plasma and markers of activation in circulating immune cells</p>	<p>Correlation coefficients between AUC and/or Cmax of each compound or a compound measure and any of the safety or efficacy variables</p> <p>Immunohistochemistry of pre- and on-treatment tumor biopsies,</p> <p>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)</p>

Study design

5 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.

10 The study is comprised of a dose escalation part followed by a dose expansion part.

15 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:



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.

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", February 1, 2007) and endorsed by numerous publications (Babb et al. 1998, Neuenschwander et al. 2008).

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.

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.

- For combinations with IO, changes in the immune infiltrate in tumors will also be characterized following combination treatment in the target disease indications.

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.

- 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

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.

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.

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 IO 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.

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.

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.

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.

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.

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.

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

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

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.

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,

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.

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

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

Patients eligible for inclusion in this study have to meet all of the following criteria:

1. Written informed consent must be obtained prior to any procedures
2. Age 18 years.
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:
 - Disease A
 - Disease B
 - Disease C
4. ECOG Performance Status 0 – 1 (or 2 TBC)
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.
6. Prior therapy with the secondary agent or related compounds (i.e. same MOA) is allowed

Exclusion criteria

Patients eligible for this study must not meet any of the following criteria:

1. History of severe hypersensitivity reactions to other mAbs (OR to same backbone mAb as in ADC OR to same IO mAb if applicable)
2. Known history of positive serum human ADA to backbone of mAb as in ADC
3. Central Nervous System (CNS) disease only (if applicable)
4. Symptomatic CNS metastases or evidence of leptomeningeal disease (brain MRI or previously documented cerebrospinal fluid (CSF) cytology)
 - 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)
 - Patients with discrete dural metastases are eligible.
5. Patient having out of range laboratory values defined as:
 - 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
 - Total bilirubin $> 1.5 \times \text{ULN}$, except for patients with Gilbert's syndrome who

are excluded if total bilirubin > 3.0 x ULN or direct bilirubin > 1.5 x ULN

- Alanine aminotransferase (ALT) > 3 x ULN, except for patients that have tumor involvement of the liver, who are excluded if ALT > 5 x ULN
- Aspartate aminotransferase (AST) > 3 x ULN, except for patients that have tumor involvement of the liver, who are excluded if AST > 5 x ULN
- Absolute neutrophil count < 1.0 x 10⁹/L
- Platelet count < 75 x 10⁹/L
- Hemoglobin (Hgb) < 8 g/dL
- Potassium, magnesium, calcium or phosphate abnormality > CTCAE grade 1 despite appropriate replacement therapy

6. Impaired cardiac function or clinically significant cardiac disease, including any of the following:

- 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.
- QTcF >470 msec for females or >450 msec for males on screening ECG using Fridericia's correction, congenital long QT syndrome
- Acute myocardial infarction or unstable angina pectoris < 3 months (months prior to study entry)
- Clinically significant valvular disease with documented compromise in cardiac function
- Symptomatic pericarditis
- History of or ongoing documented cardiomyopathy
- Left Ventricular Ejection Fraction (LVEF) <40%, as determined by echocardiogram (ECHO) or Multi gated acquisition (MUGA) scan
- 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)
- Presence of unstable atrial fibrillation (ventricular response rate > 100 bpm).
 - NOTE: Patients with stable atrial fibrillation can be enrolled provided they do not meet other cardiac exclusion criteria.
- Complete left bundle branch block (LBBB), bifascicular block
- Any clinically significant ST segment and/or T-wave abnormalities

7. Toxicity attributed to prior IO 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.

8. Patients with active, known or suspected autoimmune disease. Subjects with vitiligo, type I 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.

9. Human Immunodeficiency Virus (HIV), or active Hepatitis B (HBV) or Hepatitis C (HCV) virus infection

- 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).
- 5 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.
- 10 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.
- 15 12. Active diarrhea CTCAE grade 2 or a medical condition associated with chronic diarrhea (such as irritable bowel syndrome, inflammatory bowel disease)
- 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.
- 20 14. Active infection requiring systemic antibiotic therapy.
- 15. Active ulceration of the upper GI tract or GI bleeding
- 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)
- 17. Active autoimmune disease, motor neuropathy considered of autoimmune origin, and other CNS autoimmune disease
- 25 18. Patients requiring concomitant immunosuppressive agents or chronic treatment with corticoids except:
 - replacement dose steroids in the setting of adrenal insufficiency
 - topical, inhaled, nasal and ophthalmic steroids are allowed
- 30 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)
- 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.
- 35 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).
- 22. Radiotherapy within 2 weeks of the first dose of study drug, except for
- 40 palliative radiotherapy to a limited field, such as for the treatment of bone pain or a focally painful tumor or mass. To allow for assessment of response to treatment, patients must have remaining measurable disease that has not been irradiated
- 23. Participation in an interventional, investigational study within 2 weeks of the first dose of study treatment.
- 45 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.

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.

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.

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:

- 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
- 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
- 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.
- 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.
 - 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.
 - 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

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

A **hematologic DLT** is defined as:

- 5 ▪ Grade 3 or 4 febrile neutropenia or neutropenic infection
- Grade 4 neutropenia lasting >7 days
- Grade 4 thrombocytopenia
- Grade 3 thrombocytopenia with clinically significant bleeding, or Grade 3 thrombocytopenia requiring a platelet transfusion
- 10 ▪ Grade 3 anemia that requires transfusion
- Grade 4 anemia

A **non-hematologic DLT** is defined as:

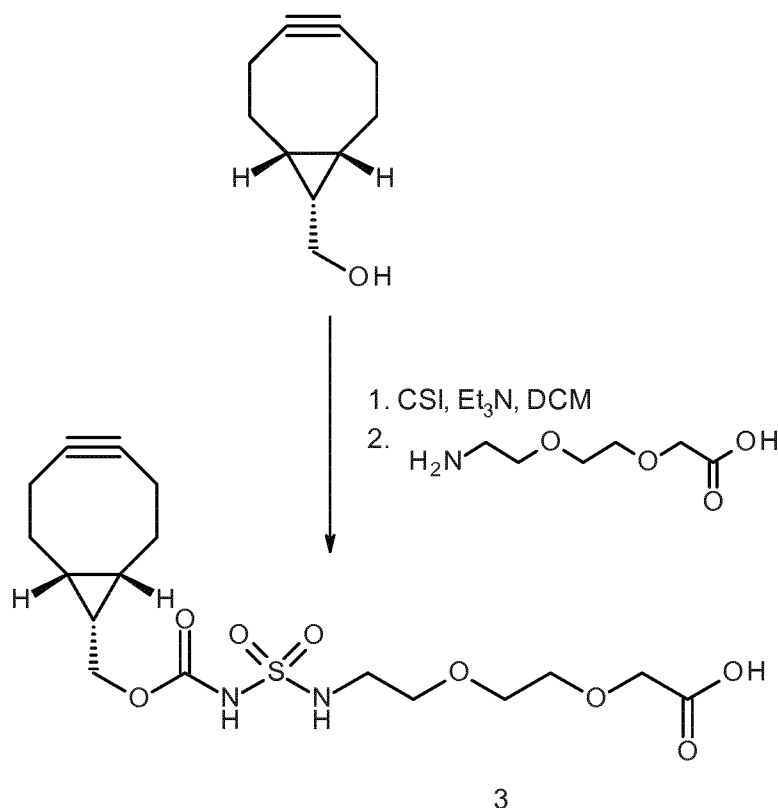
- 15 ▪ Grade 4 non-hematologic toxicity
- Grade 3 non-hematologic toxicity lasting >3 days despite optimal supportive care or medical intervention
- A case of Hy's law (AST and/or ALT > 3x ULN and bilirubin > 2x ULN, and without initial findings of cholestasis (serum alkaline phosphatase (ALP) activity < 2x 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)
- 20 ▪ Grade 3 or higher hypersensitivity/infusion-related reaction (regardless of premedication). A grade 3 hypersensitivity / infusion-related reaction that resolves within 8 hours after onset with appropriate clinical management does not qualify as a DLT.
- 25 ▪ LVEF decrease to < 40% or >20% decrease from baseline
- Grade 4 tumor lysis syndrome (Grade 3 TLS will not constitute DLT unless it leads to irreversible end-organ damage)
- 30 The following conditions are not considered non-hematologic DLT:
 - Grade 3 fatigue for ≤ 7 days
 - 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.
- 35 • AST or ALT elevation ≥ 5 x ULN but ≤ 8 x ULN, without concurrent elevation in bilirubin, that downgrades to ≤ Grade 2 within 5 days after onset.
- Grade 3 serum lipase or serum amylase for ≤ 7 days if without clinical signs or symptoms of pancreatitis

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.

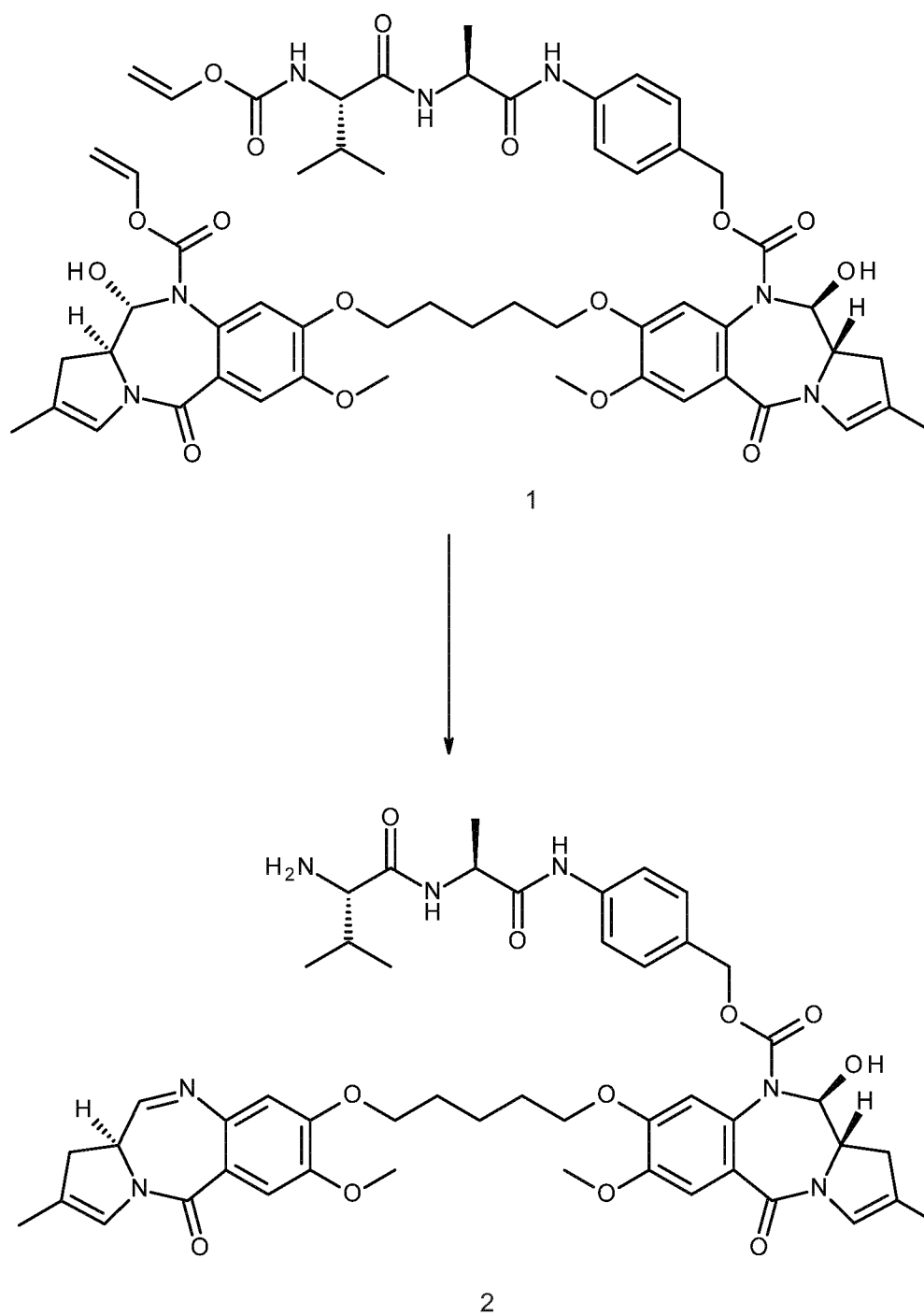
5 Dose modifications

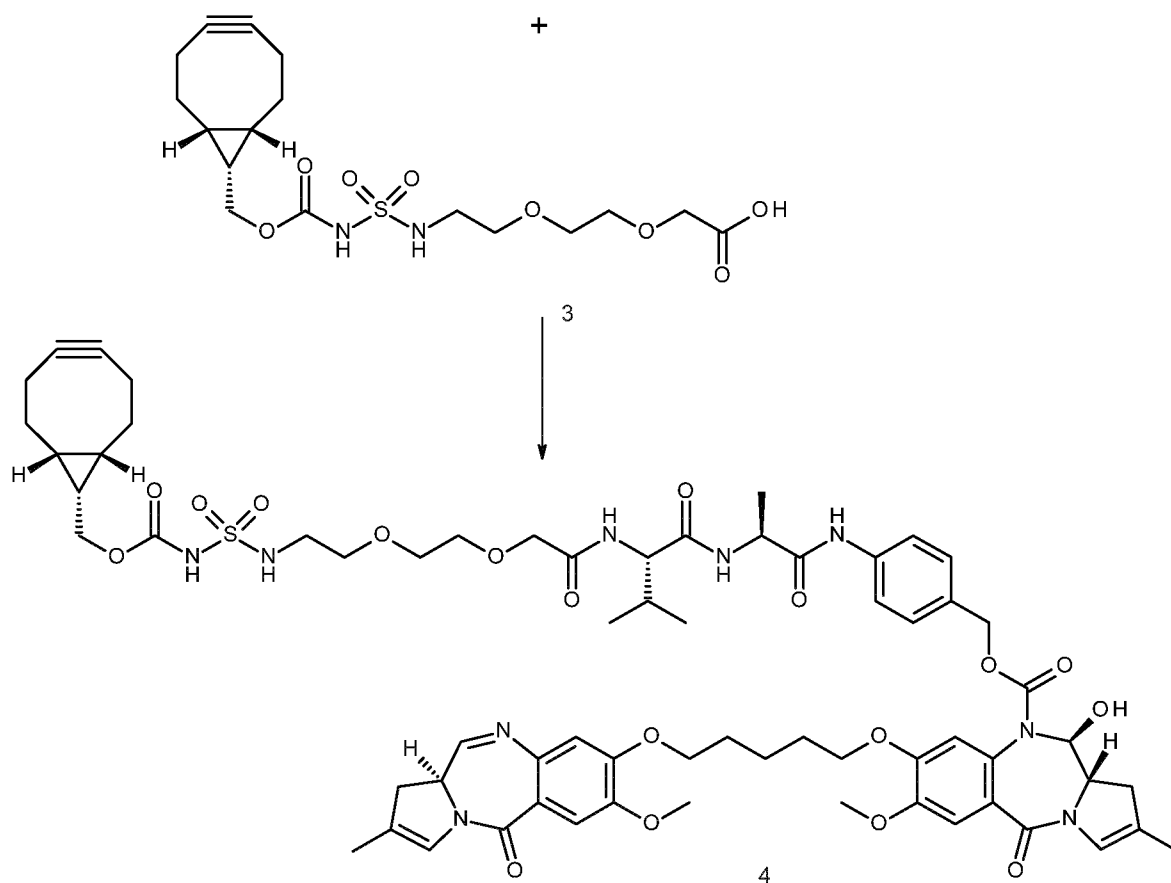
Guidelines for management of specific toxicities are detailed in the table below. For management of events not specified in the tables, the following may serve as a guidance to investigators:

AE Grade	ADC Management Guideline
1	No dose adjustment is required.
2	<p><u>First occurrence:</u> 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 \leq 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.</p> <p>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.</p> <p><u>Second occurrence:</u> 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 resolution. If improvement to \leq 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.</p> <p><u>Third occurrence:</u> Permanently discontinue one or both drugs.</p>
3	<p><u>First occurrence:</u> 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.</p> <p><u>Second occurrence:</u> Permanently discontinue one or both drugs</p>
4	Permanently discontinue one or both drugs.

EXAMPLE 4: Synthesis of Intermediate 3

A solution of BCN alcohol (0.384 g, 2.55 mmole) in MeCN (25 mL) under a N₂ atmosphere was cooled to 0 °C, and chlorosulfonyl isocyanate (CSI) was added dropwise (0.255 mL, 415 mg, 2.93 mmole, 1.15 equiv.). After stirring for 15 minutes, Et₃N 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 H₂O (5 mL) was added and the reaction mixture was stirred to room temperature for 2 h. After this time, CHCl₃ (50 mL) and H₂O (100 mL) were added, and the layers were separated. To the aqueous layer in a separatory funnel was added CH₂Cl₂ (100 mL) and the pH was adjusted to 4 with 1 N HCl, before separation of layers. The water layer was extracted twice with CH₂Cl₂ (2 × 100 mL), the organic layers were combined and dried (Na₂SO₄), filtered and concentrated. The residue was purified by flask column chromatography on silica, elution with CH₂Cl₂ to 20% MeOH in CH₂Cl₂. Yield 0.42 g (1.0 mmole, 39%) of **3** as a colorless sticky wax.

EXAMPLE 5: Synthesis of Drug Linker



Compound 1 can be synthesised as described in WO2014/057074 – see compound 22.

- 5 (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
- 10 $\text{Pd}(\text{PPh}_3)_4$ 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 (Na_2SO_4) and concentrated. The residue is purified by RP-HPLC (30–90% MeCN (0.1% formic acid) in H_2O (0.1% formic acid).
- 15 The combined fractions are passed through SPE (HCO_3^-) columns and concentrated. After addition of MeCN (50 mL) the mixture is again concentrated. The resulting residue **2** is used in the next step.

20 The conversion of the reaction can be monitored through LCMS analysis. Column: XBridge BEH C18 Intelligent Speed (IS) Column, 130Å, 3.5 μm (4.6 mm x 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.

(b) To a solution of the above residue **2** in CHCl_3 (5 mL) is added a solution of **3** (15 mg, 36 μmol , mw 418 g/mole) in CHCl_3 (0.8 mL). The resulting mixture is added to solid EDC.HCl (4.7 mg, 25 μmol), CHCl_3 (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 (Na_2SO_4) and concentrated. The residue is purified by RP-HPLC (30–90% MeCN (no acid) in H_2O (0.01% formic acid)). The HPLC collection tubes are filled with 5% aqueous $(\text{NH}_4)\text{HCO}_3$ before collection. The combined HPLC fractions are extracted with DCM (3×20 mL). The combined organic layers are dried (Na_2SO_4) and concentrated. The product **4** is obtained as slightly yellow/white oil (21 mg, 16 μmol , mw 1323 g/mole, 67% over two steps).

The conversion of the reaction can be monitored through LCMS analysis. Column: XBridge BEH C18 Intelligent Speed (IS) Column, 130Å, 3.5 μm (4.6 mm x 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

The reaction conditions for the one-pot glycan remodelling are:

15 mg/ml AXL Antibody (~0.1 mM)

0.15 mg/mL EndoSH (1% w/w) from *Streptococcus pyogenes*

1.13 mg/mL His-TnGalNAcT (7.5% w/w) Galactose-N-acetyl Transferase (GalNAcT) enzyme

2.5 mM 6-N₃GalNAc-UDP (25 eq. compared to IgG)

10mM MnCl_2

25 mM TrisHCl pH 8.0

150 mM NaCl

Incubate 16 hours at 30°C

Procedure

This example is on a 25 mg-scale, which may be altered as necessary. The individual components are added in order and mixed:

106.5 μL 25 mM Tris pH 8.0, 150 mM NaCl (to obtain a final volume of 1667 μL)

1 mL 25 mg/mL AXL Antibody in 25 mM Tris pH 8.0, 150 mM NaCl

71.4 μL 3.5 mg/mL EndoSH in 25 mM Tris pH 8.0

389 μL 4.82 mg/mL His-TnGalNAcT in 25 mM Tris pH 8.0

16.7 μL 1M MnCl_2 in MQ

83.4 μL 0.1 M 6-N₃GalNAc-UDP in MQ

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 ($\pm 10\%$ of total heavy chain), resulting from modified galactose transfer to core GlcNAc (without Fucose) substituted Ab.

5 *Purification procedure*

Buffers

Binding/wash buffer (TBS pH 7.5):

20 mM TrisHCl pH 7.5

150 mM NaCl

10 Wash buffer for endotoxin removal (TBS pH 7.5 + Triton-X100):

20 mM TrisHCl pH 7.5

150 mM NaCl

0.2% Triton X-100

Elution buffer:

15 0.1 M Glycine pH 2.7

CIP buffer:

0.5 M NaOH

Procedure

20 1. Wash the MabSelectSure 5 mL column (5 mL/min) with the following buffers in order to clean the column before applying the sample:

Wash column with at least 5 column volumes (CV) TBS pH 7.5

Wash column with 15 CV 0.5 M NaOH

Wash column with 5 CV TBS pH 7.5

25 Wash column with 5 CV Glycine pH 2.7

Wash column with TBS pH 7.5 until a natural pH is obtained

2. Remove precipitation from reaction mixture by centrifugation (5 min at 4000g) or by filtration (0.22 or 0.45 μ m filter)

3. Load sample at 2 mL/min and perform the following steps with 5 mL/min:

30 Wash with at least 20 CV TBS = 0.2% Triton X-100

Wash with at least 20 CV TBS

Elute with 0.1 M Glycine pH 2.7

4. Immediately neutralize fractions by adding 1/5 volume of 1 M Tris-HCl pH 8.0 and mixing

35 5. Dialyze sample against 3 x ≥ 50 volumes of PBS pH 7.4 at 4°C (3 x ≥ 1 hour)

6. Concentrate sample using spinfilter devices to ~ 20 mg/mL

EXAMPLE 7: Conjugation of 4 to modified antibody to produce ConjA

Reaction conditions

40 15 mg/ml azido-modified AXL antibody (0.1 M IgG)

0.5 mM 4 (5 eq. compared to IgG = 2.5 eq per azide)

10% DMF or 25% propyleneglycol

PBS pH7.4

45 *Procedure*

1. Add 9 vols of 16.67 mg/ml azido-modified antibody in PBS pH7
2. Add 1 vol of 5 mM **4** in DMF and mix immediately.
3. Incubate overnight.
4. Measure conversion by RP-HPLC and MS.

5

EXAMPLE 8: Purification of ADC*Sample preparation*

The following requirements should be met before loading onto the column:

Organic solvent $\leq 5\%$

10 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)

No precipitants

The above requirements can be accomplished using the following procedure:

- 15 1. Dilute sample with PBS pH7.4 to a final organic solvent concentration of $\leq 5\%$
2. If volume exceeds 3% of the CV, the sample was concentrated using Amicon Ultra centrifugal filters (MWCO 10kDa)
3. Potential precipitation is removed by centrifugation (10 min at 13000 rpm in a table top centrifuge)

20

Purification

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:

25 Wash column with 1 CV water

Wash column with 1 CV 0.5 M NaOH.

Equilibrate column with PBS pH 7.4 (Sigma, D8537) until neutral pH is obtained.

30 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 3x1L of formulation buffer (30 mM histidine, 200mM 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.

35 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

40 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 8 x 10-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, CA). Error bars indicate standard deviation (SD).

The EC₅₀ of ConjA was found to be 0.0554 µg/mL.

EXAMPLE 10: Antigen binding study

Maxisorp plates were coated at +4°C overnight with human Axl 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 8 x 3-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, CA). Figure 2 shows the results obtained, where ▲ 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

5 x 10⁶ 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 administrated 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, CA) was used for graphical presentations and statistical analyses. Figure 3 shows the results obtained, where ▲ is ConjA, and ● is the vehicle alone. Error bars indicate SEM.

- 5 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

- 10 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
15 sampling for pharmacokinetics. At necropsy, macroscopic observations were taken with selected organs weighed and retained for possible histopathology.

- 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
20 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.

25 EXAMPLE 13: Synergy in SN12C cells (AXL high-expressing) between ADCxAXL and each of Cytarabine, Decitabine, Gemcitabine, Olaparib, and Fludarabine

- 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
30 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.

- 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
35 (3 x cell doubling time). Absorption was analysed at 492nm on a Thermo Labsystems Multiscan Ascent plate reader using the MTT assay.

- 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
40 synergism carries a CI value of >0.7 and <1.

Results are shown in Figure 4 (Cytarabine), Figure 5 (Fludarabine), Figure 6 (Decitabine), Figure 7 (Olaparib), and Figure 8 (Gemcitabine).

EXAMPLE 14: synergy between ADCxAXL and each of MEKi and BRAFiMEKi

Examples of suitable MEK inhibitors such as Trametinib, Cobimetinib, Binimetinib, Selumetinib, U0126 or PD325901.

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

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.

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 mechanisms 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.

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.

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.

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.

After incubation the in vitro cytotoxicity of the combinations (as determined by CellTiter-Glo® or MTS assays) will be measured. 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.

BRAFi

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

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.

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.

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.

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.

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

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.

Typically, the ADC is dosed as a single dose between 0.1 and 1 mg/kg, while the PD1 antagonist is dosed Q3d x 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.

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

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.

Typically, the ADC is dosed as a single dose between 0.1 and 1 mg/kg, while the PD1 antagonist is dosed Q3d x 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.

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

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.

Typically, the ADC is dosed as a single dose between 0.1 and 1 mg/kg, while the CLTA4 antagonist is dosed Q3d x 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.

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

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. or 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.

Typically, the ADC is dosed as a single dose between 0.1 and 1 mg/kg, while the OX40 agonist is dosed Q3d x 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.

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

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.

Typically, the ADC is dosed as a single dose between 0.1 and 1 mg/kg, while the GITR agonist is dosed Q3d x 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.

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

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.

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.

Accordingly, through these two mechanisms, tumors not expressing AXL can be killed by targetting 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

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 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 PD1 antagonist, concomitantly with the PD1 antagonist, or after the PD1 antagonist, as decided by the experimenter.

Typically, the ADC is dosed as a single dose between 0.1 and 1 mg/kg, while the PD1 antagonist is dosed Q3d x 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),
5 completely responding (CR) tumor free surviving (TFS) mice is determined in each group.

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.
10

PD-L1 antagonists

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,
15 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
20 antagonist, as decided by the experimenter.

Typically, the ADC is dosed as a single dose between 0.1 and 1 mg/kg, while the PDL1 antagonist is dosed Q3d x 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
25 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.

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
30 PDL1 antagonist alone.

CTLA4 antagonists

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
35 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 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
40 before the CTLA4 antagonist, concomitantly with the CTLA4 antagonist, or after the CTLA4 antagonist, as decided by the experimenter.

Typically, the ADC is dosed as a single dose between 0.1 and 1 mg/kg, while the CTLA4 antagonist is dosed Q3d x 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.

- 5 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

- 10 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
15 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.

- 20 Typically, the ADC is dosed as a single dose between 0.1 and 1 mg/kg, while the OX40 agonist is dosed Q3d x 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.

- 25 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

- 30 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
35 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.

- 40 Typically, the ADC is dosed as a single dose between 0.1 and 1 mg/kg, while the GITR agonist is dosed Q3d x 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.

- 5 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.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method for treating a cancer in an individual, the method comprising administering to the individual an effective amount of an anti-AXL antibody drug conjugate (ADC) and a secondary agent,

wherein the secondary agent is a PD1 antagonist, a PD-L1 antagonist, a GITR (Glucocorticoid-Induced TNFR-Related protein) agonist, a OX40 agonist, a PARP inhibitor (PARPi), an AXL-kinase inhibitor (AXLi), or a BRAF inhibitor (BRAFi); and

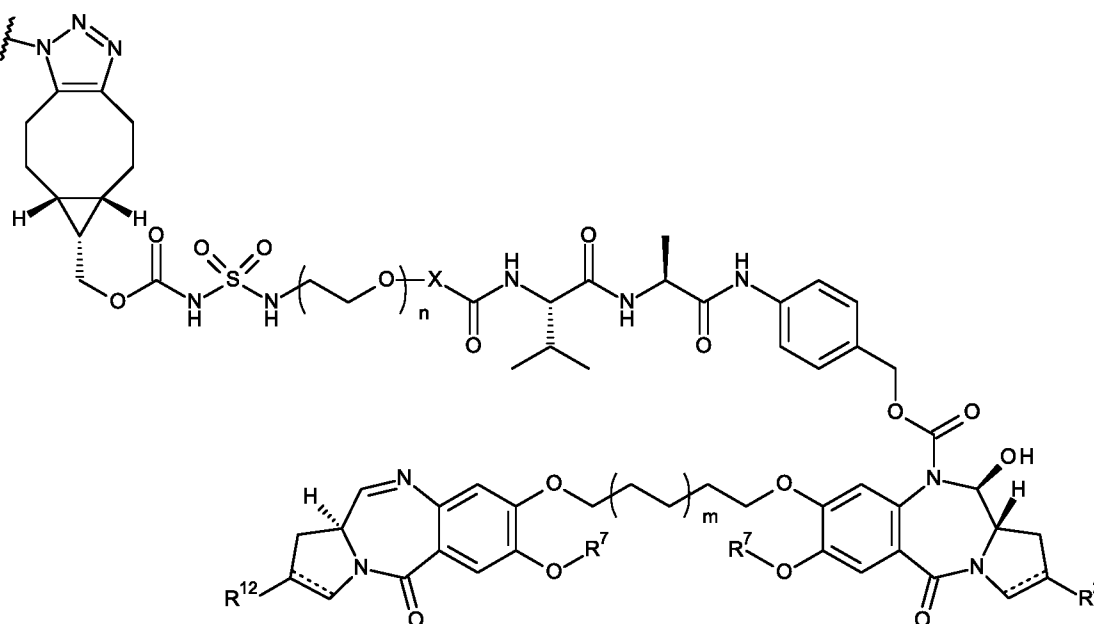
wherein the anti-AXL ADC is a conjugate of formula (I):



wherein:

Ab is an antibody that binds to AXL;

DL is



wherein:

X is selected from the group comprising: a single bond, $-\text{CH}_2-$ and $-\text{C}_2\text{H}_4-$;

n is from 1 to 8;

m is 0 or 1;

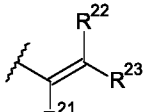
R^7 is either methyl or phenyl;

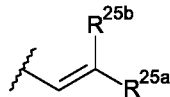
when there is a double bond between C2 and C3, R^2 is selected the group consisting of:

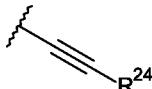
(ia) C_{5-10} aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, carboxy, ester, C_{1-7} alkyl, C_{3-7} heterocyclyl and bis-oxy- C_{1-3} alkylene;

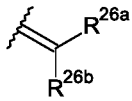
(ib) C_{1-5} saturated aliphatic alkyl;

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

(id) , 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^{12} group is no more than 5;

(ie)  , 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

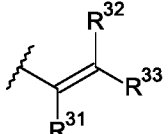
(if)  , where R^{24} is selected from: H; C_{1-3} saturated alkyl; C_{2-3} alkenyl; C_{2-3} alkynyl; cyclopropyl; phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl; when there is a single bond between C2 and C3, R^2 is

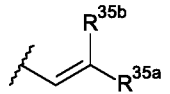
 , where R^{26a} and R^{26b} are independently selected from H, F, C_{1-4} saturated alkyl, C_{2-3} alkenyl, which alkyl and alkenyl groups are optionally substituted by a group selected from C_{1-4} alkyl amido and C_{1-4} alkyl ester; or, when one of R^{26a} and R^{26b} is H, the other is selected from nitrile and a C_{1-4} alkyl ester; when there is a double bond between C2' and C3', R^{12} is selected the group consisting of:

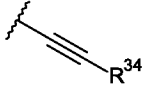
(ia) C_{5-10} aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, carboxy, ester, C_{1-7} alkyl, C_{3-7} heterocyclyl and bis-oxy- C_{1-3} alkylene;

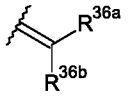
(ib) C_{1-5} saturated aliphatic alkyl;

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

(id)  , 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;

(ie)  , 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

(if)  , where R^{34} 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; when there is a single bond between C2' and C3', R^{12} is

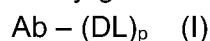
 , where R^{36a} and R^{36b} are independently selected from H, F, C_{1-4} saturated alkyl, C_{2-3} alkenyl, which alkyl and alkenyl groups are optionally substituted by a group

selected from 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.

2. Use of a first composition comprising an anti-AXL antibody drug conjugate (ADC) in the manufacture of a medicament for treating a cancer in an individual, wherein the first composition is to be administered in combination with a second composition comprising a secondary agent,

wherein the secondary agent is a PD1 antagonist, a PD-L1 antagonist, a GITR (Glucocorticoid-Induced TNFR-Related protein) agonist, a OX40 agonist, a PARP inhibitor (PARPi), an AXL-kinase inhibitor (AXLi), or a BRAF inhibitor (BRAFi); and

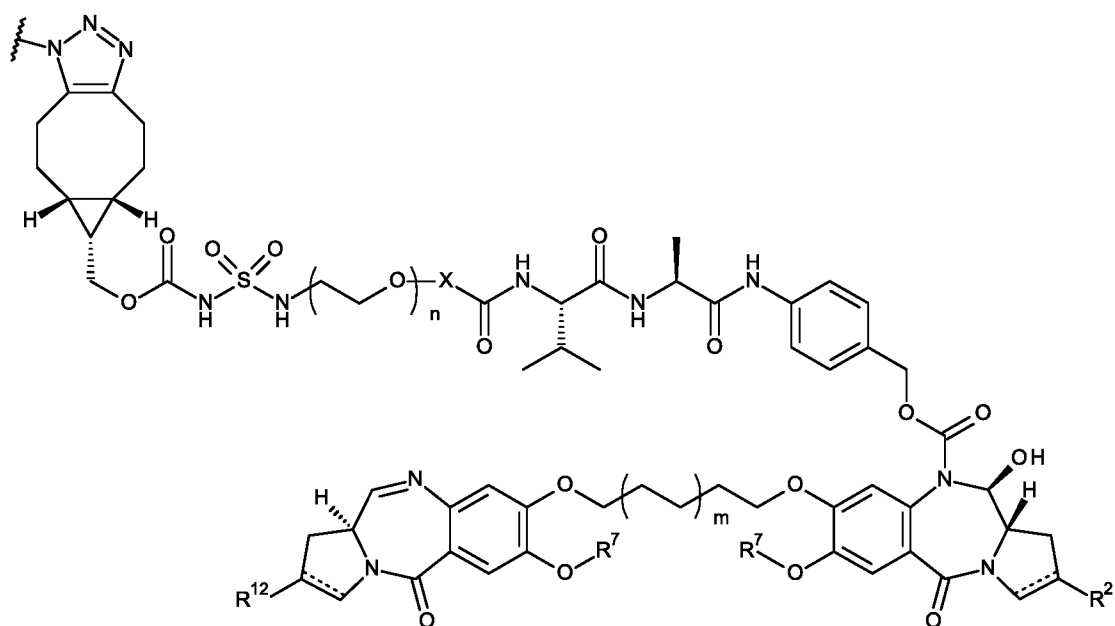
wherein the anti-AXL ADC is a conjugate of formula (I):



wherein:

Ab is an antibody that binds to AXL;

DL is



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;

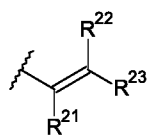
R⁷ is either methyl or phenyl;

when there is a double bond between C2 and C3, R² is selected the group consisting of:

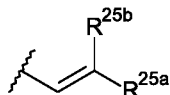
(ia) C₅₋₁₀ aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, carboxy, ester, C₁₋₇ alkyl, C₃₋₇ heterocyclyl and bis-oxy-C₁₋₃ alkylene;

(ib) C₁₋₅ saturated aliphatic alkyl;

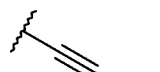
(ic) C₃₋₆ saturated cycloalkyl;



(id) , 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^{12} group is no more than 5;

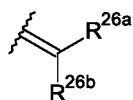


(ie) , wherein one of R^{25a} and R^{25b} is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl; and



(if) , 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;

when there is a single bond between C2 and C3, R^2 is



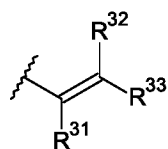
, where R^{26a} and R^{26b} are independently selected from H, F, C_{1-4} saturated alkyl, C_{2-3} alkenyl, which alkyl and alkenyl groups are optionally substituted by a group selected from C_{1-4} alkyl amido and C_{1-4} alkyl ester; or, when one of R^{26a} and R^{26b} is H, the other is selected from nitrile and a C_{1-4} alkyl ester;

when there is a double bond between C2' and C3', R^{12} is selected the group consisting of:

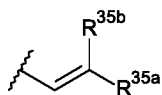
(ia) C_{5-10} aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, carboxy, ester, C_{1-7} alkyl, C_{3-7} heterocyclyl and bis-oxy- C_{1-3} alkylene;

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

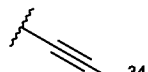
(ic) C_{3-6} saturated cycloalkyl;



(id) , 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;

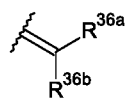


(ie) , wherein one of R^{35a} and R^{35b} is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl; and



(if) , where R^{34} 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;

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

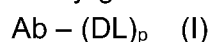


, where R^{36a} and R^{36b} are independently selected from H, F, C₁₋₄ saturated alkyl, C₂₋₃ alkenyl, which alkyl and alkenyl groups are optionally substituted by a group selected from C₁₋₄ alkyl amido and C₁₋₄ alkyl ester; or, when one of R^{36a} and R^{36b} is H, the other is selected from nitrile and a C₁₋₄ alkyl ester; and p is from 1 to 8.

3. Use of a first composition comprising a secondary agent in the manufacture of a medicament for treating a cancer in an individual, wherein the first composition is to be administered in combination with a second composition comprising an anti-AXL antibody drug conjugate (ADC),

wherein the secondary agent is a PD1 antagonist, a PD-L1 antagonist, a GITR (Glucocorticoid-Induced TNFR-Related protein) agonist, a OX40 agonist, a PARP inhibitor (PARPi), an AXL-kinase inhibitor (AXLi), or a BRAF inhibitor (BRAFi); and

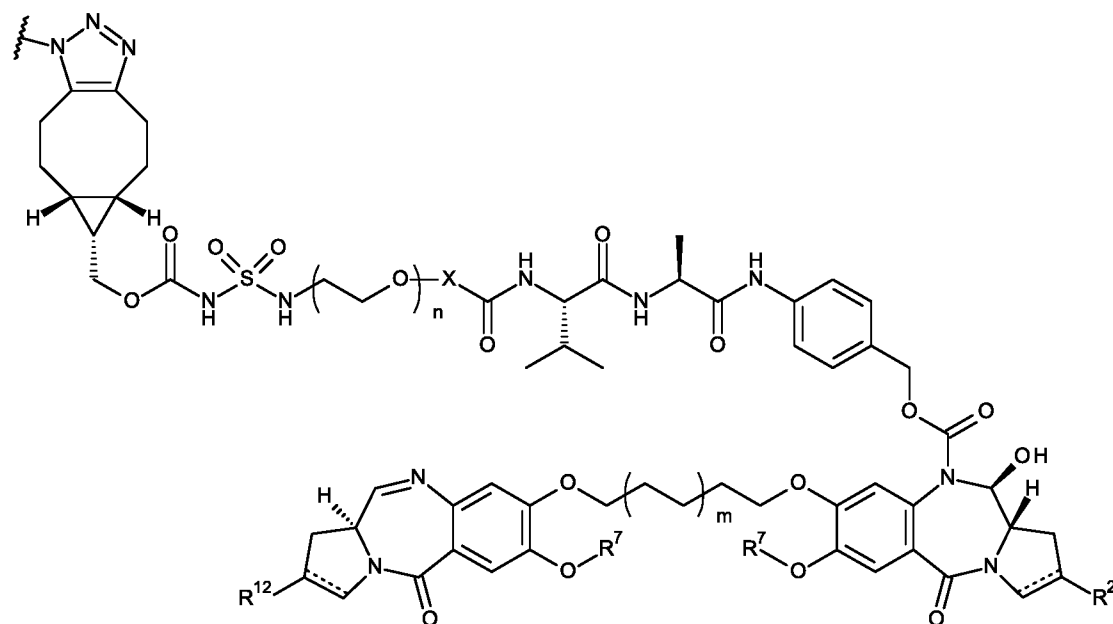
wherein the anti-AXL ADC is a conjugate of formula (I):



wherein:

Ab is an antibody that binds to AXL;

DL is



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;

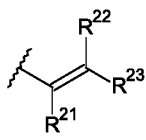
R⁷ is either methyl or phenyl;

when there is a double bond between C2 and C3, R² is selected the group consisting of:

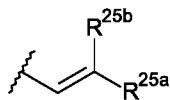
(ia) C₅₋₁₀ aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, carboxy, ester, C₁₋₇ alkyl, C₃₋₇ heterocyclyl and bis-oxy-C₁₋₃ alkylene;

(ib) C₁₋₅ saturated aliphatic alkyl;

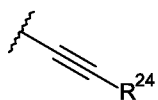
(ic) C₃₋₆ saturated cycloalkyl;



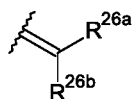
(id) , wherein each of R²¹, R²² and R²³ are independently selected from H, C₁₋₃ saturated alkyl, C₂₋₃ alkenyl, C₂₋₃ alkynyl and cyclopropyl, where the total number of carbon atoms in the R¹² group is no more than 5;



(ie) , 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) , 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

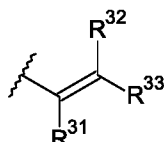


, where R^{26a} and R^{26b} are independently selected from H, F, C₁₋₄ saturated alkyl, C₂₋₃ alkenyl, which alkyl and alkenyl groups are optionally substituted by a group selected from C₁₋₄ alkyl amido and C₁₋₄ alkyl ester; or, when one of R^{26a} and R^{26b} is H, the other is selected from nitrile and a C₁₋₄ alkyl ester; when there is a double bond between C2' and C3', R¹² is selected the group consisting of:

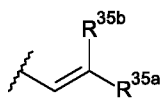
(ia) C₅₋₁₀ aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, carboxy, ester, C₁₋₇ alkyl, C₃₋₇ heterocyclyl and bis-oxy-C₁₋₃ alkylene;

(ib) C₁₋₅ saturated aliphatic alkyl;

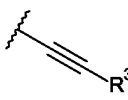
(ic) C₃₋₆ saturated cycloalkyl;

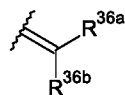


(id) , wherein each of R³¹, R³² and R³³ are independently selected from H, C₁₋₃ saturated alkyl, C₂₋₃ alkenyl, C₂₋₃ alkynyl and cyclopropyl, where the total number of carbon atoms in the R¹² group is no more than 5;



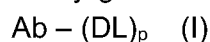
(ie) , wherein one of R^{35a} and R^{35b} is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl; and

(if) , where R³⁴ 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.

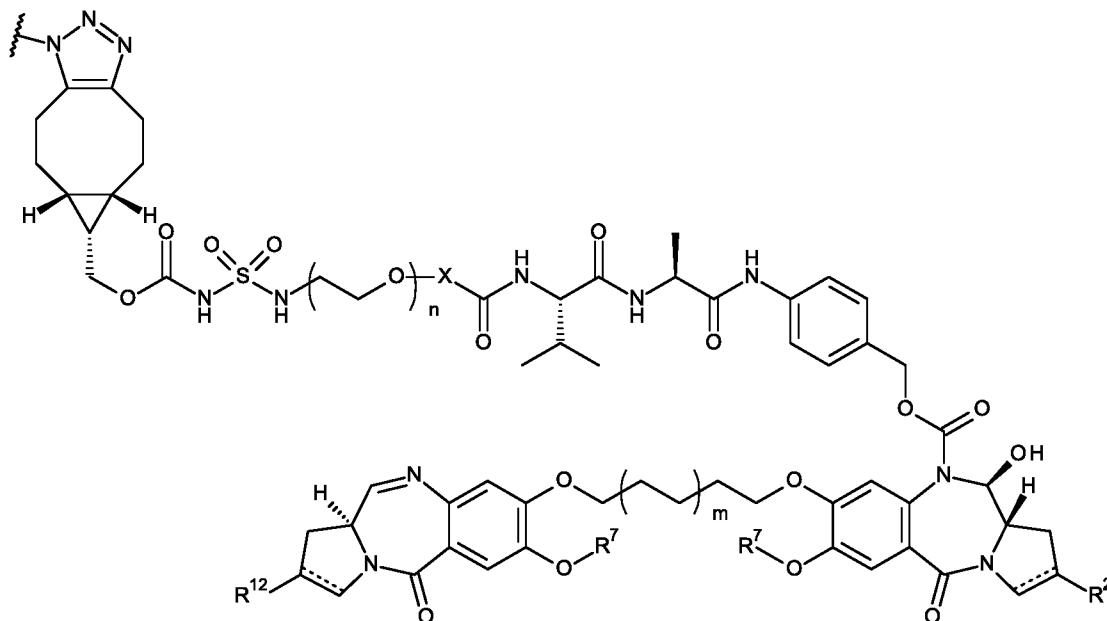
4. A kit when used for the treatment of a cancer comprising:
 a first medicament comprising an anti-AXL antibody drug conjugate (ADC);
 a second medicament comprising a secondary agent; and, optionally,
 a package insert comprising instructions for administration of the first medicament to an individual in combination with the second medicament for the treatment of cancer, wherein the secondary agent is a PD1 antagonist, a PD-L1 antagonist, a GITR (Glucocorticoid-Induced TNFR-Related protein) agonist, a OX40 agonist, a PARP inhibitor (PARPi), an AXL-kinase inhibitor (AXLi), or a BRAF inhibitor (BRAFi); and wherein the anti-AXL ADC is a conjugate of formula (I):



wherein:

Ab is an antibody that binds to AXL;

DL is



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;

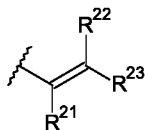
R⁷ is either methyl or phenyl;

when there is a double bond between C2 and C3, R² is selected the group consisting of:

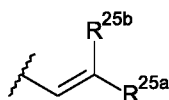
(ia) C₅₋₁₀ aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, carboxy, ester, C₁₋₇ alkyl, C₃₋₇ heterocyclyl and bis-oxy-C₁₋₃ alkylene;

(ib) C₁₋₅ saturated aliphatic alkyl;

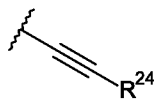
(ic) C₃₋₆ saturated cycloalkyl;



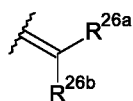
(id) , wherein each of R²¹, R²² and R²³ are independently selected from H, C₁₋₃ saturated alkyl, C₂₋₃ alkenyl, C₂₋₃ alkynyl and cyclopropyl, where the total number of carbon atoms in the R¹² group is no more than 5;



(ie) , 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) , 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



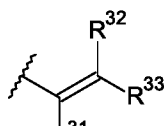
, where R^{26a} and R^{26b} are independently selected from H, F, C₁₋₄ saturated alkyl, C₂₋₃ alkenyl, which alkyl and alkenyl groups are optionally substituted by a group selected from C₁₋₄ alkyl amido and C₁₋₄ alkyl ester; or, when one of R^{26a} and R^{26b} is H, the other is selected from nitrile and a C₁₋₄ alkyl ester;

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

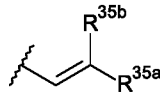
(ia) C₅₋₁₀ aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, carboxy, ester, C₁₋₇ alkyl, C₃₋₇ heterocyclyl and bis-oxy-C₁₋₃ alkylene;

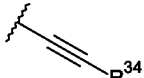
(ib) C₁₋₅ saturated aliphatic alkyl;

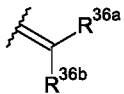
(ic) C₃₋₆ saturated cycloalkyl;



(id) , wherein each of R³¹, R³² and R³³ 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;

(ie) , 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

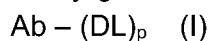
(if) , where R^{34} is selected from: H; C_{1-3} saturated alkyl; C_{2-3} alkenyl; C_{2-3} alkynyl; cyclopropyl; phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl; when there is a single bond between $C2'$ and $C3'$, R^{12} is

, where R^{36a} and R^{36b} 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^{36a} and R^{36b} is H, the other is selected from nitrile and a C_{1-4} alkyl ester; and p is from 1 to 8.

5. A pharmaceutical composition comprising an anti-AXL antibody drug conjugate (ADC) and a secondary agent,

wherein the secondary agent is a PD1 antagonist, a PD-L1 antagonist, a GITR (Glucocorticoid-Induced TNFR-Related protein) agonist, a OX40 agonist, a PARP inhibitor (PARPi), an AXL-kinase inhibitor (AXLi), or a BRAF inhibitor (BRAFi); and

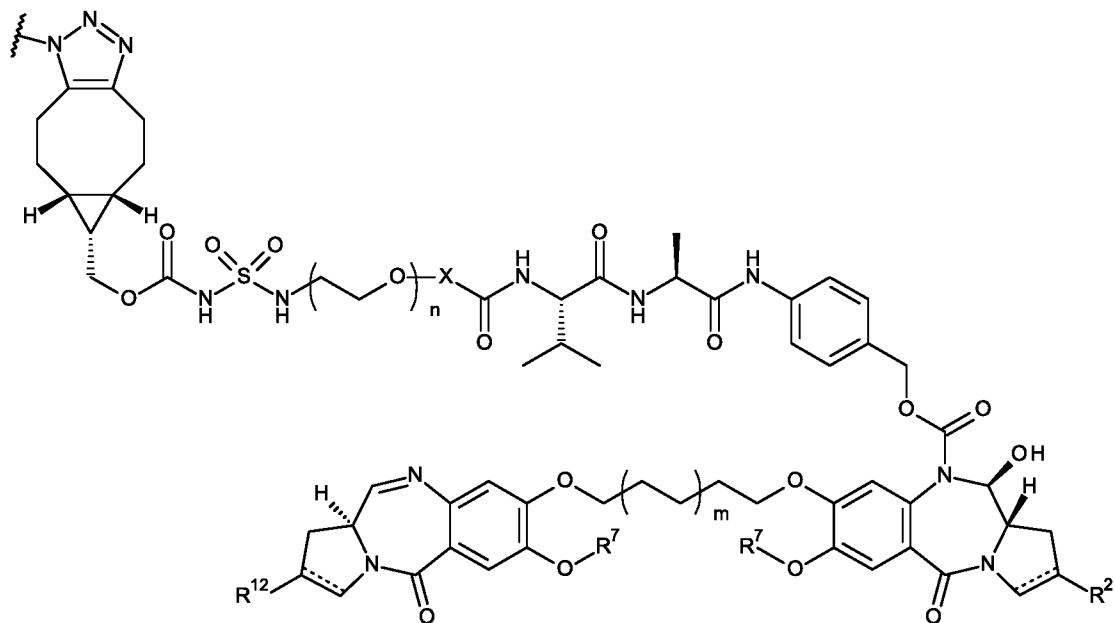
wherein the anti-AXL ADC is a conjugate of formula (I):



wherein:

Ab is an antibody that binds to AXL;

DL is



wherein:

X is selected from the group comprising: a single bond, $-\text{CH}_2-$ and $-\text{C}_2\text{H}_4-$;

n is from 1 to 8;

m is 0 or 1;

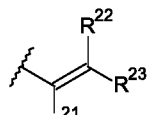
R^7 is either methyl or phenyl;

when there is a double bond between C2 and C3, R^2 is selected the group consisting of:

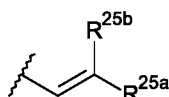
(ia) C_{5-10} aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, carboxy, ester, C_{1-7} alkyl, C_{3-7} heterocyclyl and bis-oxy- C_{1-3} alkylene;

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

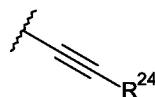
(ic) C_{3-6} saturated cycloalkyl;



(id) , 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^{12} group is no more than 5;

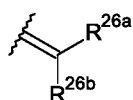


(ie) , wherein one of R^{25a} and R^{25b} is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl; and



(if) , 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;

when there is a single bond between C2 and C3, R^2 is



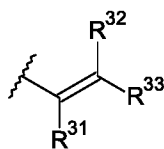
, where R^{26a} and R^{26b} are independently selected from H, F, C_{1-4} saturated alkyl, C_{2-3} alkenyl, which alkyl and alkenyl groups are optionally substituted by a group selected from C_{1-4} alkyl amido and C_{1-4} alkyl ester; or, when one of R^{26a} and R^{26b} is H, the other is selected from nitrile and a C_{1-4} alkyl ester;

when there is a double bond between C2' and C3', R^{12} is selected the group consisting of:

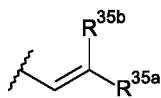
(ia) C_{5-10} aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, carboxy, ester, C_{1-7} alkyl, C_{3-7} heterocyclyl and bis-oxy- C_{1-3} alkylene;

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

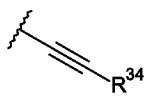
(ic) C_{3-6} saturated cycloalkyl;



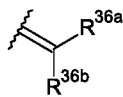
(id) , wherein each of R^{31} , R^{32} and R^{33} are independently selected from H, C_{1-3} saturated alkyl, C_{2-3} alkenyl, C_{2-3} alkynyl and cyclopropyl, where the total number of carbon atoms in the R^{12} group is no more than 5;



(ie) , 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



(if) , where R^{34} 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; when there is a single bond between $C2'$ and $C3'$, R^{12} is



, where R^{36a} and R^{36b} 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^{36a} and R^{36b} is H, the other is selected from nitrile and a C_{1-4} alkyl ester; and p is from 1 to 8.

6. A method of treating a cancer in an individual, the method comprising administering to the individual an effective amount of the composition of claim 5.

7. The use of the composition of claim 5 in the manufacture of a medicament for treating a cancer in an individual.

8. A kit comprising the composition of claim 5 and a set of instructions for administration of the medicament to an individual for the treatment of cancer.

9. The method or kit according to claim 1 or claim 4, wherein the treatment comprises administering the anti-AXL ADC before the secondary agent, simultaneous with the secondary agent, or after the secondary agent.

10. The method or kit according to any one of claims 1, 4, 6, 8 and 9, wherein the treatment further comprises administering a chemotherapeutic agent.

11. The use according to claim 2 or claim 3, wherein the anti-AXL ADC is to be administered before the secondary agent, simultaneous with the secondary agent, or after the secondary agent.

12. The use according to any one of claims 2, 3, and 7, wherein the medicament is to be administered in combination with a chemotherapeutic agent.

13. The method, use, or kit according to any one of claims 1-4 and 6-12, wherein the individual:

- (i) is human;
- (ii) has a cancer or has been determined to have cancer;
- (iii) has, or has been determined to have, a cancer characterised by the presence of a neoplasm comprising both AXL+ve and AXL-ve cells;
- (iv) has, or has been determined to have, a cancer characterised by the presence of a neoplasm comprising, or composed of, AXL-ve neoplastic cells;
- (v) has, or has been determined to have, a cancer which expresses AXL or AXL+ tumour-associated non-tumour cells, such as AXL+ infiltrating cells, optionally wherein the AXL+ infiltrating cells are dendritic cells, NK cells, or macrophages; and / or
- (vi) has, or has been determined to have, a cancer which expresses PD-L1.

14. The method, use, or kit according to any one of claims 1-4 and 6-13, wherein the treatment:

- a) effectively treats a broader range of cancers,
- b) effectively treats resistant, refractory, or relapsed cancers,
- c) has an increased response rate, and / or
- d) has increased durability;

as compared to treatment with either the anti-AXL ADC or the secondary agent alone.

15. The method, use, or kit according to any one of claims 1-4 and 6-14, wherein:

- (i) the cancer or neoplasm is all or part of a solid tumour;
- (ii) 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, and AML.

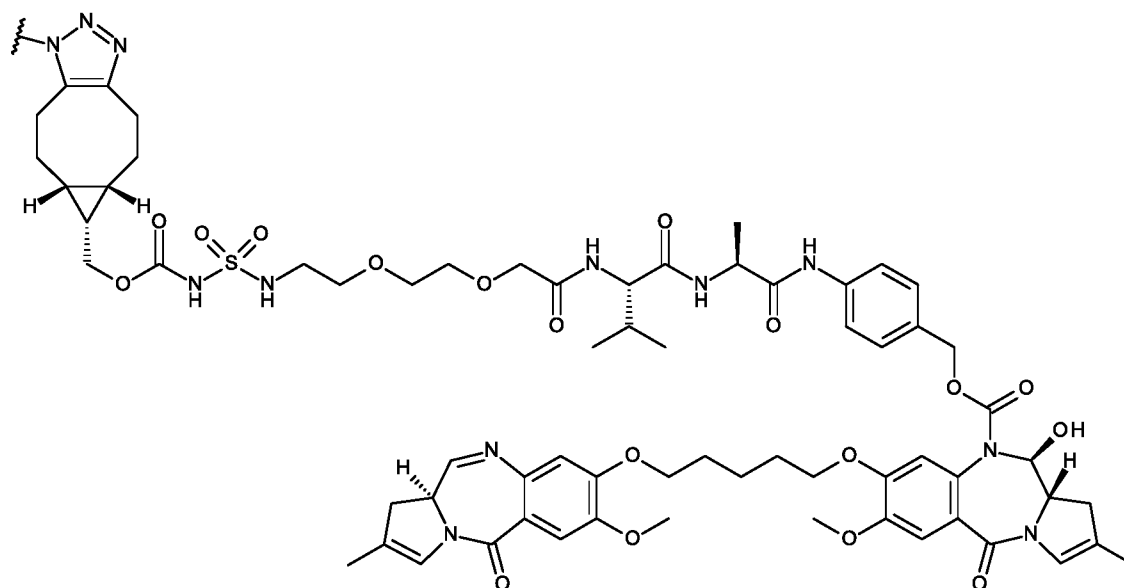
16. The method, use, kit, or pharmaceutical composition according to any one of the previous claims, wherein the anti-AXL ADC has the chemical structure:



wherein:

Ab is an antibody that binds to AXL; and

DL is:



17. The method, use, kit, or pharmaceutical composition according to any one of the previous claims, wherein Ab is an antibody comprising:

- (a) a heavy chain having the sequence according to SEQ ID NO. 3; and
- (b) a light chain having the sequence according to SEQ ID NO. 4.

18. The method, use, kit, or pharmaceutical composition according to any one of the previous claims, wherein the secondary agent is:

- (i) a PD1 antagonist selected from pembrolizumab, nivolumab, MEDI0680, PDR001 (spartalizumab), Camrelizumab, AUNP12, Pidilizumab Cemiplimab (REGN-2810), AMP-224, BGB-A317 (Tisleizumab), and BGB-108; or

- (ii) a PD-L1 antagonist selected from atezolizumab (Tecentriq), BMS-936559/MDX-1105, durvalumab/MEDI4736, and MSB0010718C (Avelumab).

19. The method, use, kit, or pharmaceutical composition according to any one of claims 1-17, wherein the secondary agent is:

- (i) a GPCR (Glucocorticoid-Induced TNFR-Related protein) agonist selected from MEDI1873, TRX518, GWN323, MK-1248, MK 4166, BMS-986156 and INCAGN1876;

- (ii) a OX40 agonist selected from MEDI0562, MEDI6383, MOXR0916, RG7888, OX40mAb24, INCAGN1949, GSK3174998, and PF-04518600;

- (iii) an AXL-kinase inhibitor (AXLi) selected from BGB324 (bemcentinib), TP0903, Gilteritinib (ASP2215), Cabozantinib (XL184), SGI7079, Merestinib, amuvatinib (MP-470), bosutinib (SKI-606), MGCD265, and foretinib (GSK1363089/XL880); or

- (iv) a BRAF inhibitor (BRAFi) selected from vemurafenib, PLX4720, dabrafenib, Sorafenib, Encorafenib, and GDC0879.

20. The method, use, kit, or pharmaceutical composition according to any one of claims 1-17, wherein the secondary agent is a PARP inhibitor (PARPi) 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.

21. The method, use, kit, or pharmaceutical composition according to any one of claims 1-17, wherein the secondary agent is Olaparib.

22. The method, use, kit, or pharmaceutical composition according to any one of claims 1-17, wherein the secondary agent is Olaparib and the anti-AXL ADC has the chemical structure:



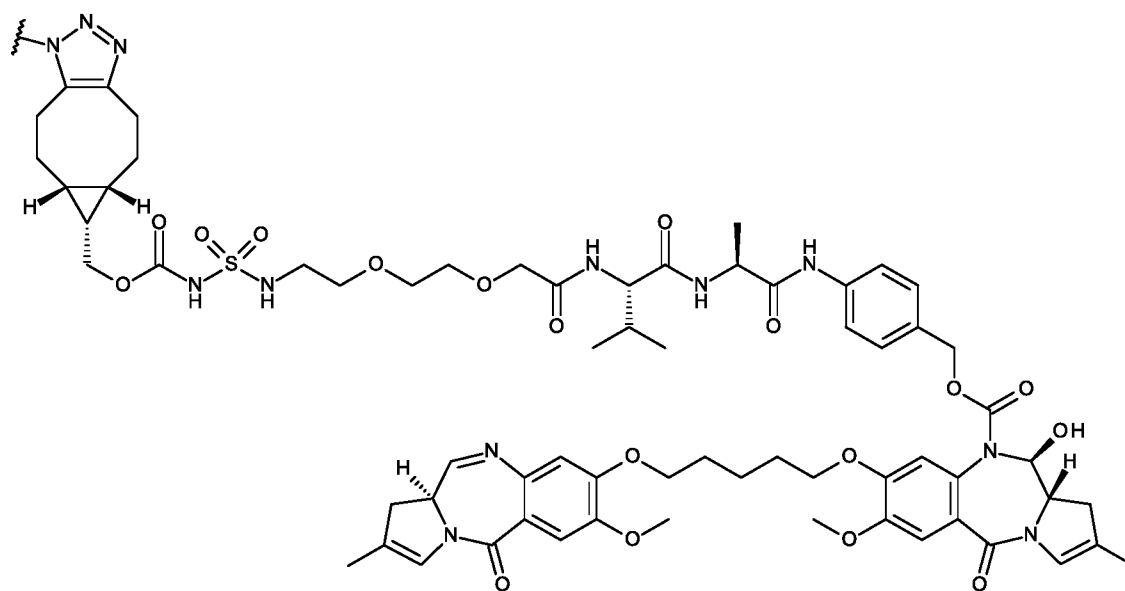
wherein:

Ab is an antibody that binds to AXL and which comprises:

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

(b) a light chain having the sequence according to SEQ ID NO. 4; and

DL is:



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

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGM^{SV}WRQAPGKGLEWVATISSGGSY
 TYYPDSVKGRFTISRDN^{SKNT}LYLQMNSLRAEDTAVYYCARHPIYYTYDDTMDYWGQG
 TTVTVSS

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

EIVLTQSPGTLSLSPGERATLSCSASSSVSSGNFHWYQQK^{PL}APRLLIYRTSNLASGIP
 ARFSGSGSGTDFTLTSSLEPEDFAVYYCQQWSGYPWTFGGGTKLEIK

SEQ ID NO.3 [1H12 Heavy Chain]

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGM^{SV}WRQAPGKGLEWVATISSGGSY
 TYYPDSVKGRFTISRDN^{SKNT}LYLQMNSLRAEDTAVYYCARHPIYYTYDDTMDYWGQG
 TTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
 FPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP
 CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNA
 KTKPREEQYN*STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
 QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFF
 LYSKLTVDKSRWQQGNV^FSCSV^MHEALHNHYTQKSLSLSPG

N* indicates Asn297 (numbering according to Kabat)

SEQ ID NO.4 [1H12 Light Chain]

EIVLTQSPGTLSLSPGERATLSCSASSSVSSGNFHWYQQK^{PL}APRLLIYRTSNLASGIP
 ARFSGSGSGTDFTLTSSLEPEDFAVYYCQQWSGYPWTFGGGTKLEIKRTVAAPSVFIFP
 PSDEQLKSGTASV^VCLLN^NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS
 TLTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

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]

EVKLLES GGGLVQPGGSLKLS CAASGFD FSRYWMSWVRQAPGKGLEWIG E INPDSSTI
NYTPSLKDKFIISRDNAKNTLYLQMSKVRSEDTALYYCAS PYYYGPFAYWGQGT LVTVS
S

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

DIVLTQSPASLAVSLGQR AII SCKASQSVSFAGTSLMHWYQQKPGQQPKLLIY RASNLEA
GFPTRFSGSGSRTDFTLNIHPVEEEDAATYYC QQSREYPRTFGGGTKLEVK

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]

QVQLVESGGGVVQPGRSLRLSCAASGFTFSRYWMSWVRQAPGKGLEWVAEINPDSST
INYTPSLKDRFAISRDN SKNTLYLQMNSLRAEDTAVYYCAS PYYYGPFAYWGQGT LVTVS
S

SEQ ID NO.20 [5F11 RHB]

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWMSWVRQAPGKGLEWVAEINPDSSTI
NYTPSLKDRFTISRDN AKNSLYLQMNSLRAEDTAVYYCAS PYYYGPFAYWGQGT LVTVS

SEQ ID NO.21 [5F11 RHC]

EVQLLES GGGLVQPGGSLRLSCAASGFTFSRYWMSWVRQAPGKGLEWVSEINPDSSTI
NYTPSLKDRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAS PYYYGPFAYWGQGT LVTVS

Figure 1B

SEQ ID NO.22 [5F11 RKA]

EIVLTQSPLSLPVTPGEPASISCKASQSVSFAGTSLMHWYLQKPGQSPQLLIYRASNLEA
GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCQQSREYPRTFGQGTKVEIK

SEQ ID NO.23 [Human Axl]

MAWRCPRMGRVPLAWCLALCGWACMAPRGTAEEESPFVGNPGNITGARGLTGTLRC
QLQVQGEPPEVHWLRDGGQILELADSTQTQVPLGEDEQDDWIVVSQLRITSLQLSDTGQY
QCLVFLGHQTFVSQPGYVGLEGLPYFLEEPEDRTVAANTPFNLSCQAQGPPEPVDLLW
LQDAVPLATAPGHGPPQRSLHVPGLNKTSSFSCEAHNAKGVTTSRTATITVLPQQPRNLH
LVSRQPTLEVAWTPGLSGIYPLTHCTLQAVLSDDGMGIQAGEPDPPEEPLTSQASVPP
HQLRLGSLHPHTPYHIRVACTSSQGPSSWTHWLPVETPEGVPLGPPENISATRNGSQAF
VHWQEPRAPLQGTLLGYRLAYQGGQDTPEVLMDIGLRQEVTLELQGDGSVSNLTVCVAA
YTAAGDGPWSLPVPLEAWRPGQAQPVHQLVKEPSTPAFSWPWWYVLLGAVVAAACVL
ILALFLVHRRKKETRYGEVFEPTVERGELVVRYRVRKSYSRRTTEATLNSLGISEELKEKL
RDVMVDRHKVALGKTLGEGEFGAVMEGQLNQDDSIKVAVKTMKIAICTRSELEDFLSE
AVCMKEFDHPNVMRLIGVCFQGGERESFPAPVVILPFMKHGDLSFLLYSRLGDQPVYL
PTQMLVKFMADIASGMEYLSTKRFIHRDLAARNCMLNENMSVCVADFGLSKKIYNGDYY
RQGRIAKMPVKWIAIESLADRVYTSKSDVWSFGVTMWEIATRGQTPYPGVENSEIYDYL
RRGNRLKQPADCLDGLYALMSRCWELNPQDRPSFTELREDLENTLKALPPAQEPDEILY
VNMDEGGGYPEPPGAAGGADPPTQPDPKDSCSCLTAAEVHPAGRYVLC PSTTPSPAQ
PADRGSPAAPGQEDGA

Figure 1C

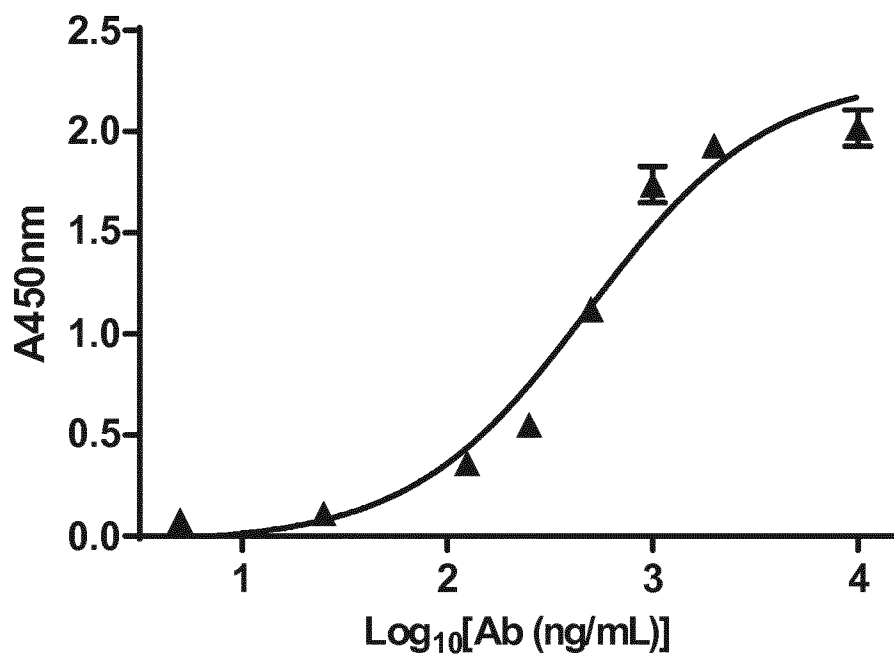


Figure 2

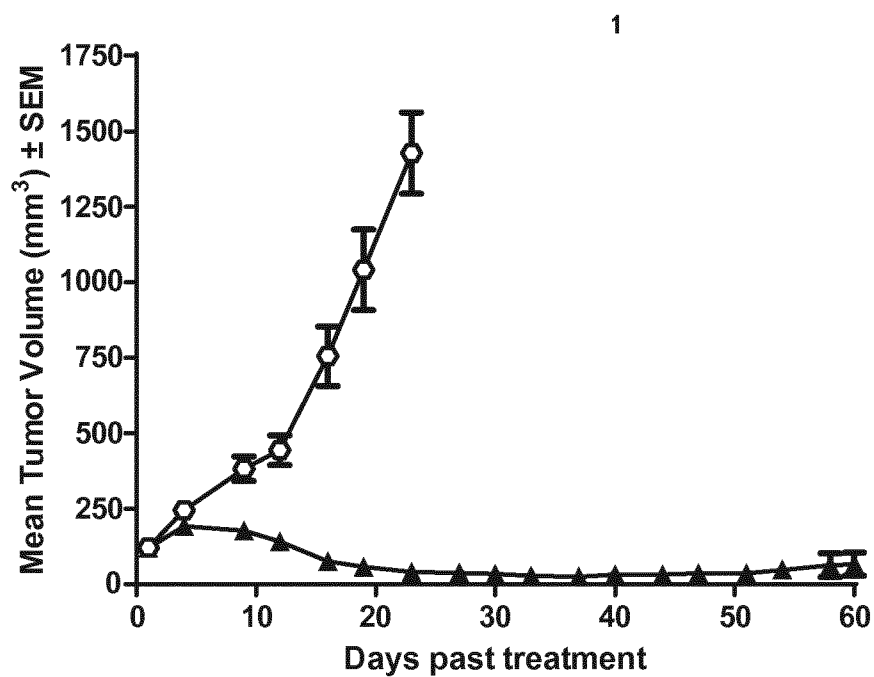


Figure 3

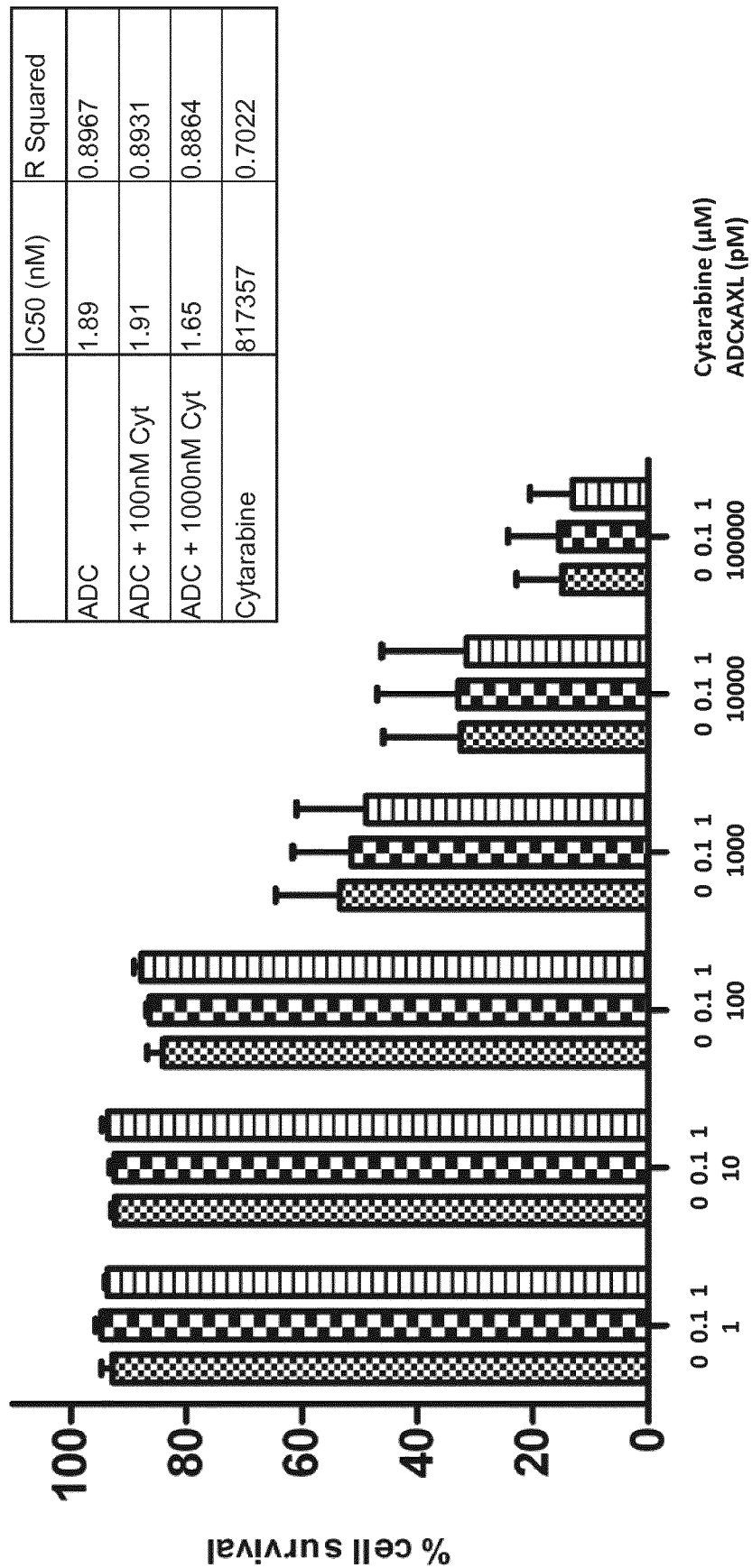


Figure 4

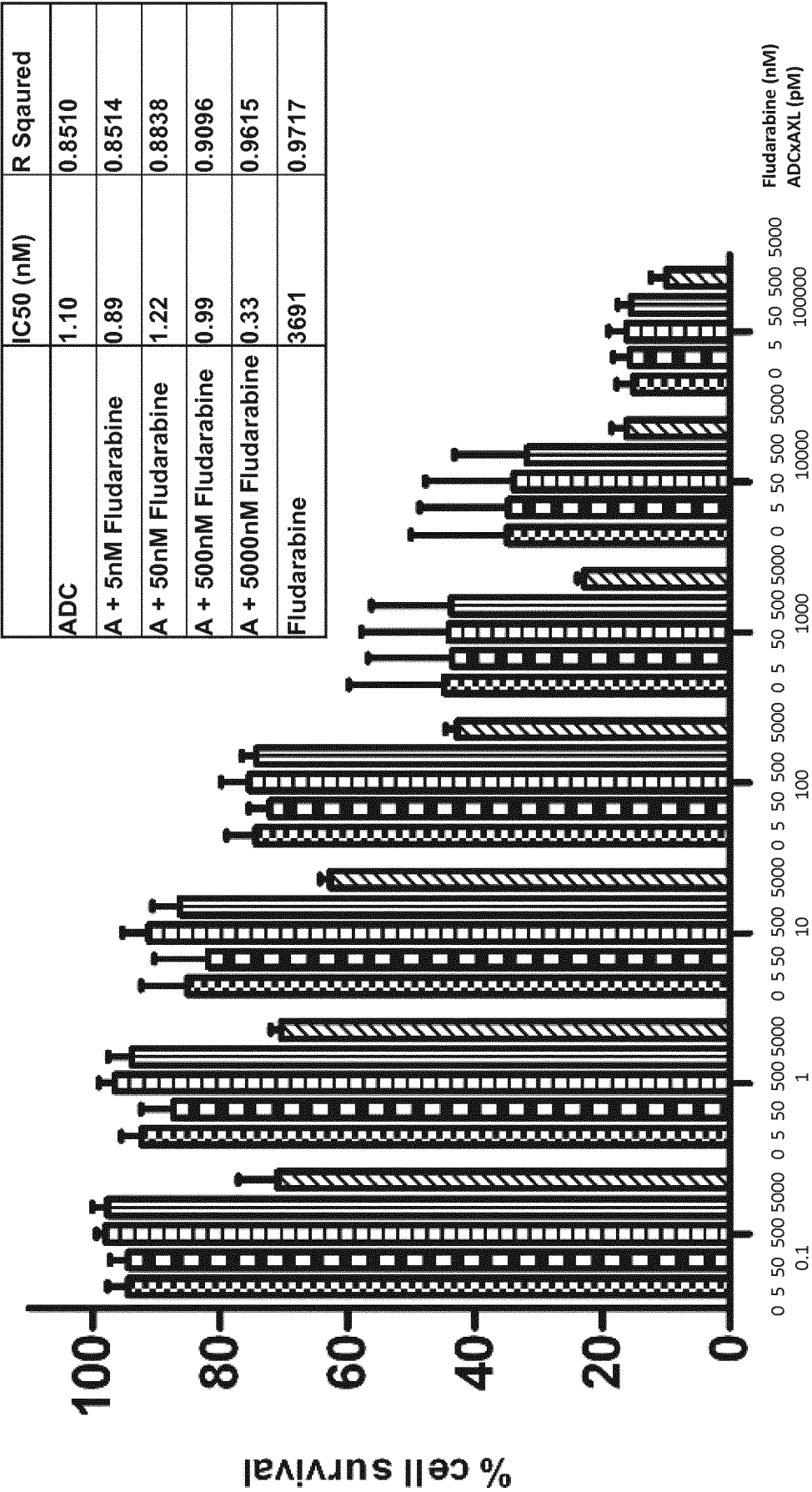


Figure 5

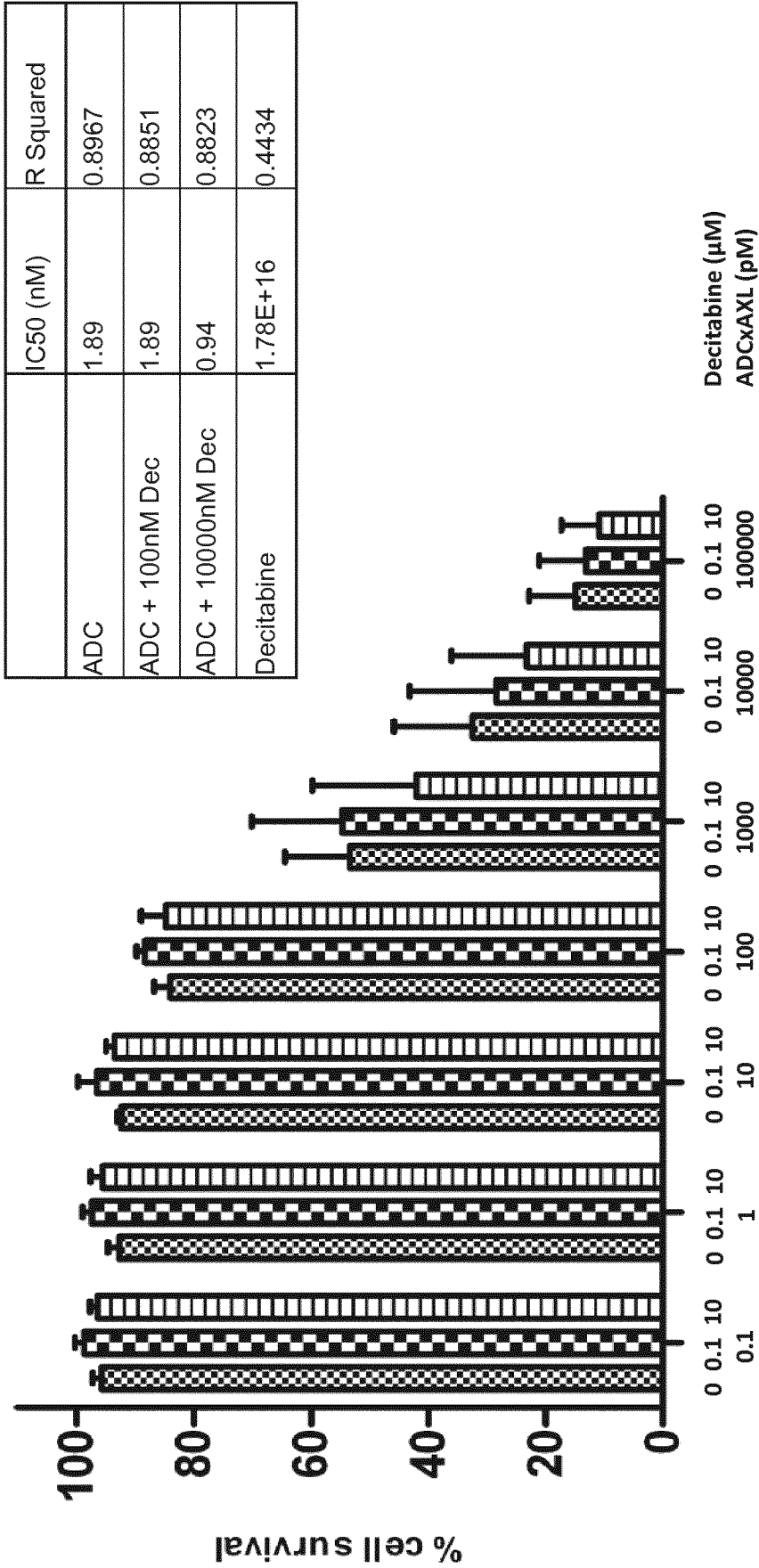


Figure 6

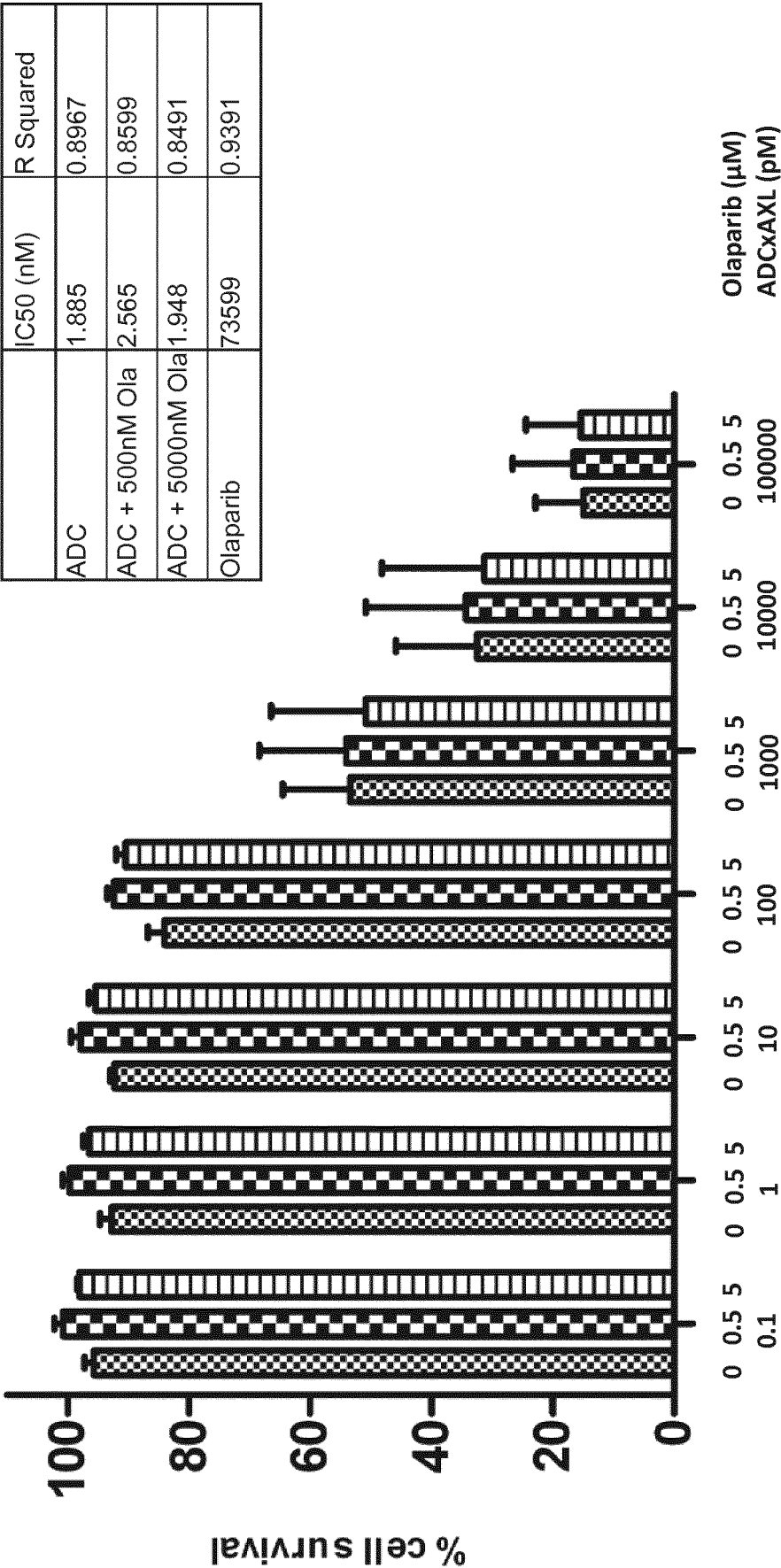


Figure 7

	IC50(nM)	R Squared
ADC	1.885	0.8967
ADC + 1nM Gemcitabine	2.543	0.8633
ADC + 10nM Gemcitabine	2.294	0.8343
Gemcitabine	883.2	0.9396

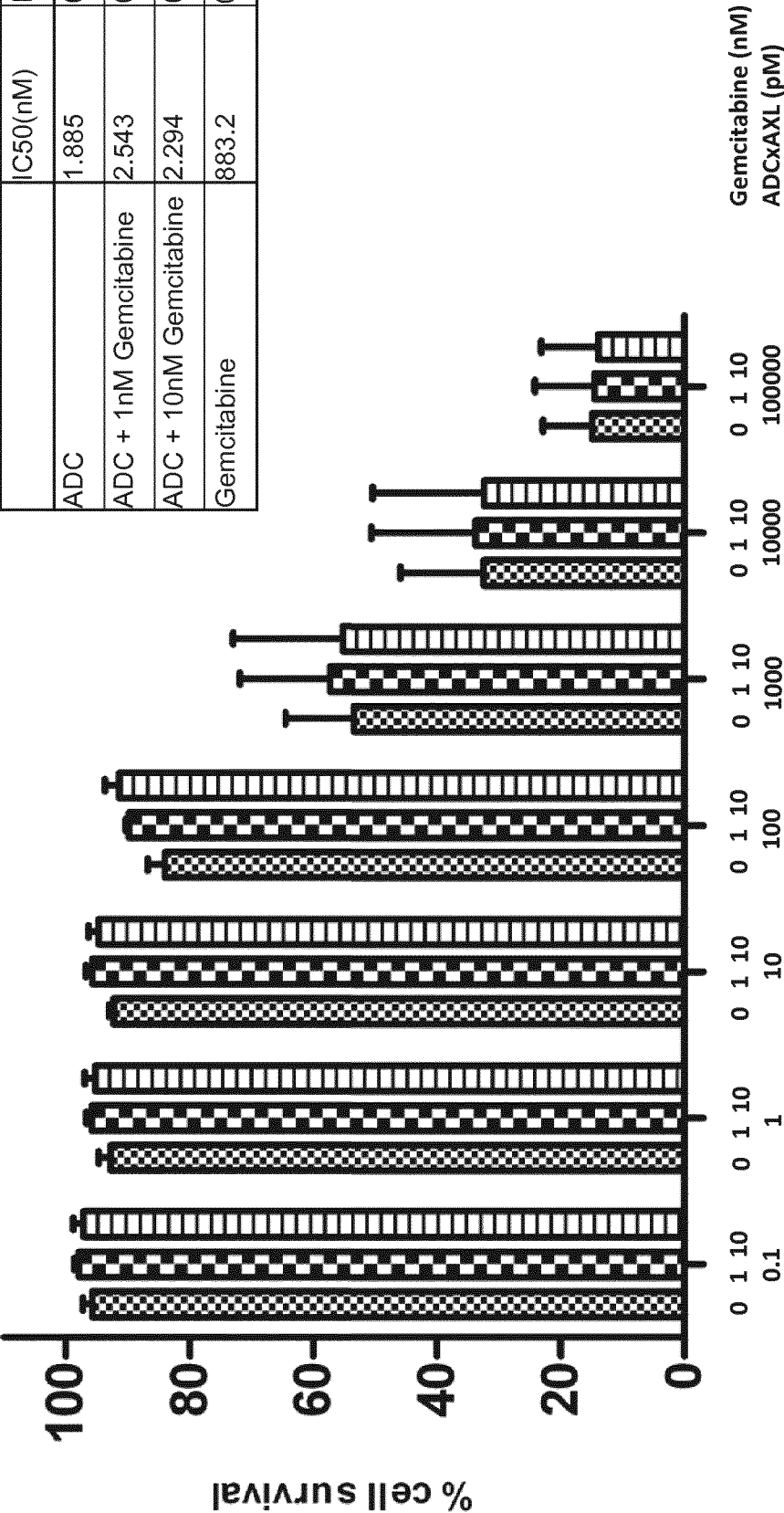


Figure 8

eolf-seql.txt
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MEDIMMUNE LIMITED

<120> COMBINATION THERAPY

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<151> 2017-04-20

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<170> PatentIn version 3.5

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<400> 1

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eolf-seql.txt

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

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
85 90 95

Ala Arg His Pro Ile Tyr Tyr Thr Tyr Asp Asp Thr Met Asp Tyr Trp
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Gly Gln Gly Thr Thr Val Thr Val Ser Ser
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Glu Arg Ala Thr Leu Ser Cys Ser Ala Ser Ser Ser Val Ser Ser Gly
20 25 30

Asn Phe His Trp Tyr Gln Gln Lys Pro Gly Leu Ala Pro Arg Leu Leu
35 40 45

eolf-seql.txt

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
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20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

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

eolf-seql.txt

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg His Pro Ile Tyr Tyr Thr Tyr Asp Asp Thr Met Asp Tyr Trp
100 105 110

Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
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
245 250 255

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
260 265 270

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
275 280 285

eolf-seql.txt

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Xaa Ser Thr
290 295 300

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
305 310 315 320

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
340 345 350

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
355 360 365

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
370 375 380

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
385 390 395 400

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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20 25 30

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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Ser Phe Asn Arg Gly Glu Cys
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Gly

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eolf-seql.txt

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Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe Ser Arg Tyr
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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
50 55 60

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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Ala Ser Pro Tyr Tyr Tyr Gly Pro Phe Ala Tyr Trp Gly Gln Gly Thr
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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

eolf-seql.txt

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
65 70 75 80

Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Ser Arg
85 90 95

Glu Tyr Pro Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Val Lys
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Asp

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Pro Tyr Tyr Tyr Gly Pro Phe Ala Tyr
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Gln Gln Ser Arg Glu Tyr Pro Arg Thr
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eolf-seql.txt

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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
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Ala Ser Pro Tyr Tyr Tyr Gly Pro Phe Ala Tyr Trp Gly Gln Gly Thr
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20 25 30

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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
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Ala Ser Pro Tyr Tyr Tyr Gly Pro Phe Ala Tyr Trp Gly Gln Gly Thr
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Leu Val Thr Val Ser
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20 25 30

eolf-seql.txt

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
115

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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

eolf-seql.txt

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Glu Tyr Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
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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
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Gly Gln Tyr Gln Cys Leu Val Phe Leu Gly His Gln Thr Phe Val Ser
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eolf-seql.txt

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Ala Val Pro Leu Ala Thr Ala Pro Gly His Gly Pro Gln Arg Ser Leu
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His Val Pro Gly Leu Asn Lys Thr Ser Ser Phe Ser Cys Glu Ala His
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Asn Ala Lys Gly Val Thr Thr Ser Arg Thr Ala Thr Ile Thr Val Leu
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eolf-seql.txt

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Tyr Gly Glu Val Phe Glu Pro Thr Val Glu Arg Gly Glu Leu Val Val
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Arg Tyr Arg Val Arg Lys Ser Tyr Ser Arg Arg Thr Thr Glu Ala Thr
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Val Met Val Asp Arg His Lys Val Ala Leu Gly Lys Thr Leu Gly Glu
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eolf-seql.txt

Ile Leu Lys Val Ala Val Lys Thr Met Lys Ile Ala Ile Cys Thr Arg
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Ser Glu Leu Glu Asp Phe Leu Ser Glu Ala Val Cys Met Lys Glu Phe
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Glu Arg Glu Ser Phe Pro Ala Pro Val Val Ile Leu Pro Phe Met Lys
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His Gly Asp Leu His Ser Phe Leu Leu Tyr Ser Arg Leu Gly Asp Gln
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Ala Asp Phe Gly Leu Ser Lys Lys Ile Tyr Asn Gly Asp Tyr Tyr Arg
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Val Glu Asn Ser Glu Ile Tyr Asp Tyr Leu Arg Arg Gly Asn Arg Leu
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eolf-seql.txt

Lys Gln Pro Ala Asp Cys Leu Asp Gly Leu Tyr Ala Leu Met Ser Arg
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785 790 795 800

Glu Asp Leu Glu Asn Thr Leu Lys Ala Leu Pro Pro Ala Gln Glu Pro
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Asp Glu Ile Leu Tyr Val Asn Met Asp Glu Gly Gly Gly Tyr Pro Glu
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Pro Pro Gly Ala Ala Gly Gly Ala Asp Pro Pro Thr Gln Pro Asp Pro
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Lys Asp Ser Cys Ser Cys Leu Thr Ala Ala Glu Val His Pro Ala Gly
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eolf-seql.txt

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Lys Phe His Phe Val Ser Gly Ser Pro Phe Gly Met Asp Val
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<223> BMS-936559/MDX-1105 Antibody 2 VL CDR1

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Arg Ala Ser Gln Ser Val Ser Ser Tyr Leu Ala
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Asp Ala Ser Asn Arg Ala Thr
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Ser Tyr Asp Val His
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<223> BMS-936559/MDX-1105 Antibody 3 VH CDR2

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Trp Leu His Ala Asp Thr Gly Ile Thr Lys Phe Ser Gln Lys Phe Gln
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<223> BMS-936559/MDX-1105 Antibody 3 VH CDR3

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Glu Arg Ile Gln Leu Trp Phe Asp Tyr
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eolf-seql.txt

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Ala Ala Ser Ser Leu Gln Ser
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Gln Gln Tyr Asn Ser Tyr Pro Tyr Thr
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<223> 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
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val

eolf-seql.txt

35

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Ala Asn Ile Lys Gln Asp Gly Ser Glu Lys Tyr Tyr Val Asp Ser Val
50 55 60

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
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser
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<212> PRT

<213> Artificial Sequence

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<223> durvalumab/MEDI4736 VL Sequence

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Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

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
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

eolf-seql.txt

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Leu Pro
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Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
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eolf-seql.txt

<400> 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> 501

<211> 118

<212> PRT

<213> Artificial Sequence

<220>

<223> TRX518 VH sequence

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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

eolf-seql.txt

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
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<210> 502
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<213> Artificial Sequence

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<223> TRX518 VH sequence

<400> 502

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 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

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
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<223> MEDI0562 Heavy chain sequence

<400> 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

eolf-seql.txt

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

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260 265 270

eolf-seql.txt

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
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<210> 601

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eolf-seql.txt

<212> PRT

<213> Artificial Sequence

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<223> MEDI0562 Light Chain sequence

<400> 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

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

eolf-seql.txt

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> 602
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<212> PRT
<213> Artificial Sequence

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<223> MEDI6383

<400> 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

eolf-seql.txt

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

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

eolf-seql.txt

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

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<212> PRT
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<223> OX40mAb24 VH sequence

<400> 603

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
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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

eolf-seql.txt

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> 604
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<212> PRT
<213> Artificial Sequence

<220>
<223> OX40mAb24 VL sequence

<400> 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

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

<210> 605
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<212> PRT
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<400> 605

Gly Ser Ala Met His
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<210> 606
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 <223> INCAGN1949 Antibody a VH CDR2

<400> 606

Arg Ile Arg Ser Lys Ala Asn Ser Tyr Ala Thr Ala Tyr Ala Ala Ser
 1 5 10 15

Val Lys Gly

<210> 607
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<220>
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<400> 607

Gly Ile Tyr Asp Ser Ser Gly Tyr Asp Tyr
 1 5 10

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<400> 608

Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp
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<211> 7

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<223> INCAGN1949 Antibody a VL CDR2

<400> 609

Leu Gly Ser Asn Arg Ala Ser
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<210> 610

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<212> PRT

<213> Artificial Sequence

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<223> INCAGN1949 Antibody a VL CDR3

<400> 610

Met Gln Ala Leu Gln Thr Pro Leu Thr
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<210> 611

<211> 121

<212> PRT

<213> Artificial Sequence

<220>

<223> INCAGN1949 Antibody b VH sequence

<400> 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

eolf-seql.txt

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> 612
<211> 112
<212> PRT
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Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
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Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
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Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
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eolf-seql.txt

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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

eolf-seql.txt

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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
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Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu
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20 25 30

Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Ser Leu Gln
35 40 45

eolf-seql.txt

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

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