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Canadian Intellectual Property Office

CA 3235132 A1 2022/10/20

(21) 3 235 132

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(22) Date de dépôt/Filing Date: 2022/04/15

(41) Mise à la disp. pub./Open to Public Insp.: 2022/10/20

(62) Demande originale/Original Application: 3 216 880

(30) Priorités/Priorities: 2021/04/16 (US63/176,046); 2021/10/08 (US63/254,031)

(51) Cl.Int./Int.Cl. *C07K 1/107* (2006.01), *A61K 39/395* (2006.01), *A61K 47/68* (2017.01), *A61P 35/00* (2006.01), *C07K 11/00* (2006.01), *C07K 16/00* (2006.01), *C12P 21/08* (2006.01)

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(54) Titre : CONJUGUES ANTICORPS-MEDICAMENT ET LEURS PROCEDES DE FABRICATION
(54) Title: ANTIBODY DRUG CONJUGATES AND METHODS FOR MAKING THEREOF

(57) Abrégé/Abstract:
This application discloses microorganisms and methods of producing GNAQ/GNA11 inhibitors and methods of making antibody drug conjugates of anti-PMEL17 antibodies or antigen binding fragments conjugated to a GNAQ/GNA11 inhibitor. The disclosure also relates to formulations comprising antibody drug conjugates of anti-PMEL17 antibodies or antigen binding fragments conjugated to a GNAQ/GNA11 inhibitor and methods of treating or preventing cancer using the formulations.

(21) 3 235 132

(13) A1

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ABSTRACT

This application discloses microorganisms and methods of producing GNAQ/GNA11 inhibitors and methods of making antibody drug conjugates of anti-PMEL17 antibodies or antigen binding fragments conjugated to a GNAQ/GNA11 inhibitor. The disclosure also relates to formulations comprising antibody drug conjugates of anti-PMEL17 antibodies or antigen binding fragments conjugated to a GNAQ/GNA11 inhibitor and methods of treating or preventing cancer using the formulations.

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ANTIBODY DRUG CONJUGATES AND METHODS FOR MAKING THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 63/176,046, filed April 16, 2021, and U.S. Provisional Application No. 63/254,031, filed October 8, 2021. The contents of the aforementioned applications are hereby incorporated by reference in their entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on April 15, 2022, is named N2067-7179WO_SL.txt and is 239,071 bytes in size.

BACKGROUND

Uveal melanoma (UM) is a rare malignancy that accounts for 3-5% of all melanomas with an incident rate of 6-10 cases per 1 million people and 2500-3000 new cases in the US per year. Despite excellent rates of local disease control (5 year-OSR 70%), up to 50% of patients will develop metastatic disease (liver 60%, lung 25%; median OS 13 months, 2 year-OSR 8%). No proven standard of care is available for patients who do develop metastatic disease. There is a high medical need for specifically approved treatments and dedicated disease management strategies in order to improve outcomes for patients with metastatic UM.

Oncogenic mutations of GNAQ or GNA11 are the hallmark mutations in uveal melanoma. Reduction of GNAQ/11 levels leads to inhibition of cell proliferation and apoptosis. The inhibitory effect can be achieved by targeted delivery of antibody drug conjugates that inhibit GNAQ/11 activities. Despite the recent progress, the need still exists for developing novel biological materials, manufacturing methods, and formulations for the antibody drug conjugates.

SUMMARY

The disclosure provides, at least in part, genetically engineered microorganisms (*e.g.*, *Chromobacterium vaccinii*) that produce a depsipeptide, *e.g.*, compound (A1), at high titers and/or isolated yields. In some embodiments, the microorganism includes a non-native promoter operably linked to the biosynthetic gene cluster (BGC) of the depsipeptide. In some embodiments, the BGC of a natural product that interferes with the isolation of the depsipeptide is disrupted. Methods of using the microorganisms described herein to produce depsipeptides, *e.g.*, compound (A1), are also described. The depsipeptides, *e.g.*, compound (A1), produced by a method described herein can be used to produce antibody drug conjugates (*e.g.*, an antibody drug conjugate described herein), *e.g.*, in accordance with a process described herein.

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The disclosure also provides, at least in part, processes for producing partially reoxidized antibodies or antigen binding fragments thereof. In some embodiments, the process comprises reducing one or more capped cysteine residues in an antibody or antigen binding fragment thereof to provide an antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues. In some embodiments, the process comprises storing an antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues under oxidation conditions sufficient to produce a partially reoxidized antibody or antigen binding fragment thereof. Also described herein are processes of making antibody drug conjugates (*e.g.*, an antibody drug conjugate described herein) that uses a process for producing partially reoxidized antibody or antigen binding fragment thereof described herein. The antibody drug conjugates made by a process described herein can be formulated to a formulation described herein.

The disclosure also provides, at least in part, formulations for antibody drug conjugates (*e.g.*, an antibody drug conjugate described herein). In some embodiments, the formulation comprises an antibody drug conjugate described herein, a buffering agent, a stabilizing agent, and a surfactant, and has a pH of about 4.5 to about 6.5. In some embodiments, the formulation is a lyophilized formulation. The formulations described herein can be used to treat or prevent a disorder, *e.g.*, a cancer. In some embodiments, the cancer expresses PMEL17 and/or contains a mutation in the GNAQ or GNA11 gene. In some embodiments, the cancer is a carcinoma (*e.g.*, hepatocellular carcinoma), sarcoma, leukemia, lymphoma, eye cancer, eye neoplasm, melanoma (*e.g.*, uveal melanoma, non-uveal melanoma, malignant melanoma, ocular melanoma, subcutaneous melanoma, cutaneous melanoma, or mucosal melanoma), or a metastatic cancer thereof.

Engineered Microorganisms and Methods of Producing Compounds

In an aspect, the disclosure features a microorganism, comprising a nucleic acid that comprises a nucleotide sequence encoding a polypeptide associated with the production of a compound having the structure of Formula (A1), wherein the nucleotide sequence is operably linked to a non-native promoter.

In some embodiments, the microorganism is a genetically engineered form of a microorganism that naturally produces the compound. In some embodiments, the microorganism is a bacterium. In some embodiments, the microorganism is a *Chromobacterium*. In some embodiments, the microorganism is *Chromobacterium vaccinii*. In some embodiments, the microorganism is *Chromobacterium vaccinii* DSM 25150 (= ATCC BAA-2314, = NRRL B-50840, = MWU205). In some embodiments, the microorganism is an isolated microorganism. In some embodiments, the microorganism is a synthetic microorganism.

In some embodiments, the nucleic acid is on a chromosome of the microorganism, *e.g.*, naturally located on the chromosome or stably integrated to the chromosome. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound is

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located in a biosynthetic gene cluster (BGC). In some embodiments, the BGC is a compound (A1)-BGC. In some embodiments, the compound (A1)-BGC has the gene organization shown in **FIG. 1**.

In some embodiments, the BGC comprises one or more (*e.g.*, two, three, four, five, six, seven, or all) of *frsA, frsB, frsC, frsD, frsE, frsF, frsG*, or *frsH*, or a homolog thereof. In some embodiments, the BGC comprises *frsA, frsB, frsC, frsD, frsE, frsF, frsG*, and *frsH*, or a homolog thereof.

In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound is located in *frsA, frsB, frsC, frsD, frsE, frsF, frsG*, or *frsH*, or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound is located in a region comprising *frsA* or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound is located in a region comprising *frsA* and *frsB*, or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound is located in a region comprising *frsA*, *frsB*, and *frsC*, or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound is located in a region comprising *frsA*, *frsB*, *frsC*, and *frsD*, or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound is located in a region comprising *frsA*, *frsB*, *frsC*, *frsD*, and *frsE*, or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound is located in a region comprising *frsA*, *frsB*, *frsC*, *frsD*, *frsE*, and *frsF*, or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound is located in a region comprising *frsA*, *frsB*, *frsC*, *frsD*, *frsE*, *frsF*, and *frsG*, or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound is located in a region comprising *frsA*, *frsB*, *frsC*, *frsD*, *frsE*, *frsF*, *frsG*, and *FrsH*, or a homolog thereof.

In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound comprises the coding sequence of *frsA, frsB, frsC, frsD, frsE, frsF, frsG*, or *frsH*, or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound comprises the coding sequence of *frsA* or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound comprises the coding sequence of *frsA* and *frsB*, or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound comprises the coding sequence of *frsA*, *frsB*, and *frsC*, or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound comprises the coding sequence of *frsA*, *frsB*, *frsC*, and *frsD*, or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound comprises the coding sequence of *frsA*, *frsB*, *frsC*, *frsD*, and *frsE*, or a homolog

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thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound comprises the coding sequence of *frsA*, *frsB*, *frsC*, *frsD*, *frsE*, and *frsF*, or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound comprises the coding sequence of *frsA*, *frsB*, *frsC*, *frsD*, *frsE*, *frsF*, and *frsG*, or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound comprises the coding sequence of *frsA*, *frsB*, *frsC*, *frsD*, *frsE*, *frsF*, *frsG*, and *FrsH*, or a homolog thereof.

In some embodiments, the nucleic acid comprises a nucleotide sequence resulted from a homologous recombination event (*e.g.*, for promoter exchange) using the nucleotide sequence of SEQ ID NO: 317, or a functional fragment thereof, or a nucleotide sequence having at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity thereto, or differing by no more than about 100, about 90, about 80, about 70, about 60, about 50, about 40, about 30, about 20, about 15, about 10, about 5, or about 2 nucleotides therefrom.

In some embodiments, the nucleic acid comprises a nucleotide sequence associated GenBank accession number: BankIt2437961 BSeq#1 MW732719, or a functional fragment thereof, or a nucleotide sequence having at least about 75%, about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity thereto.

In certain embodiments, the non-native promoter is from the same microorganism. In other embodiments, the non-native promoter is from a different microorganism.

In some embodiments, the non-native promoter comprises a *vioP* promoter, an *nptII* promoter, an *rbs* promoter, a *J23119* promoter, a *pLpp* promoter, a *PS12burk* promoter, an *ErmE** promoter, or a *Pem7* promoter. In some embodiments, the non-native promoter comprises a *vioP* promoter, an *nptII* promoter, or an *rbs* promoter.

In some embodiments, the non-native promoter comprises the nucleotide sequence of any of SEQ ID NOs: 264-266, 268-271, or 316, or a functional fragment thereof, or a nucleotide sequence having at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity thereto, or differing by no more than about 60, about 50, about 40, about 30, about 20, about 15, about 10, about 5, or about 2 nucleotides therefrom. In some embodiments, the non-native promoter comprises or consists of the nucleotide sequence of any of SEQ ID NOs: 264-266, 268-271, or 316 or a functional fragment thereof.

In some embodiments, the non-native promoter comprises a *vioP* promoter. In some embodiments, the non-native promoter comprises the nucleotide sequence of SEQ ID NO: 264, or a functional fragment thereof, or a nucleotide sequence having at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity thereto, or differing by no more than about 60, about 50, about 40, about 30, about 20, about 15, about 10, about 5, or about 2 nucleotides therefrom. In some embodiments, the non-native promoter comprises or consists of the nucleotide sequence of SEQ ID NO: 264, or a functional fragment thereof.

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In some embodiments, the non-native promoter comprises an *nptII* promoter. In some embodiments, the non-native promoter comprises the nucleotide sequence of SEQ ID NO: 265, or a functional fragment thereof, or a nucleotide sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto, or differing by no more than about 60, about 50, about 40, about 30, about 20, about 15, about 10, about 5, or about 2 nucleotides therefrom. In some embodiments, the non-native promoter comprises or consists of the nucleotide sequence of SEQ ID NO: 265, or a functional fragment thereof.

In some embodiments, the non-native promoter comprises an *rbs* promoter. In some embodiments, the non-native promoter comprises the nucleotide sequence of SEQ ID NO: 266, or a functional fragment thereof, or a nucleotide sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto, or differing by no more than 60, 50, 40, 30, 20, 15, 10, 5, or 2 nucleotides therefrom. In some embodiments, the non-native promoter comprises or consists of the nucleotide sequence of SEQ ID NO: 266, or a functional fragment thereof.

In some embodiments, the non-native promoter comprises a *J23119* promoter. In some embodiments, the non-native promoter comprises the nucleotide sequence of SEQ ID NO: 316, or a functional fragment thereof, or a nucleotide sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto, or differing by no more than 60, 50, 40, 30, 20, 15, 10, 5, or 2 nucleotides therefrom. In some embodiments, the non-native promoter comprises or consists of the nucleotide sequence of SEQ ID NO: 316, or a functional fragment thereof.

In some embodiments, the non-native promoter comprises a *pLpp* promoter. In some embodiments, the non-native promoter comprises the nucleotide sequence of SEQ ID NO: 268, or a functional fragment thereof, or a nucleotide sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto, or differing by no more than 60, 50, 40, 30, 20, 15, 10, 5, or 2 nucleotides therefrom. In some embodiments, the non-native promoter comprises or consists of the nucleotide sequence of SEQ ID NO: 268, or a functional fragment thereof.

In some embodiments, the non-native promoter comprises a *Pem7* promoter. In some embodiments, the non-native promoter comprises the nucleotide sequence of SEQ ID NO: 269, or a functional fragment thereof, or a nucleotide sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto, or differing by no more than 60, 50, 40, 30, 20, 15, 10, 5, or 2 nucleotides therefrom. In some embodiments, the non-native promoter comprises or consists of the nucleotide sequence of SEQ ID NO: 269, or a functional fragment thereof.

In some embodiments, the non-native promoter comprises a *PS12burk* promoter. In some embodiments, the non-native promoter comprises the nucleotide sequence of SEQ ID NO: 270, or a functional fragment thereof, or a nucleotide sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto, or differing by no more than 60, 50, 40, 30, 20, 15, 10, 5, or 2 nucleotides therefrom. In some embodiments, the non-native promoter comprises or consists of the nucleotide sequence of SEQ ID NO: 270, or a functional fragment thereof.

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In some embodiments, the non-native promoter comprises an *ErmE** promoter. In some embodiments, the non-native promoter comprises the nucleotide sequence of SEQ ID NO: 271, or a functional fragment thereof, or a nucleotide sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto, or differing by no more than 60, 50, 40, 30, 20, 15, 10, 5, or 2 nucleotides therefrom. In some embodiments, the non-native promoter comprises or consists of the nucleotide sequence of SEQ ID NO: 271, or a functional fragment thereof.

In some embodiments, the non-native promoter controls the transcription of one or more (*e.g.*, two, three, four, five, six, seven, or all) of *FrsA, FrsB, FrsC, FrsD, FrsE, FrsF, FrsG*, or *FrsH*, or a homolog thereof. In some embodiments, the non-native promoter controls the transcription of *FrsA, FrsB, FrsC, FrsD, FrsE, FrsF, FrsG*, and *FrsH*, or a homolog thereof. In some embodiments, the non-native promoter is inserted upstream of the coding region of *FrsA*, or a homolog thereof, *e.g.*, upstream of the coding region of all of *FrsA, FrsB, FrsC, FrsD, FrsE, FrsF, FrsG*, and *FrsH*, or a homolog thereof.

In some embodiments, the polypeptide associated with the production of the compound is a non-ribosomal peptide synthetase (NRPS) or a functional fragment thereof. In some embodiments, the NRPS is FrsA, FrsD, FrsE, FrsF, or FrsG, or a homolog thereof.

In some embodiments, the polypeptide associated with the production of the compound is a MbtH-like protein or a functional fragment thereof. In some embodiments, the MbtH-like protein is FrsB or a homolog thereof.

In some embodiments, the polypeptide associated with the production of the compound is a malate dehydrogenase. In some embodiments, the malate dehydrogenase is FrsC or a homolog thereof.

In some embodiments, the polypeptide associated with the production of the compound is a hydroxylase. In some embodiments, the hydroxylase is FrsH or a homolog thereof.

In some embodiments, the nucleic acid comprises a plurality of nucleotide sequences encoding a plurality of polypeptides associated with the production of the compound. In some embodiments, the plurality of polypeptides associated with the production of the compound comprises a plurality of NRPSs, or a functional fragment thereof. In some embodiments, the plurality of NRPSs comprise two or more (*e.g.*, three, four, or all) of FrsA, FrsD, FrsE, FrsF, or FrsG, or a homolog thereof. In some embodiments, the plurality of polypeptides associated with the production of the compound comprises an NRPS, a MbtH-like protein, a malate dehydrogenase, and a hydroxylase, or a functional fragment thereof.

In some embodiments, the plurality of polypeptides associated with the production of the compound comprises FrsA, FrsB, FrsC, FrsD, FrsE, FrsF, FrsG, and FrsH, or a homolog thereof.

In some embodiments, the microorganism has an increased titer of the compound. In some embodiments, the titer of the compound is increased by at least about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, or about 10-fold, compared to a reference microorganism,

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e.g., an otherwise identical microorganism that does not comprise the non-native promoter in the compound (A1)-BGC (*e.g.*, a wild-type), when cultured under conditions that allow production of the compound. In some embodiments, the titer of the compound is increased by at least about 100 mg/L, about 200 mg/L, about 300 mg/L, about 400 mg/L, about 500 mg/L, about 600 mg/L, about 700 mg/L, about 800 mg/L, about 900 mg/L, or about 1000 mg/L, compared to a reference microorganism, *e.g.*, an otherwise identical microorganism that does not comprise the non-native promoter in the compound (A1)-BGC (*e.g.*, a wild-type), when cultured under conditions that allow production of the compound.

In some embodiments, the BGC of a natural product that interferes with the isolation of the compound is altered, *e.g.*, disrupted. In some embodiments, the natural product comprises one or more (*e.g.*, two, three, or all) of: violacein, compound J, compound F5, compound F3, or compound D. In some embodiments, at least a portion of the BGC of the natural product interferes with the isolation of the compound is deleted. In some embodiments, the violacein-BGC is altered, *e.g.*, disrupted. In some embodiments, the compound J-BGC is altered, *e.g.*, disrupted. In some embodiments, the compound J-BGC is associated with the production of compound J, compound F5, compound F3, and compound D. In some embodiments, both the violacein-BGC and the compound J-BGC are altered, *e.g.*, disrupted. In some embodiments, the production of the natural product is reduced, *e.g.*, by at least about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%.

In some embodiments, the microorganism has an increased isolation yield of the compound. In some embodiments, the isolation yield of the compound is increased by about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or more, compared to a reference microorganism, *e.g.*, an otherwise identical microorganism in which the BGC of the natural product that interferes with the isolation of the compound is not altered (*e.g.*, a wild-type).

In some embodiments, the BGC of a natural product that interferes with the isolation of the compound is not altered, *e.g.*, not disrupted.

In another aspect, the disclosure features a microorganism that produces a compound having the structure of Formula (A1), wherein the BGC of a natural product that interferes with the isolation of the compound is altered.

In some embodiments, the natural product comprises one or more (*e.g.*, two, three, or all) of: violacein, compound J, compound F5, compound F3, or compound D. In some embodiments, at least a portion of the BGC of the natural product interferes with the isolation of the compound is deleted. In some embodiments, the violacein-BGC is altered, *e.g.*, disrupted. In some embodiments, the compound J-BGC is altered, *e.g.*, disrupted. In some embodiments, the compound J-BGC is associated with the production of compound J, compound F5, compound F3, and compound D. In some embodiments, both the violacein-BGC and the compound J-BGC are altered, *e.g.*, disrupted. In

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some embodiments, the production of the natural product is reduced, *e.g.*, by at least about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100%.

In some embodiments, the microorganism has an increased isolation yield of the compound. In some embodiments, the isolation yield of the compound is increased by about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or more, compared to a reference microorganism, *e.g.*, an otherwise identical microorganism in which the BGC of the natural product that interferes with the isolation of the compound is not altered (*e.g.*, a wild-type).

In another aspect, the disclosure features a method of producing a compound having the structure of Formula (A1), comprising culturing (*e.g.*, fermenting) a microorganism described herein under conditions that allow the expression of the compound, thereby producing the compound.

In some embodiments, the microorganism is cultured (*e.g.*, fermented) at about 50 L to about 500 L scale, *e.g.*, at about 50 L, about 100 L, about 150 L, about 200 L, about 250 L, about 300 L, about 350 L, about 400 L, about 450 L, or about 500 L scale. In some embodiments, the microorganism is cultured (*e.g.*, fermented) at about 1,000 L to about 20,000 L scale, *e.g.*, about 1,000 L to about 10,000 L or about 10,000 L to about 20,000 L, *e.g.*, about 1,000 L, about 2,000 L, about 5,000 L, about 7,500 L, about 10,000 L, about 15,000 L, or about 20,000 L scale.

In some embodiments, the culturing (*e.g.*, fermentation) yields a titer of at least about 200 mL of the compound, *e.g.*, at least about 250 mg/mL, about 300 mg/L, about 400 mg/L, about 500 mg/L, about 600 mg/L, about 700 mg/L, about 800 mg/L, about 900 mg/L, about 1000 mg/L, about 1100 mg/L, about 1200 mg/L, about 1300 mg/L, about 1400 mg/L, about 1500 mg/L, about 1600 mg/L, about 1700 mg/L, about 1800 mg/L, about 1900 mg/L, or about 2000 mg/L of the compound.

In some embodiments, the method further comprises isolating the compound, *e.g.*, to a purity of at least about 90%, *e.g.*, at least about 95%, about 96%, about 97%, about 98%, or about 99%, *e.g.*, as determined by a method described herein.

In another aspect, the disclosure features a nucleic acid that comprises a nucleotide sequence encoding a polypeptide associated with the production of a compound having the structure of Formula (A1), wherein the nucleotide sequence is operably linked to a non-native promoter.

In some embodiments, the nucleic acid is on a chromosome of the microorganism, *e.g.*, naturally located on the chromosome or stably integrated to the chromosome. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound is located in a biosynthetic gene cluster (BGC). In some embodiments, the BGC is a compound (A1)-BGC. In some embodiments, the compound (A1)-BGC has the gene organization shown in **FIG. 1**.

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In some embodiments, the BGC comprises one or more (*e.g.*, two, three, four, five, six, seven, or all) of *frsA, frsB, frsC, frsD, frsE, frsF, frsG*, or *frsH*, or a homolog thereof. In some embodiments, the BGC comprises *frsA, frsB, frsC, frsD, frsE, frsF, frsG*, and *frsH*, or a homolog thereof.

In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound is located in *frsA, frsB, frsC, frsD, frsE, frsF, frsG, frsH*, or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound is located in a region comprising *frsA* or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound is located in a region comprising *frsA* and *frsB*, or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound is located in a region comprising *frsA*, *frsB*, and *frsC*, or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound is located in a region comprising *frsA*, *frsB*, *frsC*, and *frsD*, or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound is located in a region comprising *frsA*, *frsB*, *frsC*, *frsD*, and *frsE*, or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound is located in a region comprising *frsA*, *frsB*, *frsC*, *frsD*, *frsE*, and *frsF*, or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound is located in a region comprising *frsA*, *frsB*, *frsC*, *frsD*, *frsE*, *frsF*, and *frsG*, or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound is located in a region comprising *frsA*, *frsB*, *frsC*, *frsD*, *frsE*, *frsF*, *frsG*, and *FrsH*, or a homolog thereof.

In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound comprises the coding sequence of *frsA, frsB, frsC, frsD, frsE, frsF, frsG, frsH*, or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound comprises the coding sequence of *frsA* or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound comprises the coding sequence of *frsA* and *frsB*, or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound comprises the coding sequence of *frsA*, *frsB*, and *frsC*, or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound comprises the coding sequence of *frsA*, *frsB*, *frsC*, and *frsD*, or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound comprises the coding sequence of *frsA*, *frsB*, *frsC*, *frsD*, and *frsE*, or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound comprises the coding sequence of *frsA*, *frsB*, *frsC*, *frsD*, *frsE*, and *frsF*, or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide

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associated with the production of the compound comprises the coding sequence of *frsA*, *frsB*, *frsC*, *frsD*, *frsE*, *frsF*, and *frsG*, or a homolog thereof. In some embodiments, the nucleotide sequence encoding a polypeptide associated with the production of the compound comprises the coding sequence of *frsA*, *frsB*, *frsC*, *frsD*, *frsE*, *frsF*, *frsG*, and *FrsH*, or a homolog thereof.

In some embodiments, the nucleic acid comprises the nucleotide sequence of SEQ ID NO: 317, or a functional fragment thereof, or a nucleotide sequence having at least 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity thereto, or differing by no more than about 100, about 90, about 80, about 70, about 60, about 50, about 40, about 30, about 20, about 15, about 10, about 5, or about 2 nucleotides therefrom.

In some embodiments, the nucleic acid comprises a nucleotide sequence associated GenBank accession number: BankIt2437961 BSeq#1 MW732719, or a functional fragment thereof, or a nucleotide sequence having at least about 75%, about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity thereto.

In certain embodiments, the non-native promoter is from the same microorganism. In other embodiments, the non-native promoter is from a different microorganism.

In some embodiments, the non-native promoter comprises a *vioP* promoter, an *nptII* promoter, an *rbs* promoter, a *J23119* promoter, a *pLpp* promoter, a *PS12burk* promoter, an *ErmE** promoter, or a *Pem7* promoter. In some embodiments, the non-native promoter comprises a *vioP* promoter, an *nptII* promoter, or an *rbs* promoter.

In some embodiments, the non-native promoter comprises the nucleotide sequence of any of SEQ ID NOs: 264-266, 268-271, or 316, or a functional fragment thereof, or a nucleotide sequence having at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity thereto, or differing by no more than about 60, about 50, about 40, about 30, about 20, about 15, about 10, about 5, or about 2 nucleotides therefrom. In some embodiments, the non-native promoter comprises or consists of the nucleotide sequence of any of SEQ ID NOs: 264-266, 268-271, or 316, or a functional fragment thereof.

In some embodiments, the non-native promoter comprises a *vioP* promoter. In some embodiments, the non-native promoter comprises the nucleotide sequence of SEQ ID NO: 264, or a functional fragment thereof, or a nucleotide sequence having at least 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity thereto, or differing by no more than about 60, about 50, about 40, about 30, about 20, about 15, about 10, about 5, or about 2 nucleotides therefrom. In some embodiments, the non-native promoter comprises or consists of the nucleotide sequence of SEQ ID NO: 264, or a functional fragment thereof.

In some embodiments, the non-native promoter comprises an *nptII* promoter. In some embodiments, the non-native promoter comprises the nucleotide sequence of SEQ ID NO: 265, or a functional fragment thereof, or a nucleotide sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto, or differing by no more than about 60, about 50, about 40, about

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30, about 20, about 15, about 10, about 5, or about 2 nucleotides therefrom. In some embodiments, the non-native promoter comprises or consists of the nucleotide sequence of SEQ ID NO: 265, or a functional fragment thereof.

In some embodiments, the non-native promoter comprises an *rbs* promoter. In some embodiments, the non-native promoter comprises the nucleotide sequence of SEQ ID NO: 266, or a functional fragment thereof, or a nucleotide sequence having at least 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity thereto, or differing by no more than about 60, about 50, about 40, about 30, about 20, about 15, about 10, about 5, or about 2 nucleotides therefrom. In some embodiments, the non-native promoter comprises or consists of the nucleotide sequence of SEQ ID NO: 266, or a functional fragment thereof.

In some embodiments, the non-native promoter comprises a *J23119* promoter. In some embodiments, the non-native promoter comprises the nucleotide sequence of SEQ ID NO: 316, or a functional fragment thereof, or a nucleotide sequence having at least 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity thereto, or differing by no more than about 60, about 50, about 40, about 30, about 20, about 15, about 10, about 5, or about 2 nucleotides therefrom. In some embodiments, the non-native promoter comprises or consists of the nucleotide sequence of SEQ ID NO: 316, or a functional fragment thereof.

In some embodiments, the non-native promoter comprises a *pLpp* promoter. In some embodiments, the non-native promoter comprises the nucleotide sequence of SEQ ID NO: 268, or a functional fragment thereof, or a nucleotide sequence having at least 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity thereto, or differing by no more than about 60, about 50, about 40, about 30, about 20, about 15, about 10, about 5, or about 2 nucleotides therefrom. In some embodiments, the non-native promoter comprises or consists of the nucleotide sequence of SEQ ID NO: 268, or a functional fragment thereof.

In some embodiments, the non-native promoter comprises a *Pem7* promoter. In some embodiments, the non-native promoter comprises the nucleotide sequence of SEQ ID NO: 269, or a functional fragment thereof, or a nucleotide sequence having at least 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity thereto, or differing by no more than about 60, about 50, about 40, about 30, about 20, about 15, about 10, about 5, or about 2 nucleotides therefrom. In some embodiments, the non-native promoter comprises or consists of the nucleotide sequence of SEQ ID NO: 269, or a functional fragment thereof.

In some embodiments, the non-native promoter comprises a *PS12burk* promoter. In some embodiments, the non-native promoter comprises the nucleotide sequence of SEQ ID NO: 270, or a functional fragment thereof, or a nucleotide sequence having at least 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity thereto, or differing by no more than about 60, about 50, about 40, about 30, about 20, about 15, about 10, about 5, or about 2

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nucleotides therefrom. In some embodiments, the non-native promoter comprises or consists of the nucleotide sequence of SEQ ID NO: 270, or a functional fragment thereof.

In some embodiments, the non-native promoter comprises an *ErmE** promoter. In some embodiments, the non-native promoter comprises the nucleotide sequence of SEQ ID NO: 271, or a functional fragment thereof, or a nucleotide sequence having at least 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity thereto, or differing by no more than about 60, about 50, about 40, about 30, about 20, about 15, about 10, about 5, or about 2 nucleotides therefrom. In some embodiments, the non-native promoter comprises or consists of the nucleotide sequence of SEQ ID NO: 271, or a functional fragment thereof.

In some embodiments, the non-native promoter controls the transcription of one or more (*e.g.*, two, three, four, five, six, seven, or all) of *FrsA, FrsB, FrsC, FrsD, FrsE, FrsF, FrsG*, or *FrsH*, or a homolog thereof. In some embodiments, the non-native promoter controls the transcription of *FrsA, FrsB, FrsC, FrsD, FrsE, FrsF, FrsG*, and *FrsH*, or a homolog thereof. In some embodiments, the non-native promoter is inserted upstream of the coding region of *FrsA*, or a homolog thereof, *e.g.*, upstream of the coding region of all of *FrsA, FrsB, FrsC, FrsD, FrsE, FrsF, FrsG*, and *FrsH*, or a homolog thereof.

In some embodiments, the polypeptide associated with the production of the compound is a non-ribosomal peptide synthetase (NRPS) or a functional fragment thereof. In some embodiments, the NRPS is FrsA, FrsD, FrsE, FrsF, or FrsG, or a homolog thereof.

In some embodiments, the polypeptide associated with the production of the compound is a MbtH-like protein or a functional fragment thereof. In some embodiments, the MbtH-like protein is FrsB or a homolog thereof.

In some embodiments, the polypeptide associated with the production of the compound is a malate dehydrogenase. In some embodiments, the malate dehydrogenase is FrsC or a homolog thereof.

In some embodiments, the polypeptide associated with the production of the compound is a hydroxylase. In some embodiments, hydroxylase is FrsH or a homolog thereof.

In some embodiments, the nucleic acid comprises a plurality of nucleotide sequences encoding a plurality of polypeptides associated with the production of the compound. In some embodiments, the plurality of polypeptides associated with the production of the compound comprises a plurality of NRPSs, or a functional fragment thereof. In some embodiments, the plurality of NRPSs comprise two or more (*e.g.*, three, four, or all) of FrsA, FrsD, FrsE, FrsF, or FrsG, or a homolog thereof. In some embodiments, the plurality of polypeptides associated with the production of the compound comprises an NRPS, a MbtH-like protein, a malate dehydrogenase, and a hydroxylase, or a functional fragment thereof.

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In some embodiments, the plurality of polypeptides associated with the production of the compound comprises FrsA, FrsB, FrsC, FrsD, FrsE, FrsF, FrsG, and FrsH, or a homolog thereof.

In another aspect, the disclosure features a nucleic acid comprising a nucleotide sequence encoding a polypeptide associated with the production of one or more of: compound J, compound F5, compound F3, or compound D.

In some embodiments, the nucleic acid is an isolated nucleic acid. In some embodiments, the nucleic acid is a non-naturally occurring nucleic acid. In some embodiments, the nucleic acid is a synthetic nucleic acid. In some embodiments, the nucleic acid comprises a mutation, *e.g.*, a deletion.

In some embodiments, the nucleic acid comprises a plurality of nucleotide sequences encoding a plurality of polypeptides associated with the production of one or more of: compound J, compound F5, compound F3, or compound D.

In some embodiments, the nucleotide sequence is from a BGC. In some embodiments, the BGC is a compound J-BGC. In some embodiments, the compound J-BGC has the gene organization shown in **FIG. 1**.

In some embodiments, the nucleotide sequence comprises one or more (*e.g.*, two, three, or all) of *dis1, dis2, dis3*, or *dis4*, or a homolog thereof. In some embodiments, the BGC comprises *dis1, dis2, dis3*, and *dis4*, or a homolog thereof.

In some embodiments, the nucleic acid comprises a nucleotide sequence associated GenBank accession number: BankIt2437961 BSeq#1 MW732719, or a functional fragment thereof, or a nucleotide sequence having at least about 75%, *e.g.*, at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity thereto.

In another aspect, the disclosure features a vector comprising a nucleic acid described herein. In another aspect, the disclosure features a cell comprising a nucleic acid described herein or a vector described herein.

In another aspect, the disclosure features a method of engineering a cell, comprising altering a nucleotide sequence encoding a polypeptide associated with the production of one or more of: compound J, compound F5, compound F3, or compound D, thereby engineering the cell.

In some embodiments, the cell is a microorganism that naturally produces a compound having the structure of Formula (A1). In some embodiments, the microorganism is a bacterium, *e.g.*, *Chromobacterium*. In some embodiments, the microorganism is *Chromobacterium vaccinii*, *e.g.*, *Chromobacterium vaccinii* DSM 25150 (= ATCC BAA-2314, = NRRL B-50840, = MWU205).

In some embodiments, the nucleotide sequence is disrupted, *e.g.*, at least a portion of the nucleotide sequence is deleted.

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In some embodiments, the nucleic acid comprises a plurality of nucleotide sequences encoding a plurality of polypeptides associated with the production of one or more of: compound J, compound F5, compound F3, or compound D. In some embodiments, the production of one or more (*e.g.*, two, three, or all) of: compound J, compound F5, compound F3, or compound D is reduced, *e.g.*, by at least about 50%, *e.g.*, at least about 60%, about 70%, about 80%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%.

In some embodiments, the nucleotide sequence is from a BGC. In some embodiments, the BGC is a compound J-BGC. In some embodiments, the compound J-BGC has the gene organization shown in **FIG. 1**. In some embodiments, the nucleotide sequence comprises one or more (*e.g.*, two, three, or all) of *dis1, dis2, dis3*, or *dis4*, or a homolog thereof. In some embodiments, the BGC comprises *dis1, dis2, dis3*, and *dis4*, or a homolog thereof. In some embodiments, the nucleic acid comprises a nucleotide sequence associated GenBank accession number: BankIt2437961 BSeq#1 MW732719, or a functional fragment thereof, or a nucleotide sequence having at least about 75%, about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity thereto.

In some embodiments, the alteration increases the isolation yield of the compound. In some embodiments, the isolation yield of the compound is increased by about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or more, compared to a reference microorganism, *e.g.*, an otherwise identical microorganism in which the BGC of the natural product that interferes with the isolation of the compound is not altered (*e.g.*, a wild-type).

Processes for Partial Reoxidation and Making Antibody Drug Conjugates

In another aspect, the disclosure features a process for producing a partially reoxidized antibody or antigen binding fragment thereof, comprising: providing an antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues; and storing the antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues under oxidation conditions, thereby producing the partially reoxidized antibody or antigen binding fragment thereof.

In some embodiments, the process further comprises providing an antibody or antigen binding fragment thereof comprising one or more capped cysteine residues. In some embodiments, the one or more capped cysteine residues are located in the constant domain a heavy chain of the antibody or antigen binding fragment thereof. In some embodiments, the antibody or antigen binding fragment thereof comprises four capped cysteine residues. In some embodiments, the antibody or antigen binding fragment thereof comprises two capped cysteine residues. In some embodiments, the antibody or antigen binding fragment thereof comprises a capped cysteine residue located in a CH1 region of the antibody or antigen binding fragment thereof. In some embodiments, the antibody or antigen binding fragment thereof comprises a capped cysteine residue located in each CH1 region of the antibody or antigen binding fragment thereof. In some embodiments, the capped cysteine residue is an

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engineered surface-exposed cysteine residue. In some embodiments, the antibody or antigen binding fragment thereof comprises a capped cysteine residue located at residue 152 (EU numbering) in the CH1 region of the antibody or antigen binding fragment thereof. In some embodiments, the one or more capped cysteine residues are capped with a cysteine or a glutathione.

In some embodiments, the process further comprises reducing one or more capped cysteine residues to provide an antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues. In some embodiments, the one or more decapped cysteine residues are located in the constant domain a heavy chain of the antibody or antigen binding fragment thereof. In some embodiments, the antibody or antigen binding fragment thereof comprises four decapped cysteine residues. In some embodiments, the antibody or antigen binding fragment thereof comprises two decapped cysteine residues. In some embodiments, the antibody or antigen binding fragment thereof comprises a decapped cysteine residue located in a CH1 region of the antibody or antigen binding fragment thereof. In some embodiments, the antibody or antigen binding fragment thereof comprises a decapped cysteine residue located in each CH1 region of the antibody or antigen binding fragment thereof. In some embodiments, the decapped cysteine residue is an engineered surface-exposed cysteine residue. In some embodiments, the antibody or antigen binding fragment thereof comprises a decapped cysteine residue located at residue 152 (EU numbering) in the CH1 region of the antibody or antigen binding fragment thereof.

In some embodiments, the process comprises reducing the one or more capped cysteine residues by contacting the antibody or antigen binding fragment thereof comprising one or more capped cysteine residues with a reduction wash buffer, thereby providing an antibody or antigen binding fragment comprising one or more decapped cysteine residue.

In some embodiments, the reduction wash buffer comprises about 5 mM to about 50 mM cysteine, *e.g.*, about 10 mM to about 40 mM, about 20 mM to about 30 mM, about 5 mM to about 15 mM, about 5 mM to about 25 mM, about 5 mM to about 35 mM, about 5 mM to about 45 mM, about 40 mM to about 50 mM, about 30 mM to about 50 mM, about 20 mM to about 50 mM, about 10 mM to about 50 mM, about 10 mM to about 20 mM, about 15 mM to about 25 mM, about 25 mM to about 35 mM, about 30 mM to about 40 mM, or about 35 mM to about 45 mM, *e.g.*, about 10 mM, about 12 mM, about 14 mM, about 16 mM, about 20 mM, about 25 mM, or about 30 mM. In some embodiments, the reduction wash buffer comprises about 5 mM to about 15 mM cysteine, *e.g.*, about 10 mM cysteine.

In some embodiments, the reduction wash buffer has a pH of about 6.0 to about 7.5, *e.g.*, about 6.5 to about 7.2 or about 6.8 to about 7.0. In some embodiments, the reduction wash buffer has a pH of about 6.8 to about 7.0, *e.g.*, about 6.9.

In some embodiments, contacting the antibody or antigen binding fragment thereof comprising one or more capped cysteine residues with the reduction wash buffer is performed on a column (*e.g.*, a cation exchange chromatography column).

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In some embodiments, the process further comprises collecting the antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues in an eluate after contacting with the reduction wash buffer. In some embodiments, the eluate has a pH of about 5.5 to about 6.0, *e.g.*, about 5.8. In some embodiments, the concentration of the antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues in the eluate is about 5 g/L to 25 g/L, *e.g.*, about 8 g/L to about 20 g/L, about 10 g/L to about 18 g/L, about 12 g/L to about 15 g/L, about 5 g/L to about 20 g/L, about 5 g/L to about 15 g/L, about 5 g/L to about 10 g/L, about 20 g/L to about 25 g/L, about 15 g/L to about 25 g/L, about 10 g/L to about 25 g/L, about 10 g/L to about 20 g/L, about 13 g/L to about 14 g/L, about 12 g/L to about 15 g/L, *e.g.*, about 5 g/L, about 6 g/L, about 7 g/L, about 8 g/L, about 9 g/L, about 10 g/L, about 11 g/L, about 12 g/L, about 13 g/L, about 13.5 g/L, about 14 g/L, about 15 g/L, about 16 g/L, about 17 g/L, about 18 g/L, about 19 g/L, about 20 g/L, about 21 g/L, about 22 g/L, about 23 g/L, about 24 g/L, or about 25 g/L.

In some embodiments, the antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues is stored in the eluate with stirring. In some embodiments, the antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues is stored in the eluate without stirring.

In some embodiments, the antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues is stored in a container filled to a maximum of about 50% to about 70% volume, *e.g.*, for about 12 hours to about 96 hours, *e.g.*, about 12 hours to about 84 hours, about 24 hours to about 84 hours, about 36 hours to about 72 hours, about 48 hours to about 60 hours, about 24 hours to about 72 hours, about 24 hours to about 60 hours, about 24 hours to about 48 hours, about 24 hours to about 36 hours, about 72 hours to about 84 hours, about 60 hours to about 84 hours, about 48 hours to about 84 hours, about 36 hours to about 84 hours, about 12 hours to about 36 hours, about 24 hours to about 48 hours, about 36 hours to about 60 hours, about 48 hours to about 72 hours, or about 72 hours to about 96 hours, *e.g.*, about 12 hours, about 24 hours, about 36 hours, about 48 hours, about 60 hours, about 72 hours, about 84 hours, or about 96 hours. In some embodiments, the antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues is stored in the container filled to a maximum of about 60%. In some embodiments, the antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues is stored for about 48 hours to about 72 hours, *e.g.*, without stirring. In some embodiments, the antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues is stored for about 12 hours to about 36 hours, *e.g.*, about 24 hours, *e.g.*, with stirring.

In some embodiments, the antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues is stored at room temperature. In some embodiments, the antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues is stored at about 2 °C to about 8 °C (*e.g.* about 4 °C), *e.g.*, for at least 48 hours (*e.g.*, at least about 48 hours to about 96 hours).

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In some embodiments, the antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues is stored with air overlay.

In some embodiments, the process further comprises purifying the partially reoxidized antibody or antigen binding fragment thereof.

In some embodiments, the process further comprises conjugating a linker-drug moiety (*e.g.*, a linker-drug moiety described herein) to the partially reoxidized antibody or antigen binding fragment thereof to produce an antibody drug conjugate (*e.g.*, an antibody drug conjugate described herein).

In some embodiments, the process further comprises pre-forming a linker-drug moiety of the following Formula (B):

$$R^8\text{-}L_B\text{-}(D)_n \qquad (B)$$

wherein:

D is a GNAQ inhibitor, a GNA11 inhibitor or an inhibitor of GNAQ and GNA11;

R^8 is a reactive group;

L_B is a cleavable or non-cleavable linker, and

n is 1, 2, 3 or 4;

In some embodiments, D is a GNAQ inhibitor described herein. In some embodiments, D is a GNA11 inhibitor described herein. In some embodiments, D is an inhibitor of GNAQ and GNA11 as described herein. In some embodiments, R is a reactive group described herein. In some embodiments, L_B is a cleavable linker described herein. In some embodiments, L_B is a non-cleavable linker described herein.

In some embodiments, the antibody or antigen binding fragment thereof binds to PMEL17. In some embodiments, the antibody or antigen binding fragment thereof is an antibody or antigen binding fragment thereof described herein.

In some embodiments, the process further comprises purifying the antibody drug conjugate.

In some embodiments, the process results in at least about 75%, *e.g.*, at least about 80%, about 85%, about 90%, or about 95%, on-site coupling, *e.g.*, one linker-drug moiety per heavy chain ("HC1"). In some embodiments, the process results in less than about 25%, *e.g.*, at least about 20%, about 15%, about 10%, or about 5%, off-site coupling, *e.g.*, one linker-drug moiety per light chain ("LC1") and/or two linker-drug moieties per heavy chain ("HC2"). In some embodiments, the process results in at least about 70%, *e.g.*, at least about 75%, about 80%, about 85%, about 90%, or about 95%, purity, *e.g.*, as determined by non-reduced CE-SDS.

In another aspect, the disclosure features a process for producing an anti-PMEL17 antibody drug conjugate, comprising:

providing an antibody or antigen binding fragment thereof comprising one or more capped cysteine residues with a reduction wash buffer, thereby providing an antibody or antigen binding fragment comprising one or more decapped cysteine residues;

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storing the antibody or fragment thereof comprising one or more decapped cysteine residues under oxidation conditions, thereby producing a partially reoxidized antibody or antigen binding fragment thereof; and

conjugating a linker-drug moiety to the partially reoxidized antibody or antigen binding fragment thereof, thereby producing the anti-PMEL17 antibody drug conjugate,

wherein the antibody or antigen binding fragment thereof binds to PMEL17; and

wherein the linker-drug moiety of the following Formula (B):

$$R^8\text{-}L_B\text{-}(D)_n \qquad (B)$$

wherein:

D is a GNAQ inhibitor, a GNA11 inhibitor or an inhibitor of GNAQ and GNA11;

R^8 is a reactive group;

L_B is a cleavable or non-cleavable linker, and

n is 1, 2, 3 or 4.

Formulations of Antibody Drug Conjugates

In another aspect, the disclosure features a formulation comprising an antibody drug conjugate, a buffering agent, a stabilizing agent, and a surfactant, wherein the formulation has a pH of 4.5 to 6.5, wherein the antibody drug conjugate comprises the formula (C)

$$Ab\text{-}(L_A\text{-}(D)_n)_y \qquad (C)$$

wherein:

D is a GNAQ inhibitor, a GNA11 inhibitor or an inhibitor of GNAQ and GNA11;

Ab is an antibody or antigen binding fragment thereof that binds to human PMEL17 protein;

L_A is a linker;

n is 1, 2, 3 or 4, and

y is 1, 2, 3 or 4.

In an embodiment, the D is a GNAQ inhibitor described herein, *e.g.*, a GNA11 inhibitor described herein or an inhibitor of GNAQ and GNA11 described herein.

In an embodiment, the antibody drug conjugate is present at a concentration of about 5 mg/mL to about 30 mg/mL, *e.g.,* about 10 mg to about 30 mg, about 15 mg/mL to about 25 mg/mL, about 18 mg/mL to about 22 mg/mL, about 10 mg/mL to 25 mg/mL, about 10 mg/mL to about 20 mg/mL, about 10 mg/mL to about 15 mg/mL, about 25 mg to about 30 mg/mL, about 20 mg/mL to about 30 mg/mL, about 5 mg/mL to about 15 mg/mL, about 15 mg/mL to about 25 mg/mL, or about 18 mg/mL to about 22 mg/mL, *e.g.,* about 5 mg/mL, about 6 mg/mL, about 7 mg/mL, about 8 mg/mL, about 9 mg/mL, about 10 mg/mL, about 11 mg/mL, about 12 mg/mL, about 13 mg/mL, about 14 mg/mL, about 15 mg/mL, about 16 mg/mL, about 17 mg/mL, about 18 mg/mL, about 19 mg/mL, about 20 mg/mL, about 21 mg/mL, about 22 mg/mL, about 23 mg/mL, about 24 mg/mL, or about 25 mg/mL.

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In an embodiment, the antibody drug conjugate is present at a concentration of about 15 mg/mL to about 25 mg/mL, *e.g.*, about 20 mg/mL.

In an embodiment, the buffering agent comprises histidine. In an embodiment, the buffering agent comprises succinate. In an embodiment, the buffering agent is present at a concentration of about 5 mM to about 50 mM, *e.g.*, about 10 mM to about 40 nM, about 15 mM to about 35 mM, about 20 mM to about 30 mM, about 5 mM to about 40 mM, about 5 mM to about 30 mM, about 5 mM to about 20 mM, about 5 mM to about 15 mM, about 5 mM to about 10 mM, about 40 mM to about 50 mM, about 35 mM to about 50 mM, about 30 mM to about 50 mM, about 25 mM to about 50 mM, about 20 mM to about 50 mM, about 15 mM to about 50 mM, about 10 mM to about 50 mM, about 10 mM to about 20 mM, about 15 mM to about 25 mM, about 25 mM to about 35 mM, about 30 mM to about 40 mM, or about 35 mM to about 45 mM, *e.g.*, about 5 mM, about 10 mM, about 15 mM, about 20 mM, about 25 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, or about 50 mM. In an embodiment, the buffering agent is present at a concentration of about 15 mM to about 25 mM, *e.g.*, about 20 mM.

In an embodiment, the stabilizing agent comprises sucrose. In an embodiment, the stabilizing agent comprises trehalose. In an embodiment, the stabilizing agent is present at a concentration of about 100 mM to about 500 mM, *e.g.*, about 150 mM to about 450 mM, about 200 mM to about 400 mM, about 250 mM to about 350 mM, about 100 mM to about 450 mM, about 100 mM to about 400 mM, about 100 mM to about 350 mM, about 100 mM to about 300 mM, about 100 mM to about 250 mM, about 100 mM to about 200 mM, about 100 mM to about 150 mM, about 450 mM to about 500 mM, about 400 mM to about 500 mM, about 350 mM to about 500 mM, about 300 mM to about 500 mM, about 250 mM to about 500 mM, about 200 mM to about 500 mM, about 150 mM to about 500 mM, about 150 mM to about 250 mM, about 200 mM to about 250 mM, about 200 mM to about 300 mM, about 300 mM to about 400 mM, or about 350 mM to about 450 mM, *e.g.*, about 100 mM, about 150 mM, about 200 mM, about 240 mM, about 250 mM, about 300 mM, about 350 mM, about 400 mM, about 450 mM, or about 500 mM. In an embodiment, the stabilizing agent and is present at a concentration of about 200 mM to about 300 mM, *e.g.*, about 240 mM.

In an embodiment, the surfactant comprises polysorbate 20. In an embodiment, the surfactant comprises polysorbate 80. In an embodiment, the surfactant is present at a concentration of about 0.01% to about 0.06%, *e.g.*, about 0.02% to about 0.05%, about 0.03% to about 0.04%, about 0.01% to about 0.05%, about 0.01% to about 0.04%, about 0.01% to about 0.03%, about 0.01% to about 0.02%, about 0.05% to about 0.06%, about 0.04% to about 0.06%, about 0.03% to about 0.06%, about 0.02% to about 0.06%, about 0.02% to about 0.04%, about 0.03% to about 0.05%, *e.g.*, about 0.01%, about 0.02%, about 0.03%, about 0.04%, about 0.05%, or about 0.06%. In an embodiment, the surfactant is present at a concentration of about 0.01% to about 0.03%, *e.g.*, about 0.02%.

In an embodiment, the formulation has a pH of about 4.7 to about 5.3, about 5.0 to about 6.0, about 5.2 to about 5.8, about 5.4 to about 5.6, about 5.2 to about 6.0, about 5.4 to about 6.0, about 5.6

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to about 6.0, about 5.8 to about 6.0, about 5 to about 5.8, about 5 to about 5.6.0, about 5 to about 5.4, about 5 to about 5.2, about 4.8 to about 5.2, about 5.1 to about 5.3, about 5.2 to about 5.4, about 5.3 to about 5.5, about 5.5 to about 5.7, about 5.6 to about 5.8, about 5.7 to about 5.9, about 4.9 to about 5.5, about 5.5 to about 6.1, or about 5.7 to about 6.3, *e.g.*, about 4.5, about 4.6, about 4.7, about 4.8, about 4.9, about 5, about 5.1, about 5.2, about 5.3, about 5.4, about 5.5, about 5.6, about 5.7, about 5.8, about 5.9, about 6.0, about 6.1, about 6.2, about 6.3, about 6.4, or about 6.5. In an embodiment, the formulation has a pH of about 5.0 to about 5.6, *e.g.*, about 5.3.

In an embodiment, the formulation comprises about 15 mg/mL to about 25 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine or succinate, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose or trehalose, and about 0.01% to about 0.03% of a surfactant comprising polysorbate 20 or polysorbate 80, wherein the formulation has a pH of about 5.0 to about 5.6.

In an embodiment, the formulation comprises about 15 mg/mL to about 25 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose, and about 0.01% to about 0.03% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.0 to about 5.6.

In an embodiment, the formulation comprises about 20 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine or succinate, about 240 mM of a stabilizing agent comprising sucrose or trehalose, and about 0.02% of a surfactant comprising polysorbate 20 or polysorbate 80, wherein the formulation has a pH of about 5.3.

In an embodiment, the formulation comprises about 20 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine, about 240 mM of a stabilizing agent comprising sucrose, and about 0.02% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.3.

In an embodiment, the formulation comprises about 15 mg/mL to about 25 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine or succinate, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose or trehalose, and about 0.01% to about 0.03% of a surfactant comprising polysorbate 20 or polysorbate 80, wherein the formulation has a pH of about 4.7 to about 5.3.

In an embodiment, the formulation comprises about 15 mg/mL to about 25 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose, and about 0.01% to about 0.03% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 4.7 to about 5.3.

In an embodiment, the formulation comprises about 20 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine or succinate, about 240 mM of a

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stabilizing agent comprising sucrose or trehalose, and about 0.02% of a surfactant comprising polysorbate 20 or polysorbate 80, wherein the formulation has a pH of about 5.0.

In an embodiment, the formulation comprises about 20 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine, about 240 mM of a stabilizing agent comprising sucrose, and about 0.02% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.0.

In an embodiment, the formulation comprises about 15 mg/mL to about 25 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine or succinate, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose or trehalose, and about 0.01% to about 0.03% of a surfactant comprising polysorbate 20 or polysorbate 80, wherein the formulation has a pH of about 5.2 to about 5.8.

In an embodiment, the formulation comprises about 15 mg/mL to about 25 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose, and about 0.01% to about 0.03% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.2 to about 5.8.

In an embodiment, the formulation comprises about 20 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine or succinate, about 240 mM of a stabilizing agent comprising sucrose or trehalose, and about 0.02% of a surfactant comprising polysorbate 20 or polysorbate 80, wherein the formulation has a pH of about 5.5.

In an embodiment, the formulation comprises about 20 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine, about 240 mM of a stabilizing agent comprising sucrose, and about 0.02% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.5.

In an embodiment, the formulation comprises about 15 mg/mL to about 25 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine or succinate, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose or trehalose, and about 0.03% to about 0.05% of a surfactant comprising polysorbate 20 or polysorbate 80, wherein the formulation has a pH of about 5.2 to about 5.8.

In an embodiment, the formulation comprises about 15 mg/mL to about 25 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose, and about 0.03% to about 0.05% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.2 to about 5.8.

In an embodiment, the formulation comprises about 20 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine or succinate, about 240 mM of a

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stabilizing agent comprising sucrose or trehalose, and about 0.04% of a surfactant comprising polysorbate 20 or polysorbate 80, wherein the formulation has a pH of about 5.5.

In an embodiment, the formulation comprises about 20 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine, about 240 mM of a stabilizing agent comprising sucrose, and about 0.04% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.5.

In an embodiment, the formulation comprises about 5 mg/mL to about 15 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine or succinate, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose or trehalose, and about 0.03% to about 0.05% of a surfactant comprising polysorbate 20 or polysorbate 80, wherein the formulation has a pH of about 5.2 to about 5.8.

In an embodiment, the formulation comprises about 5 mg/mL to about 15 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose, and about 0.03% to about 0.05% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.2 to about 5.8.

In an embodiment, the formulation comprises about 10 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine or succinate, about 240 mM of a stabilizing agent comprising sucrose or trehalose, and about 0.02% of a surfactant comprising polysorbate 20 or polysorbate 80, wherein the formulation has a pH of about 5.5.

In an embodiment, the formulation comprises about 10 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine, about 240 mM of a stabilizing agent comprising sucrose, and about 0.02% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.5.

In an embodiment, the formulation comprises about 15 mg/mL to about 25 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine or succinate, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose or trehalose, and about 0.01% to about 0.03% of a surfactant comprising polysorbate 20 or polysorbate 80, wherein the formulation has a pH of about 5.7 to about 6.3.

In an embodiment, the formulation comprises about 15 mg/mL to about 25 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose, and about 0.01% to about 0.03% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.7 to about 6.3.

In an embodiment, the formulation comprises about 20 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine or succinate, about 240 mM of a

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stabilizing agent comprising sucrose or trehalose, and about 0.02% of a surfactant comprising polysorbate 20 or polysorbate 80, wherein the formulation has a pH of about 6.0.

In an embodiment, the formulation comprises about 20 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine, about 240 mM of a stabilizing agent comprising sucrose, and about 0.02% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 6.0.

In an embodiment, the formulation comprises about 15 mg/mL to about 25 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine or succinate, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose or trehalose, and about 0.01% to about 0.03% of a surfactant comprising polysorbate 20 or polysorbate, wherein the formulation has a pH of about 4.9 to about 5.5.

In an embodiment, the formulation comprises about 15 mg/mL to about 25 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose, and about 0.01% to about 0.03% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 4.9 to about 5.5.

In an embodiment, the formulation comprises about 20 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine or succinate, about 240 mM of a stabilizing agent comprising sucrose or trehalose, and about 0.02% of a surfactant comprising polysorbate 20 or polysorbate 80, wherein the formulation has a pH of about 5.2.

In an embodiment, the formulation comprises about 20 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine, about 240 mM of a stabilizing agent comprising sucrose, and about 0.02% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.2.

In an embodiment, the formulation comprises about 15 mg/mL to about 25 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine or succinate, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose or trehalose, and about 0.01% to about 0.03% of a surfactant comprising polysorbate 20 or polysorbate 80, wherein the formulation has a pH of about 5.5 to about 6.1.

In an embodiment, the formulation comprises about 15 mg/mL to about 25 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose, and about 0.01% to about 0.03% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.5 to about 6.1.

In an embodiment, the formulation comprises about 20 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine or succinate, about 240 mM of a

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stabilizing agent comprising sucrose or trehalose, and about 0.02% of a surfactant comprising polysorbate 20 or polysorbate 80, wherein the formulation has a pH of about 5.8.

In an embodiment, the formulation comprises about 20 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine, about 240 mM of a stabilizing agent comprising sucrose, and about 0.02% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.8.

In another aspect, the disclosure features a lyophilized formulation, which is lyophilized from a formulation described herein.

In an embodiment, about 5 mL to about 5.5 mL of the formulation is lyophilized.

In an embodiment, the D in the lyophilized formulation is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10-fold more stable, compared to an otherwise identical formulation that is not lyophilized, after storage for about 0, about 4, or about 8 weeks, at about 5°C, 25°C, or 40°C, *e.g.*, as determined by CZE. In an embodiment, the percentage of D that has a ring-opening conformation in the lyophilized formulation is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10-fold lower, compared to an otherwise identical formulation that is not lyophilized, after storage for about 0, about 4, or about 8 weeks, at about 5°C, 25°C, or 40°C, *e.g.*, as determined by CZE.

In an embodiment, the lyophilized formulation comprises about 80 mg to about 120 mg (*e.g.*, about 107 mg) of the antibody drug conjugate.

In an embodiment, the level of monomers in the lyophilized formulation is at least about 95%, *e.g.*, at least about 96%, 97%, 98%, or 99%, after storage for about 0, about 4, or about 8 weeks, at about 5°C, 25°C, or 40°C, *e.g.*, as determined by SEC.

In an embodiment, the level of fragments in the lyophilized formulation is less than about 3%, *e.g.*, less than about 2.5%, 2%, 1.5%, 1%, or 0.5%, after storage for about 0, about 4, or about 8 weeks, at about 5°C, 25°C, or 40°C, *e.g.*, as determined by SEC.

In an embodiment, the level of aggregates in the lyophilized formulation is less than about 3%, *e.g.*, less than about 2.5%, 2%, 1.5%, 1%, or 0.5%, after storage for about 0, about 4, or about 8 weeks, at about 5°C, 25°C, or 40°C, *e.g.*, as determined by SEC.

In an embodiment, the level of degradation in the lyophilized formulation is less than about 2%, *e.g.*, less than about 1.5%, 1%, or 0.5%, after storage for about 4 weeks or about 8 weeks, at about 5°C, 25°C, or 40°C, *e.g.*, as determined by SEC.

In an embodiment, the level of particles greater than or equal to 10 μm in the lyophilized formulation is less than about 300 particles/ml, *e.g.,* less than about 280 particles/mL, less than about 260 particles/mL, less than about 240 particles/mL, less than about 220 particles/mL, less than about 200 particles/mL, less than about 180 particles/mL, less than about 160 particles/mL, less than about 140 particles/mL, 120 particles/mL, less than about 100 particles/mL, 80 particles/mL, 60

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particles/mL, 40 particles/mL, 20 particles/mL, or 10 particles/mL, after storage for about 0, about 4, or about 8 weeks, at about 5°C, 25°C, or 40°C, *e.g.*, as determined by light obscuration.

In an embodiment, the level of particles greater than 10 μm in the lyophilized formulation is increased by no more than about 3-fold, *e.g.*, no more than about 2.5, 2, 1.5, 1, or 0.5-fold, after storage for about 4 or about 8 weeks, at about 5°C, 25°C, or 40°C, *e.g.*, as determined by light obscuration.

In an embodiment, the level of particles greater than 25 μm in the lyophilized formulation is increased by no more than about 3-fold, *e.g.*, no more than about 2.5, 2, 1.5, 1, or 0.5-fold, after storage for about 4 or about 8 weeks, at about 5°C, 25°C, or 40°C, *e.g.*, as determined by light obscuration.

In an embodiment, the level of particles greater than 25 μm in the lyophilized formulation is less than about 20 particles/mL, *e.g.*, less than about 15 particles/mL, 10 particles/mL, 5 particles/mL, or 2 particles/mL, after storage for about 0, about 4, or about 8 weeks, at about 5°C, 25°C, or 40°C, *e.g.*, as determined by light obscuration.

In an embodiment, the level of impurity in the lyophilized formulation is less than about 15%, *e.g.*, less than about 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%, after storage for about 0, about 4, or about 8 weeks, at about 5°C, 25°C, or 40°C, *e.g.*, as determined by capillary electrophoresis.

In an embodiment, the level of impurity in the lyophilized formulation is increased by no more than 20%, *e.g.*, no more than about 15%, 10%, 5%, 2%, or 1%, after storage for about 4 weeks or about 8 weeks, at about 5°C, 25°C, or 40°C, *e.g.*, as determined by capillary electrophoresis.

In an embodiment, the level of neutral variants in the lyophilized formulation is greater than about 50%, *e.g.*, greater than about 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90%, after storage for about 0, about 4, or about 8 weeks, at about 5°C, 25°C, or 40°C, *e.g.*, as determined by capillary zone electrophoresis (CZE).

In an embodiment, the level of neutral variants in the lyophilized formulation is decreased by no more than about 20%, *e.g.*, no more than about 15%, 10%, 5%, or 2%, after storage for about 4 weeks or about 8 weeks, at about 5°C, 25°C, or 40°C, *e.g.*, as determined by CZE.

In an embodiment, the level of acidic variants in the lyophilized formulation is less than about 30%, *e.g.*, less than about 25%, 20%, 15%, 5%, or 2%, after storage for about 0, about 4, or about 8 weeks, at about 5°C, 25°C, or 40°C, *e.g.*, as determined by CZE.

In an embodiment, the level of acid variants in the lyophilized formulation is increased by no more than about 50%, *e.g.*, no more than about 40%, 30%, 20%, 10%, or 5%, after storage for about 4 weeks or about 8 weeks, at about 5°C, 25°C, or 40°C, *e.g.*, as determined by CZE.

In an embodiment, the level of basic variants in the lyophilized formulation is less than about 30%, *e.g.*, less than about 25%, 20%, 15%, 5%, or 2%, after storage for about 0, about 4, or about 8 weeks, at about 5°C, 25°C, or 40°C, *e.g.*, as determined by CZE.

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In an embodiment, the level of basic variants in the lyophilized formulation is increased by no more than about 50%, *e.g.*, no more than about 40%, 30%, 20%, 10%, or 5%, after storage for about 4 weeks or about 8 weeks, at about 5°C, 25°C, or 40°C, *e.g.*, as determined by CZE.

In an embodiment, the potency of the lyophilized formulation is decreased by no more than about 25%, *e.g.*, no more than about 20%, 15%, 10%, 5%, or 2%, after storage for about 8 weeks, at about 5°C, 25°C, or 40°C, *e.g.*, as determined by a bioactivity assay.

In an embodiment, the osmolality of the lyophilized formulation is about 200 mOsm/L to 400 mOsm/L, *e.g.*, 200 mOsm/L to 300 mOsm/L, 250 mOsm/L to 350 mOsm/L, 270 mOsm/L to 330 mOsm/L, 290 mOsm/L to 310 mOsm/L, 270 mOsm/L to 350 mOsm/L, 290 mOsm/L to 350 mOsm/L, 310 mOsm/L to 350 mOsm/L, 330 mOsm/L to 350 mOsm/L, 250 mOsm/L to 330 mOsm/L, 250 mOsm/L to 310 mOsm/L, 250 mOsm/L to 290 mOsm/L, 250 mOsm/L to 270 mOsm/L, 260 mOsm/L to 280 mOsm/L, 270 mOsm/L to 290 mOsm/L, 280 mOsm/L to 300 mOsm/L, 300 mOsm/L to 320 mOsm/L, 310 mOsm/L to 330 mOsm/L, or 320 mOsm/L to 340 mOsm/L, *e.g.*, 200 mOsm/L, 250 mOsm/L, 260 mOsm/L, 270 mOsm/L, 280 mOsm/L, 290 mOsm/L, 300 mOsm/L, 310 mOsm/L, 320 mOsm/L, 330 mOsm/L, 340 mOsm/L, 350 mOsm/L, or 400 mOsm/L.

In another aspect, the disclosure features a liquid formulation, which is reconstituted from a lyophilized formulation described herein.

In another aspect, the disclosure features a container comprising a lyophilized formulation described herein.

In an embodiment, the container is a vial, *e.g.*, a 25 R glass vial. In an embodiment, the container further comprises a stopper and a cap (*e.g.*, a flip-off aluminum cap).

Methods of Treatment

In another aspect, the disclosure features a method of treating a cancer, comprising administering to a subject in need thereof an antibody drug conjugate (ADC) described herein, or a formulation described herein, at a dose of 1 mg/kg to 16 mg/kg, thereby treating the cancer.

In an embodiment, the ADC is administered at a dose of 2 mg/kg to 15 mg/kg. In an embodiment, the ADC is administered at a dose of 1 mg/kg, 2 mg/kg, 4 mg/kg, 8 mg/kg, 12 mg/kg, or 16 mg/kg. In an embodiment, the ADC is administered once a week, once every two weeks, or once every four weeks. In an embodiment, the ADC is administered once every two weeks. In an embodiment, the ADC is administered intravenously.

In an embodiment, the subject has been treated with tebentafusp prior to the administration of the ADC. In an embodiment, the subject has not been treated with tebentafusp prior to the administration of the ADC.

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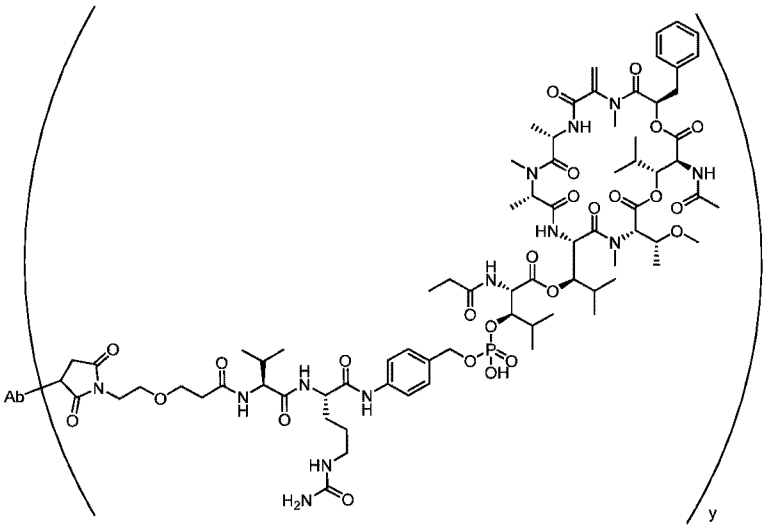
In an embodiment, the cancer expresses PMEL17, contains a mutation of the GNAQ or GNA11 gene, or the melanoma expresses PMEL17 and contains a mutation of GNAQ, GNA11, or both. In an embodiment, the cancer is a melanoma. In an embodiment, the cancer is a malignant melanoma, uveal melanoma, ocular melanoma, an eye cancer, an eye neoplasm, a cutaneous melanoma, or mucosal melanoma.

In an embodiment, the ADC has the following structure:



wherein:

Ab is an antibody or antigen binding fragment thereof that binds to human PMEL17 protein and y is 1, 2, 3, or 4. In an embodiment, y is 2.

In an embodiment, the Ab comprises:

(a) a heavy chain CDR1 of SEQ ID NO: 1, a heavy chain CDR2 of SEQ ID NO: 2, a heavy chain CDR3 of SEQ ID NO: 3, a light chain CDR1 of SEQ ID NO: 14, a light chain CDR2 of SEQ ID NO: 15, and a light chain CDR3 of SEQ ID NO: 16;

(b) a heavy chain CDR1 of SEQ ID NO: 4, a heavy chain CDR2 of SEQ ID NO: 2, a heavy chain CDR3 of SEQ ID NO: 3, a light chain CDR1 of SEQ ID NO: 14, a light chain CDR2 of SEQ ID NO:15, and a light chain CDR3 of SEQ ID NO: 16;

(c) a heavy chain CDR1 of SEQ ID NO: 5, a heavy chain CDR2 of SEQ ID NO: 6, a heavy chain CDR3 of SEQ ID NO: 3, a light chain CDR1 of SEQ ID NO: 17, a light chain CDR2 of SEQ ID NO: 18, and a light chain CDR3 of SEQ ID NO: 19; or

(d) a heavy chain CDR1 of SEQ ID NO: 7, a heavy chain CDR2 of SEQ ID NO: 8, a heavy chain CDR3 of SEQ ID NO: 9, a light chain CDR1 of SEQ ID NO: 20, a light chain CDR2 of SEQ ID NO: 18, and a light chain CDR3 of SEQ ID NO: 16.

In an embodiment, the Ab comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 10 and a light chain variable region (VL) comprising the amino

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acid sequence of SEQ ID NO: 25. In an embodiment, the Ab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 283 and a light chain comprising the amino acid sequence of SEQ ID NO: 27.

In an embodiment, the Ab comprises a heavy chain amino acid sequence comprising an N-glycosylation site. In an embodiment, the N-glycosylation site is located at Asn306.

In an embodiment, the method further comprises determining the expression of one or more biomarkers. In an embodiment, the one or more biomarkers is selected from premelanosome protein 17 (PMEL17), phosphorylated ERK (pERK), cluster of differentiation 8 (CD8), programmed death-ligand 1 (PD-L1), Dual specificity phosphatase 6 (DUSP6), Ras guanyl-releasing protein 3 (RASGRP3), or any combination thereof.

In an embodiment, the expression of the one or more biomarkers is determined in a sample from the subject. In an embodiment, the sample is obtained from the subject before, during, and/or after administration of the ADC. In an embodiment, the sample is a tumor sample. For example, the tumor sample can be an archived tissue block or obtained by a needle biopsy. In an embodiment, the mRNA expression of the one or more biomarkers is determined. In an embodiment, the protein expression of the one or more biomarkers is determined. In an embodiment, the method further comprises detecting one or more cancer-related genes by next-generation DNA sequencing, performing a whole transcriptome analysis, determining the expression of one or more immune and cancer-related genes, or a combination thereof.

In an embodiment, the method further comprises evaluating cell-free DNA (cfDNA) in a sample from the subject. In an embodiment, the sample is obtained from the subject before, during, and/or after administration of the ADC. In an embodiment, the sample is a blood sample. In an embodiment, evaluating the cell-free DNA determines one or more of: driver mutation status, predictive pharmacodynamics, tumor mutational burden (TMB), or resistance markers.

In an aspect, the disclosure features a method of evaluating a treatment (*e.g.*, a treatment described herein) of a disease (*e.g.*, a disease described herein) in a subject (*e.g.*, a subject described herein), comprising determining the expression of one or more biomarkers and/or evaluating cell-free DNA (cfDNA) in a sample (*e.g.*, a sample described herein) from the subject, in accordance with a method described herein.

In an aspect, the disclosure features a method of evaluating progression of a disease (*e.g.*, a disease described herein) in a subject (*e.g.*, a subject described herein), comprising determining the expression of one or more biomarkers and/or evaluating cell-free DNA (cfDNA) in a sample (*e.g.*, a sample described herein) from the subject, in accordance with a method described herein.

In an aspect, the disclosure features a method of selecting a treatment (*e.g.*, a treatment described herein) for a disease (*e.g.*, a disease described herein) in a subject (*e.g.*, a subject described herein), comprising determining the expression of one or more biomarkers and/or evaluating cell-free

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DNA (cfDNA) in a sample (*e.g.*, a sample described herein) from the subject, in accordance with a method described herein.

In an aspect, the disclosure features a method of selecting a subject (*e.g.*, a subject described herein) for a treatment (*e.g.*, a treatment described herein) for a disease (*e.g.*, a disease described herein), comprising determining the expression of one or more biomarkers and/or evaluating cell-free DNA (cfDNA) in a sample (*e.g.*, a sample described herein) from the subject, in accordance with a method described herein.

Other Embodiments

The aspects and embodiments described herein may include any of the embodiments below.

In some embodiments, the antibody or antigen binding fragment thereof that binds PMEL17 comprises:

(a) a heavy chain variable region that comprises a heavy chain CDR1 (Complementarity Determining Region 1) of SEQ ID NO:1, 4, 5 or 7, a heavy chain CDR2 (Complementarity Determining Region 2) of SEQ ID NO:2, 6 or 8, and a heavy chain CDR3 (Complementarity Determining Region 3) of SEQ ID NO:3 or 9; and a light chain variable region that comprises a light chain CDR1 (Complementarity Determining Region 1) of SEQ ID NO:14, 17 or 20, a light chain CDR2 (Complementarity Determining Region 2) of SEQ ID NO:15 or 18, and a light chain CDR3 (Complementarity Determining Region 3) of SEQ ID NO:16 or 19;

(b) a heavy chain variable region that comprises a heavy chain CDR1 of SEQ ID NO:33, 36, 37 or 39, a heavy chain CDR2 of SEQ ID NO:34, 38 or 40; a heavy chain CDR3 of SEQ ID NO:35 or 41; a light chain CDR1 of SEQ ID NO:46, 49 or 52; a light chain CDR2 of SEQ ID NO:47 or 50; and a light chain CDR3 of SEQ ID NO:48 or 51;

(c) a heavy chain variable region that comprises a heavy chain CDR1 of SEQ ID NO:5, 7, 57 or 60, a heavy chain CDR2 of SEQ ID NO:58, 61 or 62; a heavy chain CDR3 of SEQ ID NO:59 or 63; a light chain CDR1 of SEQ ID NO:68, 71 or 74; a light chain CDR2 of SEQ ID NO:69 or 72; and a light chain CDR3 of SEQ ID NO:70 or 73;

(d) a heavy chain variable region that comprises a heavy chain CDR1 of SEQ ID NO:79, 82, 83 or 85, a heavy chain CDR2 of SEQ ID NO:80, 84 or 86; a heavy chain CDR3 of SEQ ID NO:81 or 87; a light chain CDR1 of SEQ ID NO:92, 95 or 98; a light chain CDR2 of SEQ ID NO:93 or 96; and a light chain CDR3 of SEQ ID NO:94 or 97;

(e) a heavy chain variable region that comprises a heavy chain CDR1 of SEQ ID NO:103, 106, 107 or 109, a heavy chain CDR2 of SEQ ID NO:104, 108 or 110; a heavy chain CDR3 of SEQ ID NO:105 or 111; a light chain CDR1 of SEQ ID NO:49, 52 or 116; a light chain CDR2 of SEQ ID NO:47 or 50; and a light chain CDR3 of SEQ ID NO:117 or 118;

(f) a heavy chain variable region that comprises a heavy chain CDR1 of SEQ ID NO:123, 126, 127 or 129, a heavy chain CDR2 of SEQ ID NO:124, 128 or 130; a heavy chain CDR3 of SEQ

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ID NO:125 or 131; a light chain CDR1 of SEQ ID NO:136, 139 or 142; a light chain CDR2 of SEQ ID NO:137 or 140; and a light chain CDR3 of SEQ ID NO:138 or 141;

(g) a heavy chain variable region that comprises a heavy chain CDR1 of SEQ ID NO:123, 126, 127 or 129, a heavy chain CDR2 of SEQ ID NO:124, 128 or 130; a heavy chain CDR3 of SEQ ID NO:147 or 148; a light chain CDR1 of SEQ ID NO:153, 156 or 158; a light chain CDR2 of SEQ ID NO:50 or 154; and a light chain CDR3 of SEQ ID NO:155 or 157;

(h) a heavy chain variable region that comprises a heavy chain CDR1 of SEQ ID NO:103, 106, 107 or 109, a heavy chain CDR2 of SEQ ID NO:104, 108 or 110; a heavy chain CDR3 of SEQ ID NO:163 or 164; a light chain CDR1 of SEQ ID NO:49, 52 or 116; a light chain CDR2 of SEQ ID NO:47 or 50; and a light chain CDR3 of SEQ ID NO:169 or 170;

(i) a heavy chain variable region that comprises a heavy chain CDR1 of SEQ ID NO:175, 178, 179 or 181, a heavy chain CDR2 of SEQ ID NO:176, 180 or 182; a heavy chain CDR3 of SEQ ID NO:177 or 183; a light chain CDR1 of SEQ ID NO:49, 52 or 116; a light chain CDR2 of SEQ ID NO:47 or 50; and a light chain CDR3 of SEQ ID NO:188 or 189;

(j) a heavy chain variable region that comprises a heavy chain CDR1 of SEQ ID NO: 103, 106, 107 or 109, a heavy chain CDR2 of SEQ ID NO: 104, 108 or 110; a heavy chain CDR3 of SEQ ID NO:194 or 195; a light chain CDR1 of SEQ ID NO: 49, 52 or 116; a light chain CDR2 of SEQ ID NO: 47 or 50; and a light chain CDR3 of SEQ ID NO:200 or 201;

(k) a heavy chain variable region that comprises a heavy chain CDR1 of SEQ ID NO:206, 209, 210 or 212, a heavy chain CDR2 of SEQ ID NO:207, 211 or 213; a heavy chain CDR3 of SEQ ID NO:208 or 214; a light chain CDR1 of SEQ ID NO:153, 156 or 158; a light chain CDR2 of SEQ ID NO:50 or 154; and a light chain CDR3 of SEQ ID NO:219 or 220;

(l) a heavy chain variable region that comprises a heavy chain CDR1 of SEQ ID NO: 206, 209, 210 or 212, a heavy chain CDR2 of SEQ ID NO: 207, 211 or 213; a heavy chain CDR3 of SEQ ID NO:225 or 226; a light chain CDR1 of SEQ ID NO:136, 139 or 142; a light chain CDR2 of SEQ ID NO:137 or 140; and a light chain CDR3 of SEQ ID NO:231 or 232;

(m) a heavy chain variable region that comprises a heavy chain variable region that comprises an HCDR1 of SEQ ID NO: 206, 209, 210 or 212, an HCDR2 of SEQ ID NO: 207, 211 or 213, and an HCDR3 of SEQ ID NO:237 or 238; and a light chain variable region that comprises an LCDR1 of SEQ ID NO:243, 245 or 247, an LCDR2 of SEQ ID NO:47 or 50, and an LCDR3 of SEQ ID NO:244 or 246;

(n) a heavy chain variable region that comprises a heavy chain variable region that comprises an HCDR1 of SEQ ID NO: 206, 209, 210 or 212, an HCDR2 of SEQ ID NO: 207, 211 or 213, and an HCDR3 of SEQ ID NO:252 or 253; and a light chain variable region that comprises an LCDR1 of SEQ ID NO:153, 156 or 158, an LCDR2 of SEQ ID NO:50 or 154, and an LCDR3 of SEQ ID NO:258 or 259;

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(o) a heavy chain CDR1 of SEQ ID NO:1, a heavy chain CDR2 of SEQ ID NO:2, a heavy chain CDR3 of SEQ ID NO:3, a light chain CDR1 of SEQ ID NO:14, a light chain CDR2 of SEQ ID NO:15, and a light chain CDR3 of SEQ ID NO:16;

(p) a heavy chain CDR1 of SEQ ID NO: 4, a heavy chain CDR2 of SEQ ID NO:2, a heavy chain CDR3 of SEQ ID NO:3, a light chain CDR1 of SEQ ID NO:14, a light chain CDR2 of SEQ ID NO:15, and a light chain CDR3 of SEQ ID NO:16;

(q) a heavy chain CDR1 of SEQ ID NO:5, a heavy chain CDR2 of SEQ ID NO:6, a heavy chain CDR3 of SEQ ID NO:3, a light chain CDR1 of SEQ ID NO:17, a light chain CDR2 of SEQ ID NO: 18, and a light chain CDR3 of SEQ ID NO: 19;

(r) a heavy chain CDR1 of SEQ ID NO:7, a heavy chain CDR2 of SEQ ID NO:8, a heavy chain CDR3 of SEQ ID NO:9, a light chain CDR1 of SEQ ID NO:20, a light chain CDR2 of SEQ ID NO:18, and a light chain CDR3 of SEQ ID NO:16;

(s) a heavy chain CDR1 of SEQ ID NO:33, a heavy chain CDR2 of SEQ ID NO:34, a heavy chain CDR3 of SEQ ID NO:35, a light chain CDR1 of SEQ ID NO:46, a light chain CDR2 of SEQ ID NO:47, and a light chain CDR3 of SEQ ID NO:48;

(t) a heavy chain CDR1 of SEQ ID NO:36, a heavy chain CDR2 of SEQ ID NO:34, a heavy chain CDR3 of SEQ ID NO:35, a light chain CDR1 of SEQ ID NO:46, a light chain CDR2 of SEQ ID NO:47, and a light chain CDR3 of SEQ ID NO:48;

(u) a heavy chain CDR1 of SEQ ID NO:37, a heavy chain CDR2 of SEQ ID NO:38, a heavy chain CDR3 of SEQ ID NO:35, a light chain CDR1 of SEQ ID NO:49, a light chain CDR2 of SEQ ID NO:50, and a light chain CDR3 of SEQ ID NO:51;

(v) a heavy chain CDR1 of SEQ ID NO: 39, a heavy chain CDR2 of SEQ ID NO:40, a heavy chain CDR3 of SEQ ID NO:41, a light chain CDR1 of SEQ ID NO:52, a light chain CDR2 of SEQ ID NO:50, and a light chain CDR3 of SEQ ID NO:48;

(w) a heavy chain CDR1 of SEQ ID NO:57, a heavy chain CDR2 of SEQ ID NO:58, a heavy chain CDR3 of SEQ ID NO:59, a light chain CDR1 of SEQ ID NO:68, a light chain CDR2 of SEQ ID NO:69, and a light chain CDR3 of SEQ ID NO:70;

(x) a heavy chain CDR1 of SEQ ID NO:60, a heavy chain CDR2 of SEQ ID NO:58, a heavy chain CDR3 of SEQ ID NO:59, a light chain CDR1 of SEQ ID NO:68, a light chain CDR2 of SEQ ID NO:69, and a light chain CDR3 of SEQ ID NO:70;

(y) a heavy chain CDR1 of SEQ ID NO:5, a heavy chain CDR2 of SEQ ID NO:61, a heavy chain CDR3 of SEQ ID NO:59, a light chain CDR1 of SEQ ID NO:71, a light chain CDR2 of SEQ ID NO:72, and a light chain CDR3 of SEQ ID NO:73;

(z) a heavy chain CDR1 of SEQ ID NO:7, a heavy chain CDR2 of SEQ ID NO:62, a heavy chain CDR3 of SEQ ID NO:63, a light chain CDR1 of SEQ ID NO:74, a light chain CDR2 of SEQ ID NO:72, and a light chain CDR3 of SEQ ID NO:70;

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(aa) a heavy chain CDR1 of SEQ ID NO:79, , a heavy chain CDR2 of SEQ ID NO:80, a heavy chain CDR3 of SEQ ID NO:81, a light chain CDR1 of SEQ ID NO:92, a light chain CDR2 of SEQ ID NO:93, and a light chain CDR3 of SEQ ID NO:94;

(bb) a heavy chain CDR1 of SEQ ID NO:82, a heavy chain CDR2 of SEQ ID NO:80, a heavy chain CDR3 of SEQ ID NO:81, a light chain CDR1 of SEQ ID NO:92, a light chain CDR2 of SEQ ID NO:93, and a light chain CDR3 of SEQ ID NO:94;

(cc) a heavy chain CDR1 of SEQ ID NO:83, a heavy chain CDR2 of SEQ ID NO:84, a heavy chain CDR3 of SEQ ID NO:81, a light chain CDR1 of SEQ ID NO:95, a light chain CDR2 of SEQ ID NO:96, and a light chain CDR3 of SEQ ID NO: 97;

(dd) a heavy chain CDR1 of SEQ ID NO: 85, a heavy chain CDR2 of SEQ ID NO:86, a heavy chain CDR3 of SEQ ID NO:87, a light chain CDR1 of SEQ ID NO:98, a light chain CDR2 of SEQ ID NO:96, and a light chain CDR3 of SEQ ID NO:94;

(ee) a heavy chain CDR1 of SEQ ID NO:103, a heavy chain CDR2 of SEQ ID NO:104, a heavy chain CDR3 of SEQ ID NO:105, a light chain CDR1 of SEQ ID NO: 116; a light chain CDR2 of SEQ ID NO:47; and a light chain CDR3 of SEQ ID NO:117;

(ff) a heavy chain CDR1 of SEQ ID NO:106, a heavy chain CDR2 of SEQ ID NO:104, a heavy chain CDR3 of SEQ ID NO:105, a light chain CDR1 of SEQ ID NO: 116, a light chain CDR2 of SEQ ID NO:47, and a light chain CDR3 of SEQ ID NO:117;

(gg) a heavy chain CDR1 of SEQ ID NO:107, a heavy chain CDR2 of SEQ ID NO:108, a heavy chain CDR3 of SEQ ID NO:105, a light chain CDR1 of SEQ ID NO:49, a light chain CDR2 of SEQ ID NO:50, and a light chain CDR3 of SEQ ID NO:118;

(hh) a heavy chain CDR1 of SEQ ID NO:109, a heavy chain CDR2 of SEQ ID NO:110, a heavy chain CDR3 of SEQ ID NO:111, a light chain CDR1 of SEQ ID NO:52 a light chain CDR2 of SEQ ID NO:50, and a light chain CDR3 of SEQ ID NO:117;

(ii) a heavy chain CDR1 of SEQ ID NO:123, a heavy chain CDR2 of SEQ ID NO:124, a heavy chain CDR3 of SEQ ID NO:125, a light chain CDR1 of SEQ ID NO:136, a light chain CDR2 of SEQ ID NO:137,and a light chain CDR3 of SEQ ID NO:138;

(jj) a heavy chain CDR1 of SEQ ID NO:126, a heavy chain CDR2 of SEQ ID NO:124, a heavy chain CDR3 of SEQ ID NO:125, a light chain CDR1 of SEQ ID NO:136, a light chain CDR2 of SEQ ID NO:137, and a light chain CDR3 of SEQ ID NO:138;

(kk) a heavy chain CDR1 of SEQ ID NO:127, a heavy chain CDR2 of SEQ ID NO:128, a heavy chain CDR3 of SEQ ID NO:125, a light chain CDR1 of SEQ ID NO:139, a light chain CDR2 of SEQ ID NO:140, and a light chain CDR3 of SEQ ID NO: 141;

(ll) a heavy chain CDR1 of SEQ ID NO: 129, a heavy chain CDR2 of SEQ ID NO:130, a heavy chain CDR3 of SEQ ID NO:131, a light chain CDR1 of SEQ ID NO:142, a light chain CDR2 of SEQ ID NO:140, and a light chain CDR3 of SEQ ID NO:138;

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(mm) a heavy chain CDR1 of SEQ ID NO:123, a heavy chain CDR2 of SEQ ID NO:124, a heavy chain CDR3 of SEQ ID NO:147, a light chain CDR1 of SEQ ID NO:153, a light chain CDR2 of SEQ ID NO:154, and a light chain CDR3 of SEQ ID NO:155;

(nn) a heavy chain CDR1 of SEQ ID NO:126, a heavy chain CDR2 of SEQ ID NO:124, a heavy chain CDR3 of SEQ ID NO:147, a light chain CDR1 of SEQ ID NO:153, a light chain CDR2 of SEQ ID NO: 154, and a light chain CDR3 of SEQ ID NO:155;

(oo) a heavy chain CDR1 of SEQ ID NO:127, a heavy chain CDR2 of SEQ ID NO:128, a heavy chain CDR3 of SEQ ID NO:147, a light chain CDR1 of SEQ ID NO:156, a light chain CDR2 of SEQ ID NO:50, and a light chain CDR3 of SEQ ID NO:157;

(pp) a heavy chain CDR1 of SEQ ID NO: 129, a heavy chain CDR2 of SEQ ID NO:130, a heavy chain CDR3 of SEQ ID NO:148, a light chain CDR1 of SEQ ID NO:158, a light chain CDR2 of SEQ ID NO:50, and a light chain CDR3 of SEQ ID NO:155;

(qq) a heavy chain CDR1 of SEQ ID NO:103, a heavy chain CDR2 of SEQ ID NO:104, a heavy chain CDR3 of SEQ ID NO:163, a light chain CDR1 of SEQ ID NO: 116, a light chain CDR2 of SEQ ID NO:47, and a light chain CDR3 of SEQ ID NO:169;

(rr) a heavy chain CDR1 of SEQ ID NO:106, a heavy chain CDR2 of SEQ ID NO:104, a heavy chain CDR3 of SEQ ID NO:163, a light chain CDR1 of SEQ ID NO:116, a light chain CDR2 of SEQ ID NO:47, and a light chain CDR3 of SEQ ID NO:169;

(ss) a heavy chain CDR1 of SEQ ID NO:107, a heavy chain CDR2 of SEQ ID NO:108, a heavy chain CDR3 of SEQ ID NO:163, a light chain CDR1 of SEQ ID NO:49, a light chain CDR2 of SEQ ID NO:50, and a light chain CDR3 of SEQ ID NO:170;

(tt) a heavy chain CDR1 of SEQ ID NO: 109, a heavy chain CDR2 of SEQ ID NO:110, a heavy chain CDR3 of SEQ ID NO:164, a light chain CDR1 of SEQ ID NO:52, a light chain CDR2 of SEQ ID NO:50, and a light chain CDR3 of SEQ ID NO:169;

(uu) a heavy chain CDR1 of SEQ ID NO:175, a heavy chain CDR2 of SEQ ID NO:176, a heavy chain CDR3 of SEQ ID NO:177, a light chain CDR1 of SEQ ID NO:116, a light chain CDR2 of SEQ ID NO:47, and a light chain CDR3 of SEQ ID NO:188;

(vv) a heavy chain CDR1 of SEQ ID NO:178, a heavy chain CDR2 of SEQ ID NO:176, a heavy chain CDR3 of SEQ ID NO:177, a light chain CDR1 of SEQ ID NO:116, a light chain CDR2 of SEQ ID NO:47, and a light chain CDR3 of SEQ ID NO:188;

(ww) a heavy chain CDR1 of SEQ ID NO:179, a heavy chain CDR2 of SEQ ID NO:180, a heavy chain CDR3 of SEQ ID NO:177, a light chain CDR1 of SEQ ID NO:49, a light chain CDR2 of SEQ ID NO:50, and a light chain CDR3 of SEQ ID NO:189;

(xx) a heavy chain CDR1 of SEQ ID NO: 181, a heavy chain CDR2 of SEQ ID NO:182; a heavy chain CDR3 of SEQ ID NO:183, a light chain CDR1 of SEQ ID NO:52, a light chain CDR2 of SEQ ID NO:50, and a light chain CDR3 of SEQ ID NO:188;

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(yy) a heavy chain CDR1 of SEQ ID NO: 103, a heavy chain CDR2 of SEQ ID NO: 104, a heavy chain CDR3 of SEQ ID NO:194, a light chain CDR1 of SEQ ID NO: 116, a light chain CDR2 of SEQ ID NO: 47, and a light chain CDR3 of SEQ ID NO:200;

(zz) a heavy chain CDR1 of SEQ ID NO: 106, a heavy chain CDR2 of SEQ ID NO: 104, a heavy chain CDR3 of SEQ ID NO:194, a light chain CDR1 of SEQ ID NO: 116, a light chain CDR2 of SEQ ID NO: 47, and a light chain CDR3 of SEQ ID NO:200;

(aaa) a heavy chain CDR1 of SEQ ID NO: 107, a heavy chain CDR2 of SEQ ID NO: 108, a heavy chain CDR3 of SEQ ID NO:194, a light chain CDR1 of SEQ ID NO: 49, a light chain CDR2 of SEQ ID NO: 50, and a light chain CDR3 of SEQ ID NO: 201;

(bbb) a heavy chain CDR1 of SEQ ID NO: 109, a heavy chain CDR2 of SEQ ID NO: 110, a heavy chain CDR3 of SEQ ID NO:195, a light chain CDR1 of SEQ ID NO: 52, a light chain CDR2 of SEQ ID NO: 50, and a light chain CDR3 of SEQ ID NO:200;

(ccc) a heavy chain CDR1 of SEQ ID NO:206, a heavy chain CDR2 of SEQ ID NO:207, a heavy chain CDR3 of SEQ ID NO:208, a light chain CDR1 of SEQ ID NO:153, a light chain CDR2 of SEQ ID NO:154, and a light chain CDR3 of SEQ ID NO:219;

(ddd) a heavy chain CDR1 of SEQ ID NO:209, a heavy chain CDR2 of SEQ ID NO:207, a heavy chain CDR3 of SEQ ID NO:208, a light chain CDR1 of SEQ ID NO:153, a light chain CDR2 of SEQ ID NO: 154, and a light chain CDR3 of SEQ ID NO:219;

(eee) a heavy chain CDR1 of SEQ ID NO:210, a heavy chain CDR2 of SEQ ID NO:211, a heavy chain CDR3 of SEQ ID NO:208, a light chain CDR1 of SEQ ID NO:156, a light chain CDR2 of SEQ ID NO:50, and a light chain CDR3 of SEQ ID NO:220;

(fff) a heavy chain CDR1 of SEQ ID NO: 212, a heavy chain CDR2 of SEQ ID NO:213, a heavy chain CDR3 of SEQ ID NO:214, a light chain CDR1 of SEQ ID NO:158, a light chain CDR2 of SEQ ID NO:50, and a light chain CDR3 of SEQ ID NO:219;

(ggg) a heavy chain CDR1 of SEQ ID NO: 206, a heavy chain CDR2 of SEQ ID NO: 207, a heavy chain CDR3 of SEQ ID NO:225, a light chain CDR1 of SEQ ID NO:136, a light chain CDR2 of SEQ ID NO:137,and a light chain CDR3 of SEQ ID NO:231;

(hhh) a heavy chain CDR1 of SEQ ID NO: 209, a heavy chain CDR2 of SEQ ID NO: 207, a heavy chain CDR3 of SEQ ID NO:225, a light chain CDR1 of SEQ ID NO:136, a light chain CDR2 of SEQ ID NO:137, and a light chain CDR3 of SEQ ID NO:231;

(iii) a heavy chain CDR1 of SEQ ID NO: 210, a heavy chain CDR2 of SEQ ID NO: 211, a heavy chain CDR3 of SEQ ID NO:225, a light chain CDR1 of SEQ ID NO:139, a light chain CDR2 of SEQ ID NO:140, and a light chain CDR3 of SEQ ID NO: 232;

(jjj) a heavy chain CDR1 of SEQ ID NO: 212, a heavy chain CDR2 of SEQ ID NO: 213, a heavy chain CDR3 of SEQ ID NO: 226, a light chain CDR1 of SEQ ID NO:142; a light chain CDR2 of SEQ ID NO: 140; and a light chain CDR3 of SEQ ID NO:231;

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(kkk) a heavy chain variable region that comprises an HCDR1 of SEQ ID NO: 206, an HCDR2 of SEQ ID NO: 207, and an HCDR3 of SEQ ID NO:237,and a light chain variable region that comprises an LCDR1 of SEQ ID NO:243, an LCDR2 of SEQ ID NO:47, and an LCDR3 of SEQ ID NO:244;

(lll) a heavy chain variable region that comprises an HCDR1 of SEQ ID NO: 209, an HCDR2 of SEQ ID NO: 207, and an HCDR3 of SEQ ID NO:237, and a light chain variable region that comprises an LCDR1 of SEQ ID NO:243, an LCDR2 of SEQ ID NO:47, and an LCDR3 of SEQ ID NO:244;

(mmm) a heavy chain variable region that comprises an HCDR1 of SEQ ID NO: 210, an HCDR2 of SEQ ID NO: 211, and an HCDR3 of SEQ ID NO:237, and a light chain variable region that comprises an LCDR1 of SEQ ID NO:245, an LCDR2 of SEQ ID NO:50, and an LCDR3 of SEQ ID NO:246;

(nnn) a heavy chain variable region that comprises an HCDR1 of SEQ ID NO: 212, an HCDR2 of SEQ ID NO: 213, and an HCDR3 of SEQ ID NO:238; and a light chain variable region that comprises an LCDR1 of SEQ ID NO:247, an LCDR2 of SEQ ID NO: 50, and an LCDR3 of SEQ ID NO:244;

(ooo) a heavy chain variable region that comprises an HCDR1 of SEQ ID NO: 206, an HCDR2 of SEQ ID NO: 207, and an HCDR3 of SEQ ID NO:252,and a light chain variable region that comprises an LCDR1 of SEQ ID NO:153, an LCDR2 of SEQ ID NO: 154, and an LCDR3 of SEQ ID NO:258;

(ppp) a heavy chain variable region that comprises an HCDR1 of SEQ ID NO: 209, an HCDR2 of SEQ ID NO: 207, and an HCDR3 of SEQ ID NO:252, and a light chain variable region that comprises an LCDR1 of SEQ ID NO:153, an LCDR2 of SEQ ID NO:154, and an LCDR3 of SEQ ID NO:258;

(qqq) a heavy chain variable region that comprises an HCDR1 of SEQ ID NO: 210, an HCDR2 of SEQ ID NO: 211, and an HCDR3 of SEQ ID NO:252, and a light chain variable region that comprises an LCDR1 of SEQ ID NO:156, an LCDR2 of SEQ ID NO:50, and an LCDR3 of SEQ ID NO:259; or

(rrr) a heavy chain variable region that comprises an HCDR1 of SEQ ID NO: 212, an HCDR2 of SEQ ID NO: 213, and an HCDR3 of SEQ ID NO: 253; and a light chain variable region that comprises an LCDR1 of SEQ ID NO:158, an LCDR2 of SEQ ID NO:50, and an LCDR3 of SEQ ID NO:258.

In some embodiments, the antibody or antigen binding fragment thereof that binds PMEL17 comprises:

(a) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:10, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:21;

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(b) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:10, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:25;

(c) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:10, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:29;

(d) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:42, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:53;

(e) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:64, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:75;

(f) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:88, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:99;

(g) heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:112, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:119;

(h) heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:132, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:143;

(i) heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:149, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:159;

(j) heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:165, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:171;

(k) heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:184, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:190;

(l) heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:196, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:202;

(m) heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:215, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:221;

(n) heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:227, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:233;

(o) heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:239, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:248; or

(p) heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:254, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:260.

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In some embodiments, the antibody or antigen binding fragment thereof that binds PMEL17 comprises:

(a) a heavy chain comprising the amino acid sequence of SEQ ID NO:12, and a light chain comprising the amino acid sequence of SEQ ID NO:23;

(b) a heavy chain comprising the amino acid sequence of SEQ ID NO:12, and a light chain comprising the amino acid sequence of SEQ ID NO:27;

(c) a heavy chain comprising the amino acid sequence of SEQ ID NO:12, and a light chain comprising the amino acid sequence of SEQ ID NO:31;

(d) a heavy chain comprising the amino acid sequence of SEQ ID NO:44, and a light chain comprising the amino acid sequence of SEQ ID NO:55;

(e) a heavy chain comprising the amino acid sequence of SEQ ID NO:66, and a light chain comprising the amino acid sequence of SEQ ID NO:77;

(f) a heavy chain comprising the amino acid sequence of SEQ ID NO:90, and a light chain comprising the amino acid sequence of SEQ ID NO:101;

(g) a heavy chain comprising the amino acid sequence of SEQ ID NO:114, and a light chain comprising the amino acid sequence of SEQ ID NO:121.;

(h) a heavy chain comprising the amino acid sequence of SEQ ID NO:134, and a light chain comprising the amino acid sequence of SEQ ID NO:145;

(i) a heavy chain comprising the amino acid sequence of SEQ ID NO:151, and a light chain comprising the amino acid sequence of SEQ ID NO:161;

(j) a heavy chain comprising the amino acid sequence of SEQ ID NO:167, and a light chain comprising the amino acid sequence of SEQ ID NO:173;

(k) a heavy chain comprising the amino acid sequence of SEQ ID NO:186, and a light chain comprising the amino acid sequence of SEQ ID NO:192;

(l) a heavy chain comprising the amino acid sequence of SEQ ID NO:198, and a light chain comprising the amino acid sequence of SEQ ID NO:204;

(m) a heavy chain comprising the amino acid sequence of SEQ ID NO:217, and a light chain comprising the amino acid sequence of SEQ ID NO:223;

(n) a heavy chain comprising the amino acid sequence of SEQ ID NO:229, and a light chain comprising the amino acid sequence of SEQ ID NO:235;

(o) a heavy chain comprising the amino acid sequence of SEQ ID NO:241, and a light chain comprising the amino acid sequence of SEQ ID NO:250;

(p) heavy chain comprising the amino acid sequence of SEQ ID NO:256, and a light chain comprising the amino acid sequence of SEQ ID NO:262;

(q) a heavy chain comprising the amino acid sequence of SEQ ID NO:283, and a light chain comprising the amino acid sequence of SEQ ID NO:27;

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(r) a heavy chain comprising the amino acid sequence of SEQ ID NO:283, and a light chain comprising the amino acid sequence of SEQ ID NO:31;

(s) a heavy chain comprising the amino acid sequence of SEQ ID NO:315, and a light chain comprising the amino acid sequence of SEQ ID NO:101.

In some embodiments, the antibody or antigen binding fragment thereof comprises one or more cysteine substitutions. In some embodiments, the antibody or antigen binding fragment thereof comprises one or more cysteine substitutions selected from E152C, S375C, or both E152C and S375C of the heavy chain of the antibody or antigen binding fragment thereof, wherein the position is numbered according to the EU system. In some embodiments, the antibody or antigen binding fragment thereof comprises an E152C substitution of the heavy chain of the antibody or antigen binding fragment thereof, wherein the position is numbered according to the EU system.

In some embodiments, the antibody molecule is a monoclonal antibody. In some embodiments, the antibody molecule is an isolated antibody. In some embodiments, the antibody molecule is a synthetic antibody. In some embodiments, the antibody molecule is an engineered antibody.

In some embodiments, the antibody or antigen binding fragment is encoded by a nucleic acid described herein. In some embodiments, the nucleic acid comprises the nucleotide sequence of SEQ ID NOs: 13, 24, 28, 32, 45, 56, 67, 78, 91, 102, 115, 122, 135, 146, 152, 162, 168, 174, 187, 193, 199, 205, 218, 224, 230, 236, 242, 251, 257, 263, 319, or 320. In some embodiments, the nucleic acid is present in a vector, *e.g.*, a vector described herein. In some embodiments, the nucleic acid or vector is present in a host cell, *e.g.*, a host cell described herein.

In some embodiments, the antibody or antigen binding fragment is produced by a method comprising cultivating the host cell and recovering the antibody or antigen binding fragment from cell culture. In some embodiments, recovering the antibody from cell culture comprises the steps of: removing cells and filtering the culture; purifying the culture by affinity chromatography; inactivating any viruses in the culture by adjusting the pH to 3.4-3.6, then readjusting the pH to 5.8-6.2 and filtering the culture; purifying the culture by cation exchange chromatography and performing on-column reduction of the culture; performing anion exchange chromatography on the culture; removing viruses by nanofiltration; filtering the culture containing the antibody; and obtaining purified antibody.

In some embodiments, the antibody drug conjugate is an antibody drug conjugate made by a process described herein.

In some embodiments, the antibody drug conjugate comprises the formula (C)

$$Ab\text{-}(L_A\text{-}(D)_n)_y \qquad (C)$$

wherein:

D is a GNAQ inhibitor, a GNA11 inhibitor or an inhibitor of GNAQ and GNA11;

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Ab is an antibody or antigen binding fragment thereof that binds to human PMEL17 protein;

L_A is a linker;

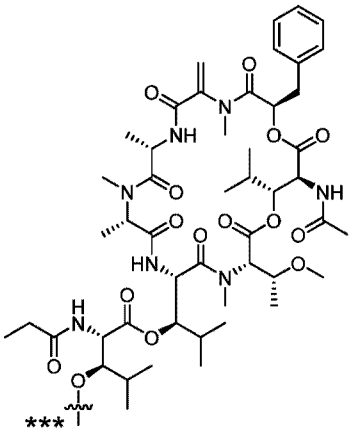
n is 1, 2, 3 or 4, and

y is 1, 2, 3 or 4.

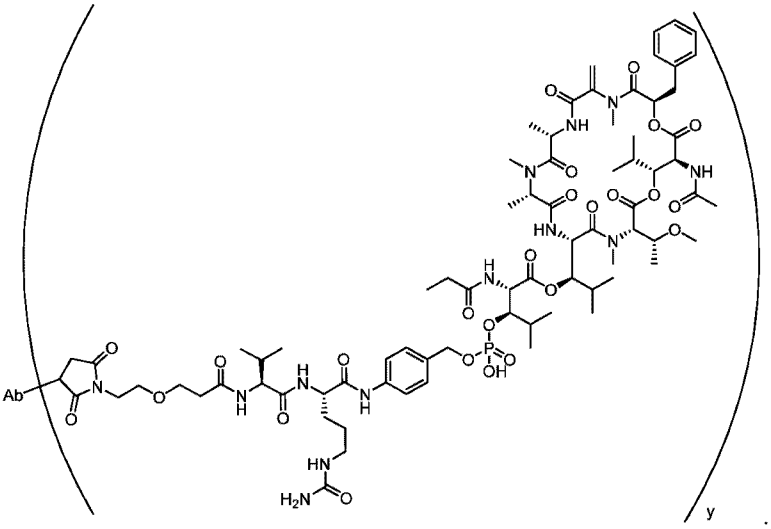
In some embodiments, said n is 1. In some embodiments, said y is about 1 to about 4. In some embodiments, said linker is a cleavable linker or a non-cleavable linker. In some embodiments, the linker comprises a ValCit peptide linker. In some

In some embodiments, said drug moiety is an inhibitor of GNAQ and GNA11.

In some embodiments, the D is



In some embodiments, the antibody drug conjugate has the following structure,



In some embodiments, the antibody drug conjugate has the following Formula (C-2):

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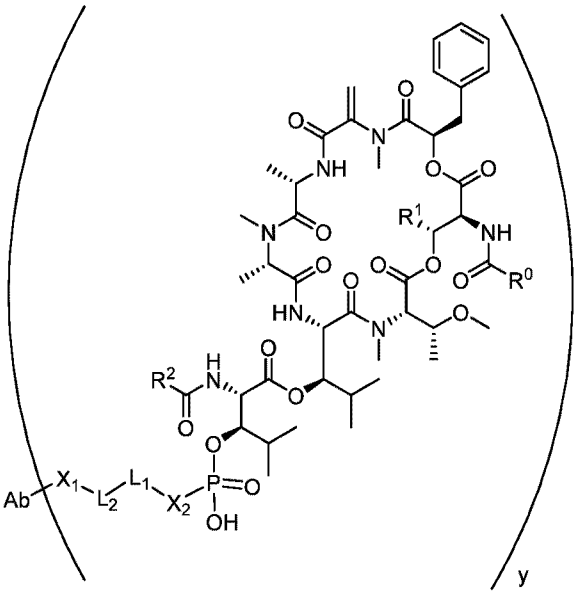

(C-2),

wherein:

R^0 is methyl or ethyl;

R^1 is methyl or isopropyl;

R^2 is methyl or ethyl;

Ab is an antibody or antigen binding fragment thereof that binds to human PMEL17 protein;

X_1 is a bivalent coupling group;

X_2 is a self-immolative spacer;

L_1 is a bivalent peptide linker;

L_2 is a bond or a linker, and

y is 1, 2, 3 or 4.

In some embodiments, the antibody drug conjugate is present in a pharmaceutical composition comprising the antibody drug conjugate and a pharmaceutically acceptable carrier. In some embodiments, the antibody drug conjugate is present in a formulation described herein.

In some embodiments, the antibody drug conjugate or formulation is used as a medicament.

In some embodiments, the antibody drug conjugate or formulation is used in a method of treating or preventing cancer in a patient in need thereof, comprising administering to said patient the antibody drug conjugate or formulation, wherein the cancer expresses PMEL17, contains a mutation of the GNAQ or GNA11 gene, or the cancer expresses PMEL17 and contains a mutation of GNAQ, GNA11, or both. In some embodiments, the cancer is a carcinoma (*e.g.*, hepatocellular carcinoma), sarcoma, leukemia, lymphoma, eye cancer, eye neoplasm, melanoma (*e.g.*, uveal melanoma, non-uveal melanoma, malignant melanoma, ocular melanoma, subcutaneous melanoma, cutaneous melanoma, or mucosal melanoma), or a metastatic cancer thereof.

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In some embodiments, the antibody drug conjugate or formulation is administered to the patient in combination with one or more additional therapeutic compounds.

In some embodiments, the one or more additional therapeutic compounds is selected from a standard of care chemotherapeutic, an MDM2 inhibitor, an MRC2 inhibitor, a PKC inhibitor, a MAPK inhibitor, a costimulatory molecule, or a checkpoint inhibitor.

In some embodiments, the costimulatory molecule is selected from an agonist of OX40, CD2, CD27, CDS, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), 4-1BB (CD137), GITR, CD30, CD40, BAFFR, HVEM, CD7, LIGHT, NKG2C, SLAMF7, NKp80, CD160, B7-H3, STING, or CD83 ligand.

In some embodiments, the checkpoint inhibitor is selected from an inhibitor of PD-1, PD-L1, PD-L2, CTLA4, TIM3, LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4 and/or TGFR beta.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts the gene organization and transposable element containing BGCs of compound (A1) (*frsA-H*) and compound J (*dis1-4*) in *C. vaccinii* (76974 bp).

FIG. 2 depicts the scheme of the insertion strategy used for promoter exchanges.

FIG. 3 depicts the production of compound J in disruption mutants compared to wild type.

FIG. 4 depicts the MS/MS spectrum of compound J.

FIG. 5 depicts the MS/MS spectrum of compound F5.

FIG. 6 depicts the MS/MS spectrum of compound F3.

FIG. 7 depicts the MS/MS spectrum of compound D.

FIG. 8 depicts the schematic view of compound J biosynthesis. Figure discloses "Ser Val His Val Leu Val" as SEQ ID NO: 324.

FIG. 9 depicts the exemplary on-site and off-side conjugations.

FIG. 10A depicts the organization of the compound (A1)-BGC (white: NRPS genes, black: genes encoding modifying enzymes) and the representation of exchanged promoters.

FIG. 10B depicts a bar chart of the titers of compound (A1) from different *C. vaccinii* promoter exchange mutants including fold changes compared to wild type.

FIG. 11A depicts the HPLC-MS extracted ion chromatograms (EIC) 1002.535 – 1002.545 Da of culture extracts of *C. vaccinii* wild type and *C. vaccinii vioP* mutant.

FIG. 11B depicts the HPLC-MS EIC 1020.49 – 1020.50 Da of culture extract of *C. vaccinii vioP* mutant fed with L-Met revealing three novel analogs of compound (A1).

FIG. 12A depicts the HPLC-MS extracted ion chromatograms (EIC) of compound (A1) at *m/z* 1002.52 – 1002.57 following incubation in human plasma at 5 µg/mL and 37 °C for 8 h (bottom) and the same sample spiked with additional 100 ng of compound 5 (final conc. 6 µg/mL).

FIG. 12B depicts the absolute quantification and fractions of compounds (A1) and 5 following incubation of compound (A1) in human plasma at 5 µg/mL and 37 °C for 24 h.

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FIG. 13A depicts the effects of compounds (A1) and 2-8 on proliferation of Gq or G11 mutant uveal melanoma (UM) cell lines and Gq or G11 wild type skin melanoma cell lines. Growth of UM cell lines driven by constitutively active Gq or G11 mutants was inhibited by compound (A1) with sub nM GI50s for the 92.1 and MP41 cell lines, whereas the growth of skin melanoma cell lines, harboring homozygous BRAF V600E mutations, A375, and heterozygous NRAS Q61K mutations, SK-MEL-30 was unaffected by compound (A1).

FIG. 13B depicts the effects of compounds 9-15 on proliferation of Gq or G11 mutant UM cell lines and Gq or G11 wild type skin melanoma cell lines. Growth of UM cell lines driven by constitutively active Gq or G11 mutants was inhibited by compound (A1) with sub nM GI50s for the 92.1 and MP41 cell lines, whereas the growth of skin melanoma cell lines, harboring homozygous BRAF V600E mutations, A375, and heterozygous NRAS Q61K mutations, SK-MEL-30 was unaffected by compound (A1).

FIG. 14 depicts SEC data of exemplary ADC formulations.

FIG. 15 depicts light obscuration data of exemplary ADC formulations.

FIG. 16 depicts capillary electrophoresis analysis of exemplary ADC formulations.

FIG. 17 depicts capillary zone electrophoresis (CZE) analysis of exemplary ADC formulations.

FIG. 18 depicts analysis of exemplary lyophilisate ADC formulations.

FIG. 19 depicts data for pH and ring-opening of the payload using (CZE).

FIG. 20 depicts potency decay data for exemplary ADC formulations after a 2-month storage period.

FIG. 21 depicts the mean exposure profiles of total antibody (tmAb), active ADC (tADCa), and inactive ADC (tADCi) after a single i.v. dose of 3 mg/kg of compound (E) in the cynomolgus monkey.

DETAILED DESCRIPTION

This disclosure is based, at least in part, on the discovery that certain microorganisms (*e.g.*, Chromobacterium) can be genetically engineered to increase the production of certain depsipeptides (*e.g.*, compound (A1)). Compound (A1) can be isolated from the leaves of *A. crenata*, but the very low quantities and the complex matrix may prevent access to sufficient amounts of compound (A1) to allow drug development efforts and commercial manufacturing. The genetically engineered microorganisms (*e.g.*, *Chromobacterium*), nucleic acids, and methods described herein provide developable production routes for making compound (A1) with elevated titers and/or increased isolated yields to grant access to sufficient amounts of the drug substance precursor.

This disclosure is also based, at least in part, on the discovery that by targeting a higher overreduction and designing a robust, partial reoxidation step efficient decapping of engineered cysteine residues and desired drug-to-antibody ratios can be achieved without comprising on antibody purity. Overreduction and opening of intrachain disulfide bridges can lead to undesired off-site coupling. In some embodiments, an inherent LC-HC instability can result in susceptibility to

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overreduction. Without wishing to be bound by theory, it is believed that in some embodiments efficient, partial reoxidation of opened intrachain disulfide bridges is important for minimizing off-site conjugation to achieve high efficacy *in vivo* and to reduce linker payload release. Factors that can be assessed for the partial reoxidation step include, but are not limited to, one or more of: different vessel geometry, defined headspace (*e.g.*, 60%) or not, mixing (*e.g.*, 100 rpm) or not, or room temperature or cold room. In some embodiments, by adding a partial reoxidation step described herein into the GMP manufacturing process the purity of final drug substance can be increased to about 95%, *e.g.*, as determined by non-reduced CE SDS. In certain embodiments, a drug-to-antibody ratio of about 2 is achieved. In other embodiments, a drug-to-antibody ratio of about 4 is achieved.

Definitions

Unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings.

As used herein, the singular form "a" or "an" includes plural references unless indicated otherwise.

The term "or" is used herein to mean, and is used interchangeably with, the term "and/or" unless context clearly indicates otherwise.

"About" and "approximately" shall generally mean an acceptable degree of error for the quantity measured given the nature or precision of the measurements. Exemplary degrees of error are within 20 percent (%), typically, within 10%, and more typically, within 5% of a given value or range of values.

The term "biosynthetic gene cluster" or "BGC" interchangeably refers to a locally clustered group of two or more genes that together encode a biosynthetic pathway for the production of a metabolite. In some embodiments, the metabolite is a secondary or specialized metabolite, which is not directly involved in the normal growth, development, or reproduction of the organism that produce the metabolite.

The term "alkyl" refers to a monovalent saturated hydrocarbon chain having the specified number of carbon atoms. For example, C_1-C_6alkyl refers to an alkyl group having from 1 to 6 carbon atoms. Alkyl groups may be straight or branched. Representative branched alkyl groups have one, two, or three branches. Examples of alkyl groups include, but are not limited to, methyl, ethyl, propyl (n-propyl and isopropyl), butyl (n-butyl, isobutyl, sec-butyl, and t-butyl), pentyl (n-pentyl, isopentyl, and neopentyl), and hexyl.

"Cleavable" as used herein refers to a linking group or linker component that connects two moieties by covalent connections, but breaks down to sever the covalent connection between the moieties under physiologically relevant conditions, typically a cleavable linking group is severed in vivo more rapidly in an intracellular environment than when outside a cell, causing release of the payload to preferentially occur inside a targeted cell. Cleavage may be enzymatic or non-enzymatic,

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but generally releases a payload from an antibody without degrading the antibody. Cleavage may leave some portion of a linking group or linker component attached to the payload, or it may release the payload without any residue of the linking group.

"Non-cleavable" as used herein refers to a linking group or linker component that is not especially susceptible to breaking down under physiological conditions, *e.g.*, it is at least as stable as the antibody or antigen binding fragment portion of the conjugate. Such linking groups are sometimes referred to as 'stable', meaning they are sufficiently resistant to degradation to keep the payload connected to antibody or antigen binding fragment until the antibody or antigen binding fragment is itself at least partially degraded, *i.e.*, the degradation of the antibody or antigen binding fragment precedes cleavage of the linking group *in vivo*. Degradation of the antibody portion of an ADC having a stable or non-cleavable linking group may leave some or all of the linking group, *e.g.*, one or more amino acid groups from an antibody, attached to the payload or drug moiety that is delivered in vivo.

The term "antibody" as used herein refers to a polypeptide of the immunoglobulin family that is capable of binding a corresponding antigen non-covalently, reversibly, and in a specific manner. For example, a naturally occurring IgG antibody is a tetramer comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (C1q) of the classical complement system.

The term "antibody" includes, but is not limited to, monoclonal antibodies, human antibodies, humanized antibodies, chimeric antibodies, and anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to antibodies of the disclosure). The antibodies can be of any isotype/class (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), or subclass (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2).

"Complementarity-determining domains" or "complementary-determining regions ("CDRs") interchangeably refer to the hypervariable regions of VL and VH. The CDRs are the target protein-binding site of the antibody chains that harbors specificity for such target protein. There are three CDRs (CDR1-3, numbered sequentially from the N-terminus) in each human VL or VH, constituting

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about 15-20% of the variable domains. The CDRs are structurally complementary to the epitope of the target protein and are thus directly responsible for the binding specificity. The remaining stretches of the VL or VH, the so-called framework regions, exhibit less variation in amino acid sequence (Kuby, Immunology, 4th ed., Chapter 4. W.H. Freeman & Co., New York, 2000).

The positions of the CDRs and framework regions can be determined using various well known definitions in the art, *e.g.*, Kabat, Chothia, international ImMunoGeneTics database (IMGT) (on the worldwide web at www.imgt.org/), and AbM (see, *e.g.*, Johnson *et al.*, Nucleic Acids Res., 29:205-206 (2001); Chothia and Lesk, J. Mol. Biol., 196:901-917 (1987); Chothia *et al.*, Nature, 342:877-883 (1989); Chothia *et al.*, J. Mol. Biol., 227:799-817 (1992); Al-Lazikani *et al.*, J. Mol. Biol., 273:927-748 (1997)). Definitions of antigen combining sites are also described in the following: Ruiz *et al.*, Nucleic Acids Res., 28:219–221 (2000); and Lefranc, M.P., Nucleic Acids Res., 29:207-209 (2001); MacCallum *et al.*, J. Mol. Biol., 262:732-745 (1996); and Martin *et al.*, Proc. Natl. Acad. Sci. USA, 86:9268–9272 (1989); Martin *et al.*, Methods Enzymol., 203:121–153 (1991); and Rees *et al.*, In Sternberg M.J.E. (ed.), Protein Structure Prediction, Oxford University Press, Oxford, 141–172 (1996).

Both the light and heavy chains are divided into regions of structural and functional homology. The terms "constant" and "variable" are used functionally. In this regard, it will be appreciated that the variable domains of both the light (VL) and heavy (VH) chain portions determine antigen recognition and specificity. Conversely, the constant domains of the light chain (CL) and the heavy chain (CH1, CH2 or CH3) confer important biological properties such as secretion, transplacental mobility, Fc receptor binding, complement binding, and the like. By convention, the numbering of the constant region domains increases as they become more distal from the antigen binding site or amino-terminus of the antibody. The N-terminus is a variable region and at the C-terminus is a constant region; the CH3 and CL domains actually comprise the carboxy-terminal domains of the heavy and light chain, respectively.

The term "antigen binding fragment", as used herein, refers to one or more portions of an antibody that retain the ability to specifically interact with (*e.g.*, by binding, steric hindrance, stabilizing/destabilizing, spatial distribution) an epitope of an antigen. Examples of binding fragments include, but are not limited to, single-chain Fvs (scFv), camelid antibodies, disulfide-linked Fvs (sdFv), Fab fragments, F(ab') fragments, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; a F(ab)2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consisting of the VH and CH1 domains; a Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a dAb fragment (Ward *et al.*, Nature 341:544-546, 1989), which consists of a VH domain; and an isolated complementarity determining region (CDR), or other epitope-binding fragments of an antibody.

Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables

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them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv ("scFv"); *see, e.g.*, Bird *et al.*, Science 242:423-426, 1988; and Huston *et al.*, Proc. Natl. Acad. Sci. 85:5879-5883, 1988). Such single chain antibodies are also intended to be encompassed within the term "antigen binding fragment." These antigen binding fragments are obtained using conventional techniques known to those of skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

Antigen binding fragments can also be incorporated into single domain antibodies, maxibodies, minibodies, single domain antibodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv (*see, e.g.*, Hollinger and Hudson, Nature Biotechnology 23:1126-1136, 2005). Antigen binding fragments can be grafted into scaffolds based on polypeptides such as fibronectin type III (Fn3) (*see* U.S. Pat. No. 6,703,199, which describes fibronectin polypeptide monobodies).

Antigen binding fragments can be incorporated into single chain molecules comprising a pair of tandem Fv segments (VH-CH1-VH-CH1) which, together with complementary light chain polypeptides, form a pair of antigen binding regions (Zapata *et al.*, Protein Eng. 8:1057-1062, 1995; and U.S. Pat. No. 5,641,870).

The term "monoclonal antibody" or "monoclonal antibody composition" as used herein refers to polypeptides, including antibodies and antigen binding fragments that have substantially identical amino acid sequence or are derived from the same genetic source. This term also includes preparations of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope.

The term "human antibody", as used herein, includes antibodies having variable regions in which both the framework and CDR regions are derived from sequences of human origin. Furthermore, if the antibody contains a constant region, the constant region also is derived from such human sequences, *e.g.*, human germline sequences, or mutated versions of human germline sequences or antibody containing consensus framework sequences derived from human framework sequences analysis, for example, as described in Knappik *et al.*, J. Mol. Biol. 296:57-86, 2000). Also included are antibodies derived from human sequences wherein one or more CDRs has been mutated for affinity maturation or for manufacturing/payload conjugation purposes. *See* Kilpatrick *et al.*, "Rapid development of affinity matured monoclonal antibodies using RIMMS," Hybridoma. 1997 Aug;16(4):381-9.

The human antibodies of the disclosure may include amino acid residues not encoded by human sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*, or a conservative substitution to promote stability or manufacturing).

The term "recognize" as used herein refers to an antibody or antigen binding fragment thereof that finds and interacts (*e.g.*, binds) with its epitope, whether that epitope is linear or conformational. The term "epitope" refers to a site on an antigen to which an antibody or antigen binding fragment of the disclosure specifically binds. Epitopes can be formed both from contiguous amino acids or

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noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include techniques in the art, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance (*see, e.g.*, Epitope Mapping Protocols in Methods in Molecular Biology, Vol. 66, G. E. Morris, Ed. (1996)).

The term "affinity" as used herein refers to the strength of interaction between antibody and antigen at single antigenic sites. Within each antigenic site, the variable region of the antibody "arm" interacts through weak non-covalent forces with antigen at numerous sites; the more interactions, the stronger the affinity.

The term "isolated antibody" refers to an antibody that is substantially free of other antibodies having different antigenic specificities. An isolated antibody that specifically binds to one antigen may, however, have cross-reactivity to other antigens. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

The term "corresponding human germline sequence" refers to the nucleic acid sequence encoding a human variable region amino acid sequence or subsequence that shares the highest determined amino acid sequence identity with a reference variable region amino acid sequence or subsequence in comparison to all other all other known variable region amino acid sequences encoded by human germline immunoglobulin variable region sequences. The corresponding human germline sequence can also refer to the human variable region amino acid sequence or subsequence with the highest amino acid sequence identity with a reference variable region amino acid sequence or subsequence in comparison to all other evaluated variable region amino acid sequences. The corresponding human germline sequence can be framework regions only, complementarity determining regions only, framework and complementarity determining regions, a variable segment (as defined above), or other combinations of sequences or subsequences that comprise a variable region. Sequence identity can be determined using the methods described herein, for example, aligning two sequences using BLAST, ALIGN, or another alignment algorithm known in the art. The corresponding human germline nucleic acid or amino acid sequence can have at least about 90%, 91, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the reference variable region nucleic acid or amino acid sequence. Corresponding human germline sequences can be determined, for example, through the publicly available international ImMunoGeneTics database (IMGT) (on the worldwide web at www.imgt.org/) and V-base (on the worldwide web at vbase.mrc-cpe.cam.ac.uk).

The phrase "specifically binds" or "selectively binds," when used in the context of describing the interaction between an antigen (*e.g.*, a protein) and an antibody, antibody fragment, or antibody-derived binding agent, refers to a binding reaction that is determinative of the presence of the antigen

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in a heterogeneous population of proteins and other biologics, *e.g.*, in a biological sample, *e.g.*, a blood, serum, plasma or tissue sample. Thus, under certain designated immunoassay conditions, the antibodies or binding agents with a particular binding specificity bind to a particular antigen at least two times the background and do not substantially bind in a significant amount to other antigens present in the sample. In one embodiment, under designated immunoassay conditions, the antibody or binding agent with a particular binding specificity binds to a particular antigen at least ten (10) times the background and does not substantially bind in a significant amount to other antigens present in the sample. Specific binding to an antibody or binding agent under such conditions may require the antibody or agent to have been selected for its specificity for a particular protein. As desired or appropriate, this selection may be achieved by subtracting out antibodies that cross-react with molecules from other species (*e.g.*, mouse or rat) or other subtypes. Alternatively, in some embodiments, antibodies or antibody fragments are selected that cross-react with certain desired molecules.

A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (*see, e.g.*, Harlow & Lane, Using Antibodies, A Laboratory Manual (1998), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity). Typically a specific or selective binding reaction will produce a signal at least twice over the background signal and more typically at least 10 to 100 times over the background.

The term "equilibrium dissociation constant (KD, M)" refers to the dissociation rate constant (kd, time-1) divided by the association rate constant (ka, time-1, M-1). Equilibrium dissociation constants can be measured using any known method in the art. The antibodies of the present disclosure generally will have an equilibrium dissociation constant of less than about 10^{-7} or 10^{-8} M, for example, less than about 10^{-9} M or 10^{-10} M, in some embodiments, less than about 10^{-11} M, 10^{-12} M or 10^{-13} M.

The term "bioavailability" refers to the systemic availability (*i.e.*, blood/plasma levels) of a given amount of drug administered to a patient. Bioavailability is an absolute term that indicates measurement of both the time (rate) and total amount (extent) of drug that reaches the general circulation from an administered dosage form.

As used herein, the phrase "consisting essentially of" refers to the genera or species of active pharmaceutical agents included in a method or composition, as well as any excipients inactive for the intended purpose of the methods or compositions. In some embodiments, the phrase "consisting essentially of" expressly excludes the inclusion of one or more additional active agents other than an antibody drug conjugate of the disclosure. In some embodiments, the phrase "consisting essentially of" expressly excludes the inclusion of one or more additional active agents other than an antibody drug conjugate of the disclosure and a second co-administered agent.

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The term "amino acid" refers to naturally occurring, synthetic, and unnatural amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, *e.g.*, hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Amino acid analogs refer to compounds that have the same basic chemical structure as a naturally occurring amino acid, *i.e.*, an α-carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, *e.g.*, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (*e.g.*, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

The term "conservatively modified variant" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refer to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid that encodes a polypeptide is implicit in each described sequence.

For polypeptide sequences, "conservatively modified variants" include individual substitutions, deletions or additions to a polypeptide sequence which result in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the disclosure. The following eight groups contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (*see, e.g.*, Creighton, Proteins (1984)). In some embodiments, the term "conservative sequence modifications" are used to refer to amino acid modifications that do not

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significantly affect or alter the binding characteristics of the antibody containing the amino acid sequence.

The term "optimized" as used herein refers to a nucleotide sequence that has been altered to encode an amino acid sequence using codons that are preferred in the production cell or organism, generally a eukaryotic cell, for example, a yeast cell, a Pichia cell, a fungal cell, a Trichoderma cell, a Chinese Hamster Ovary cell (CHO) or a human cell. The optimized nucleotide sequence is engineered to retain completely or as much as possible the amino acid sequence originally encoded by the starting nucleotide sequence, which is also known as the "parental" sequence.

The terms "percent identical" or "percent identity," in the context of two or more nucleic acids or polypeptide sequences, refers to the extent to which two or more sequences or subsequences that are the same. Two sequences are "identical" if they have the same sequence of amino acids or nucleotides over the region being compared. Two sequences are "substantially identical" if two sequences have a specified percentage of amino acid residues or nucleotides that are the same (*i.e.*, 60% identity, optionally 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identity over a specified region, or, when not specified, over the entire sequence), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Optionally, the identity exists over a region that is at least about 30 nucleotides (or 10 amino acids) in length, or more preferably over a region that is 100 to 500 or 1000 or more nucleotides (or 20, 50, 200 or more amino acids) in length.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

A "comparison window", as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith and Waterman, Adv. Appl. Math. 2:482c (1970), by the homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package,

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Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (*see, e.g.*, Brent *et al.*, Current Protocols in Molecular Biology, 2003).

Two examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.*, Nuc. Acids Res. 25:3389-3402, 1977; and Altschul *et al.*, J. Mol. Biol. 215:403-410, 1990, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al.*, *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a word length (W) of 11, an expectation (E) or 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a word length of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff and Henikoff, (1989) Proc. Natl. Acad. Sci. USA 89:10915) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (*see, e.g.*, Karlin and Altschul, Proc. Natl. Acad. Sci. USA 90:5873-5787, 1993). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

The percent identity between two amino acid sequences can also be determined using the algorithm of E. Meyers and W. Miller, Comput. Appl. Biosci. 4:11-17 (1988) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid

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sequences can be determined using the Needleman and Wunsch, J. Mol. Biol. 48:444-453 (1970) algorithm which has been incorporated into the GAP program in the GCG software package (available at www.gcg.com), using either a BLOSUM62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

Other than percentage of sequence identity noted above, another indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the antibodies raised against the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules or their complements hybridize to each other under stringent conditions, as described below. Yet another indication that two nucleic acid sequences are substantially identical is that the same primers can be used to amplify the sequence.

The term "nucleic acid" is used herein interchangeably with the term "polynucleotide" and refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs).

Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (*e.g.*, degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, as detailed below, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al.*, (1991) Nucleic Acid Res. 19:5081; Ohtsuka *et al.*, (1985) J. Biol. Chem. 260:2605-2608; and Rossolini *et al.*, (1994) Mol. Cell. Probes 8:91-98).

The term "operably linked" in the context of nucleic acids refers to a functional relationship between two or more polynucleotide (*e.g.*, DNA) segments. Typically, it refers to the functional relationship of a transcriptional regulatory sequence to a transcribed sequence. For example, a promoter or enhancer sequence is operably linked to a coding sequence if it stimulates or modulates the transcription of the coding sequence in an appropriate host cell or other expression system. Generally, promoter transcriptional regulatory sequences that are operably linked to a transcribed sequence are physically contiguous to the transcribed sequence, *i.e.*, they are cis-acting. However, some transcriptional regulatory sequences, such as enhancers, need not be physically contiguous or located in close proximity to the coding sequences whose transcription they enhance.

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The terms "polypeptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer. Unless otherwise indicated, a particular polypeptide sequence also implicitly encompasses conservatively modified variants thereof.

The term "antibody drug conjugate" or "immunoconjugate" as used herein refers to the linkage of an antibody or an antigen binding fragment thereof with another agent, such as a chemotherapeutic agent, a toxin, an immunotherapeutic agent, an imaging probe, and the like. The linkage can be covalent bonds, or non-covalent interactions such as through electrostatic forces. Various linkers, known in the art, can be employed in order to form the antibody drug conjugate. Additionally, the antibody drug conjugate can be provided in the form of a fusion protein that may be expressed from a polynucleotide encoding the immunoconjugate. As used herein, "fusion protein" refers to proteins created through the joining of two or more genes or gene fragments which originally coded for separate proteins (including peptides and polypeptides). Translation of the fusion gene results in a single protein with functional properties derived from each of the original proteins.

The term "subject" includes human and non-human animals. Non-human animals include all vertebrates, *e.g.*, mammals and non-mammals, such as non-human primates, sheep, dog, cow, chickens, amphibians, and reptiles. Except when noted, the terms "patient" or "subject" are used herein interchangeably.

The term "cytotoxin", or "cytotoxic agent" as used herein, refers to any agent that is detrimental to the growth and proliferation of cells and may act to reduce, inhibit, or destroy a cell or malignancy.

The term "anti-cancer agent" as used herein refers to any agent that can be used to treat or prevent a cell proliferative disorder such as cancer, including but not limited to, cytotoxic agents, chemotherapeutic agents, radiotherapy and radiotherapeutic agents, targeted anti-cancer agents, and immunotherapeutic agents.

The term "drug moiety" or "payload" as used herein refers to a chemical moiety that is conjugated to an antibody or antigen binding fragment of the disclosure, and can include any therapeutic or diagnostic agent, for example, an anti-cancer, anti-inflammatory, anti-infective (*e.g.*, anti-fungal, antibacterial, anti-parasitic, anti-viral), or an anesthetic agent. For example, the drug moiety can be an anti-cancer agent, such as a cytotoxin. In certain embodiments, a drug moiety is a target inhibitor compound. In addition, a payload can be a biophysical probe, a fluorophore, a spin label, an infrared probe, an affinity probe, a chelator, a spectroscopic probe, a radioactive probe, a lipid molecule, a polyethylene glycol, a polymer, a spin label, DNA, RNA, a protein, a peptide, a surface, an antibody, an antibody fragment, a nanoparticle, a quantum dot, a liposome, a PLGA particle, a saccharide or a polysaccharide.

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In some embodiments, the drug moiety or payload is a GNAQ inhibitor, a GNA11 inhibitor or an inhibitor of GNAQ and GNA11 (GNAQ/GNA11 inhibitor). In some embodiments, a GNAQ/11 inhibitor is a molecule that inhibits GNAQ/11-mediated production of IP3 and/or exhibits a dose-response antiproliferative effect in cells dependent on GNAQ/11 signaling (*i.e.*, GNAQ/11 mutant uveal melanoma cells). In some embodiments, a GNAQ/11 inhibitor is a compound that stabilizes GNAQ/11 in the inactive GDP-bound state and prevents GDP release, or binds to the active GTP-bound state and prevents GNAQ/11 interaction with downstream effectors. In some embodiments, a GNAQ/11 inhibitor functions by inhibiting a mutant GNAQ and/or GNA11, such as one comprising a Q209L/P mutation. Methods for attaching such drug moieties to a linker compatible with the targeting moiety are given in the present disclosure, along with the methods known in the art. See, *e.g.*, Singh *et al.*, (2009) Therapeutic Antibodies: Methods and Protocols, vol. 525, 445-457.

GNAQ (Guanine nucleotide-binding protein G(q) subunit alpha, also known as CMC1, G-ALPHA-q, GAQ, SWS, and G protein subunit alpha q) and GNA11 (Guanine nucleotide-binding protein subunit alpha-11, also known as FBH, FBH2, FHH2, GNA-11, HHC2, HYPOC2, and G protein subunit alpha 11) are closely related GTPases that constitute α subunits of heterotrimeric G proteins acting downstream of G protein-coupled receptors (GPCRs). The α subunits act as a switch for activation of G proteins by exchanging the guanosine diphosphate (GDP) for guanosine triphosphate (GTP), leading to the activation of distinct downstream effectors. The activation is terminated by the intrinsic GTPase activity, as GTP is hydrolyzed to GDP (Van Raamsdonk *et al.*, 2010, N Engl J Med.; 363(23):2191-9). The classical activation of Gq protein cascade occurs via phospholipase C-β (PLC-β), which hydrolyses phospholipid phosphatidylinositol 4,5-biphosphate to release two potent second messengers: D-myo-inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG). Following the transient increase of intracellular Ca^{2+}, IP3 is rapidly transformed into IP2, IP1, and myo-inositol. On the other hand, DAG activates protein kinase C (PKC), leading to a cascade of phosphorylation of RAF, MEK, and ERK, which translocate to the nucleus to regulate cell proliferation and survival (Krantz *et al.*, 2017, Clin Ophthalmol.; 11:279-289).

Exemplary amino acid and nucleotide sequences of human GNAQ have been published in GenBank with Accession Nos. NP_002063 and NM_002072, respectively. Exemplary amino acid and nucleotide sequences of human GNAQ are also described in International Application Publication No. WO 2020/128612 as SEQ ID NOs: 268 and 269, respectively.

Exemplary amino acid and nucleotide sequences of human GNA11 have been published in GenBank with Accession Nos. NP_002058 and NM_002067, respectively. Exemplary amino acid and nucleotide sequences of human GNA11 are also described in International Application Publication No. WO 2020/128612 as SEQ ID NOs: 270 and 271, respectively.

"Tumor" refers to neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues.

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The term "anti-tumor activity" means a reduction in the rate of tumor cell proliferation, viability, or metastatic activity. For example, anti-tumor activity can be shown by a decline in growth rate of abnormal cells that arises during therapy or tumor size stability or reduction, or longer survival due to therapy as compared to control without therapy. Such activity can be assessed using accepted in vitro or in vivo tumor models, including but not limited to xenograft models, allograft models, MMTV models, and other known models known in the art to investigate anti-tumor activity.

The term "malignancy" refers to a non-benign tumor or a cancer. As used herein, the term "cancer" includes a malignancy characterized by deregulated or uncontrolled cell growth. Exemplary cancers include, but are not limited to, carcinomas (*e.g.*, hepatocellular carcinoma), sarcomas, leukemias, lymphomas, eye cancers, eye neoplasms, and melanomas (*e.g.*, uveal melanoma, non-uveal melanoma, malignant melanoma, ocular melanoma, subcutaneous melanoma, cutaneous melanoma, or mucosal melanoma).

The term "cancer" includes primary malignant tumors (*e.g.*, those whose cells have not migrated to sites in the subject's body other than the site of the original tumor) and secondary malignant tumors (*e.g.*, those arising from metastasis, the migration of tumor cells to secondary sites that are different from the site of the original tumor).

The term "PMEL17" (also referred to as premelanosome protein (PMEL), D12S53E, ME20, ME20-M, ME20M, P1, P100, gp100, SI, SIL, and silver locus protein homolog (SILV)) refers to a single-pass Type I transmembrane protein produced by melanocytes and involved in melanin synthesis. Exemplary amino acid and nucleotide sequences of human PMEL17 have been published in GenBank with the following Accession Nos.: NP_008859, NP_001307050, NP_001307051, NP_001186982, NP_001186983 (amino acid sequences), and NM_006928, NM_001200053, NM_001200054, NM_001320121, NM_001320122 (nucleotide sequences). Exemplary amino acid and nucleotide sequences of human PMEL17 are also described in International Application Publication No. WO 2020/128612 as SEQ ID NOs: 272-276 (amino acid sequences) and SEQ ID NOs: 277-281 (nucleotide sequences). As used herein, the term "PMEL17" is used to refer collectively to all naturally occurring isoforms of PMEL17 protein, or a variant thereof.

The term "variant" refers to a polypeptide that has a substantially identical amino acid sequence to a reference polypeptide, or is encoded by a substantially identical nucleotide sequence, and is capable of having one or more activities of the reference polypeptide. For example, a variant can have about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher sequence identity to a reference polypeptide, while retain one or more activities of the reference polypeptide.

As used herein, the terms "treat," "treating," or "treatment" of any disease or disorder refers in one embodiment, to ameliorating the disease or disorder (*i.e.*, slowing or arresting or reducing the development of the disease or at least one of the clinical symptoms thereof). In another embodiment, "treat," "treating," or "treatment" refers to alleviating or ameliorating at least one physical parameter including those which may not be discernible by the patient. In yet another embodiment, "treat,"

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"treating," or "treatment" refers to modulating the disease or disorder, either physically, (*e.g.*, stabilization of a discernible symptom), physiologically, (*e.g.*, stabilization of a physical parameter), or both.

As used herein, the term "prevent", "preventing" or "prevention" of any disease or disorder refers to the prophylactic treatment of the disease or disorder; or delaying the onset or progression of the disease or disorder

The term "therapeutically acceptable amount" or "therapeutically effective dose" interchangeably refers to an amount sufficient to effect the desired result (*i.e.*, a reduction in tumor size, inhibition of tumor growth, prevention of metastasis, inhibition or prevention of viral, bacterial, fungal or parasitic infection). In some embodiments, a therapeutically acceptable amount does not induce or cause undesirable side effects. In some embodiments, a therapeutically acceptable amount induces or causes side effects but only those that are acceptable by the healthcare providers in view of a patient's condition. A therapeutically acceptable amount can be determined by first administering a low dose, and then incrementally increasing that dose until the desired effect is achieved. A "prophylactically effective dosage," and a "therapeutically effective dosage," of the molecules of the disclosure can prevent the onset of, or result in a decrease in severity of, respectively, disease symptoms, including symptoms associated with cancer.

The term "co-administer" refers to the presence of two active agents in the blood of an individual. Active agents that are co-administered can be concurrently or sequentially delivered.

The term "inhibition," "inhibitor," "binding antagonist" or "antagonist" includes a reduction in a certain parameter, *e.g.*, an activity, of a given molecule. For example, inhibition of an activity of a given molecule, of at least 5%, 10%, 20%, 30%, 40% or more is included by this term. Thus, inhibition need not be 100%.

The term "activation," "activator," or "agonist" includes an increase in a certain parameter, *e.g.*, an activity, of a given molecule. For example, increase of an activity of a given molecule of at least 5%, 10%, 25%, 50%, 75% or more is included by this term.

Chromobacterium

Chromobacterium is a genus of Gram-negative rod-shaped bacteria. Currently, eleven species within the genus are known, which are *C. alkanivorans*, *C. amazonense*, *C. aquaticum*, *C. haemolyticum*, *C. phragmitis*, *C. piscinae*, *C. pseudoviolaceum*, *C. rhizoryzae*, *C. subtsugae*, *C. vaccinii*, and *C. violaceum*.

In some embodiments, the *Chromobacterium* is *C. vaccinii*. *C. vaccinii* was isolated from soil of a cranberry tree (Soby *et al.* (2013) Int J Syst Evol Microbiol.;63(5):1840-6) and deposited at both the DSMZ and the ATCC strain collections (*Chromobacterium vaccinii* DSM 25150 = ATCC BAA-2314, = NRRL B-50840, = MWU205). The GenBank accession number for the 16S rRNA gene sequence of the strain is JN120869. A draft genome of *C. vaccinii* DSM 25150 was published by

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Vöing *et al.* (2015) *Genome Announc* 3, e00477–15 (GenBank accession number: NZ_JZJL00000000).

C. vaccinii grows readily on nutrient agar, producing distinctive smooth low convex colonies with a dark violet metallic sheen, due to production of violacein, upon incubation at 28 °C for 2 days. *C. vaccinii* is unlikely to cause any disease in healthy adult humans and is of minimal potential hazard to laboratory personnel and the environment.

The microorganisms described herein include, for example, a mutant form of *C. vaccinii* that naturally produces a depsipeptide, *e.g.*, compound (A1). In some embodiments, the *C. vaccinii* mutant comprise a mutation that increases the titer of compound (A1). In some embodiments, the *C. vaccinii* mutant comprises a mutation that increases the isolated yield of compound (A1). In some embodiments, the *C. vaccinii* mutant comprise a mutation that increases the titer of compound (A1) and a mutation that increases the isolated yield of compound (A1). In some embodiments, the *C. vaccinii* mutant comprises a promoter insertion mutation (*e.g.*, a promoter insertion mutation described herein). In some embodiments, the *C. vaccinii* mutant comprises a disruption mutation (*e.g.*, a disruption mutation described herein). In some embodiments, the *C. vaccinii* mutant comprises a plurality of disruption mutations (*e.g.*, a plurality of disruption mutations described herein). In some embodiments, the *C. vaccinii* mutant comprises a promoter insertion mutation (*e.g.*, a promoter insertion mutation described herein) and a disruption mutation (*e.g.*, a disruption mutation described herein). In some embodiments, the *C. vaccinii* mutant comprises a promoter insertion mutation (*e.g.*, a promoter insertion mutation described herein) and a plurality of disruption mutations (*e.g.*, a plurality of disruption mutations described herein).

Promoter insertion mutants

In some embodiments, the *C. vaccinii* mutant has an increased titer of compound (A1) compared to a reference *C. vaccinii*, *e.g.*, a wild type *C. vaccinii*, when cultured under identical or similar conditions. For example, the native promoter upstream of the compound (A1)-BGC can be altered, *e.g.*, replaced with a non-native promoter, *e.g.*, a stronger, non-native promoter. Without wishing to be bound by theory, it is believed that the compound (A1)-BGC contains a single operon and its transcription is likely driven by a single promoter.

The non-native promoter may be any promoter sequence which is not naturally operably linked to the compound (A1)-BGC and which, when so operably linked, increases the transcriptional activity of the compound (A1)-BGC.

In some embodiments, the non-native promoter is from *C. vaccinii*, *e.g.*, a promoter that controls the expression of a BGC other than the compound (A1)-BGC, *e.g.*, a *vioP* promoter. In other embodiments, the non-native promoter is not from *C. vaccinii*. In some embodiments, the non-native promoter is from *E. coli*.

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In some embodiments, the non-native promoter is a constitutive promoter. In some embodiments, the non-native promoter is an inducible promoter. In some embodiments, the promoter, *e.g.*, a vioP promoter, is induced by the production of homoserine lactones. In some embodiments, the non-native promoter is a violacein promoter. In some embodiments, the non-native promoter is a ribosomal promoter. In some embodiments, the non-native promoter is located in a transposon.

In certain embodiments, the non-native promoter is naturally occurring promoter. In other embodiments, the non-native promoter is a synthetic promoter. In other embodiments, the non-native promoter is a consensus promoter, *e.g.*, comprising a consensus sequence of a group of related naturally occurring promoters.

In some embodiments, the non-native promoter is a *vioP* promoter. In some embodiments, the non-native promoter is a *nptII* promoter. In some embodiments, the non-native promoter is an *rbs* promoter.

Other non-native promoters include, but are not limited to, a J23119 promoter (*e.g.*, as described at parts.igem.org), a pLpp promoter (*e.g.*, as described at parts.igem.org), a PS12burk promoter (*e.g.*, as described in Choi *et al.* (2008) App Environ Microbiol 74(4):1064-75), an Erme* promoter (*e.g.*, as described in Bibb *et al.* (1994) Mol Microbiol 14(3):533-45), or a Pem7 promoter (*e.g.*, as described in Zobel *et al.* (2015) ACS Synthetic Biology 4:1341-51).

In some embodiments, the non-native promoter is a promoter described in **Table 1**. In some embodiments, the heterologous promoter comprises a nucleotide sequence described in **Table 1**, or a functional fragment thereof. In some embodiments, the heterologous promoter comprises the nucleotide sequence of SEQ ID NO: 264, or a sequence having at least about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity thereto, or a functional fragment thereof. In some embodiments, the heterologous promoter comprises the nucleotide sequence of SEQ ID NO: 265, or a sequence having at least about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity thereto, or a functional fragment thereof. In some embodiments, the heterologous promoter comprises the nucleotide sequence of SEQ ID NO: 266, or a sequence having at least about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity thereto, or a functional fragment thereof. In some embodiments, the heterologous promoter comprises the nucleotide sequence of SEQ ID NO: 316, or a sequence having at least about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity thereto, or a functional fragment thereof. In some embodiments, the heterologous promoter comprises the nucleotide sequence of SEQ ID NO: 268, or a sequence having at least about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity thereto, or a functional fragment thereof. In some

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embodiments, the heterologous promoter comprises the nucleotide sequence of SEQ ID NO: 269, or a sequence having at least about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity thereto, or a functional fragment thereof. In some embodiments, the heterologous promoter comprises the nucleotide sequence of SEQ ID NO: 270, or a sequence having at least about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity thereto, or a functional fragment thereof. In some embodiments, the heterologous promoter comprises the nucleotide sequence of SEQ ID NO: 271, or a sequence having at least about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity thereto, or a functional fragment thereof.

Table 1. Nucleotide sequences of exemplary promoters

Promoter Name	Nucleotide Sequence	SEQ ID NO.
vioP	CGTCGTTGATCCCAGGCAGCCCTTTGTCTCCGTTTCTCCACGTCATGCCC TGACCCTTGGAACAGGATGGGCCGTCCTGTCAGACAATATGCTTGATGAA TTAGTTCGGTTGAACTAACGTGGCTGAAAATTACAAAGGCATTTAATTTT AAAAATATAAATGCCTTATAAATTTCATGCCGGGAAACCGGTCATGACGA GGATACAAGAGGCTGCATCCCGATTTCGAGGCGAGAGTGCCAAGCATTTA CGTCGTCCATGCCCGTTCGTTGTTGCCGCGCGGCGGGCGTGAATTGAACA GTCAAAGGGACATTCGCG	264
nptII	TGGACAGCAAGCGAACCGGAATTGCCAGCTGGGGCGCCCTCTGGTAAGGT TGGGAAGCCCTGCAAAGTAAACTGGATGGCTTTCTTGCCGCCAAGGATCT GATGGCGCAGGGGATCAAGATCTGATCAAGAGACAGGATAAGGAGGTACA GATTCAT	265
rbs	CACCGGCCTGACGGCCAAGTGTTGAAAAAACGCTGCTCCATCAACGGTTA ACGTTGGCGGGGCGGCGTTTTTTATTTGACGCTGGGCCGTTGACTAAAAT ATAATCCTCCGTCCTTTCTGGAGGCGTGTTGTCTTCGGGAAGAATCAACT AGGAACTGATAGTA	266
J23119	TTGACAGCTAGCTCAGTCCTAGGTATAATGCTAGC	316
pLpp	ATCAAAAAAATATTCTCAACATAAAAAACTTTGTGTAATACTTGTAACG	268
Pem7	GTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACAA GGTGAGGAACTAAACCATG	269
PS12burk	GAGCTGTTGACTCGCTTGGGATTTTCGGAATATCATGCCGGGTGGGCCCG GGAAAGCCACGTTGTGTCTCAAAATCTCTGATGTTACATTGCACAAGATA AAAATATATCATCATGAACAATAAAACTGTCTGCTTACATAAACAGTAAT ACAAGGGGTGTT	270
*ErmE**	GGATCCGACGTCCATGCGAGTGTCCGTTCGAGTGGCGGCTTGCGCCCGAT GCTAGTCGCGGTTGATCGGCGATCGCAGGTGCACGCGGTCCATCTTGACG GCTGGCGAGAGGTGCGGGGAGGATCTGACCGACGCGGTCCACACGTGGCA CCGCGATGCTGTTGACAGCCAATCGTGCCGGTTGGTAGAATACAGAACCA CTCCACA	271

For example, the strain *Chromobacterium vaccinii vioP-frsA* is a mutant generated from strain *Chromobacterium vaccinii* DSM 25150 by insertion of the promoter *vioP* in front of the compound

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(A1)-BGC, creating a translational fusion. Promoter *vioP* was extracted from the strain's inherent violacein BGC. The nucleotide sequence of promoter *vioP* is shown in **Table 1**. The generation of this mutant is described in Example 1.

The violacein promoter from *C. vaccinii* and the one from *C. violaceum* same essentially the same function and about 82% sequence identify. The vioP promoter from *C. violaceum* is described, *e.g.*, in Morohoshi *et al.* (2010) Bioscie. Biotechnol. Biochem.;74 (10),2116-2119 and Zhang *et al.* (2011) Appl Microbiol Biotechnol;90:1963-1971.

As another example, the strain *Chromobacterium vaccinii nptII-frsA* is a mutant generated from strain *Chromobacterium vaccinii* DSM 25150 by insertion of the promoter *nptII* in front of the compound (A1)-BGC, creating a translational fusion. Promoter *nptII* is a constitutive promoter. The nucleotide sequence of promoter *nptII* is shown in **Table 1**. The generation of this mutant IS described in Example 1.

As yet another example, the strain *Chromobacterium vaccinii rbs-frsA* is a mutant generated from strain *Chromobacterium vaccinii* DSM 25150 by insertion of the promoter *rbs* in front of the Compound (A1)-BGC, creating a translational fusion. The ribosomal promoter *rbs* is a constitutive promoter. The nucleotide sequence of promoter *rbs* is shown in **Table 1**. The generation of this mutant is described in Example 1.

As still another example, promoter *vioP*, *nptII* or *rbs* can be replaced with promoter *J23119*, P_{S12}*burk*, *Erme**, or *pLpp*. Promoter *J23119* is a synthetic promoter. The nucleotide sequence of promoter *J23119* is shown in **Table 1**. Promoter P_{S12}*burk* is a ribosomal promoter from *Burkholderia thailandensis*. The nucleotide sequence of promoter P_{S12}*burk* is shown in **Table 1**. Promoter P_{S12}*burk* is also described in Choi *et al.* (2008) App Environ Microbiol 74(4):1064-75). P_{S12}*burk* may also be known as *S12burk*. Promoter *ErmE** is a 23S rRNA methylase promoter from *Saccharopolyspora erythraea*. The nucleotide sequence of promoter *ErmE** is shown in **Table 1**. Promoter *ErmE** is also described in described in Bibb *et al.* (1994) Mol Microbiol 14(3):533-45. Promoter *pLpp* is a synthetic promoter. The nucleotide sequence of promoter *pLpp* is shown in **Table 1**. Promoter *pLpp* is also described in parts.igem.org. *pLpp* may also be known as *Plpp*.

In some embodiments, the titer of compound (A1) of the *C. vaccinii* promoter insertion mutant is increased by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10-fold, compared to the titer of a reference *C. vaccinii*, *e.g.*, a *C. vaccinii* that does not have the promoter insertion mutation in the compound (A1)-BGC or a wild type *C. vaccinii*, when cultured under identical conditions. In some embodiments, the titer of compound (A1) of the *C. vaccinii* promoter insertion mutant is increased at least about 3-fold, *e.g.*, at least about 3.5-fold, compared to the titer of compound (A1) of a wild type *C. vaccinii*.

In some embodiments, the titer of compound (A1) of the *C. vaccinii* promoter insertion mutant is increased by at least about 100 mg/L, about 200 mg/L, about 300 mg/L, about 400 mg/L, about 500 mg/L, about 600 mg/L, about 700 mg/L, about 800 mg/L, about 900 mg/L, or about 1000

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mg/L, compared to the titer of a reference *C. vaccinii*, *e.g.*, a *C. vaccinii* that does not have the promoter insertion mutation in the compound (A1)-BGC or a wild type *C. vaccinii*, when cultured under identical conditions. In some embodiments, the titer of compound (A1) of the *C. vaccinii* promoter insertion mutant is increased, *e.g.*, from no more than about 300 mg/L to at least about 600 mg/L or at least about 800 mg/L, compared to the titer of compound (A1) of a wild type *C. vaccinii*. In some embodiments, the titer of compound (A1) of the *C. vaccinii* promoter insertion mutant is at least about 200 mg/L, about 250 mg/L, about 300 mg/L, about 350 mg/L, about 400 mg/L, about 450 mg/L, about 500 mg/L, about 550 mg/L, about 600 mg/L, about 650 mg/L, about 700 mg/L, about 750 mg/L, about 800 mg/L, about 850 mg/L, about 900 mg/L, about 950 mg/L, about 1000 mg/L, about 1100 mg/L, about 1200 mg/L, about 1300 mg/L, about 1400 mg/L, about 1500 mg/L, about 1600 mg/L, about 1700 mg/L, about 1800 mg/L, about 1900 mg/L, or about 2000 mg/L. In some embodiments, the titer of compound (A1) of the *C. vaccinii* mutant is at least about 800 mg/L, *e.g.*, about 800 mg/L to about 850 mg/L. In some embodiment, the titer of compound (A1) of the *C. vaccinii* promoter insertion mutant is at least about 600 mg/L, *e.g.*, about 600 mg/L to about 650 mg/L, *e.g.*, when cultured in an animal component-free medium.

The *C. vaccinii* promoter insertion mutants described herein can be used to produce compound (A1) in a small scale (*e.g.*, a research scale) or in a large scale (*e.g.*, a production scale). In some embodiments, compound (A1) is produced in a fermenter or a bioreactor. In some embodiments, compound (A1) is produced in a fermentation volume of about 50 L to about 250 L, *e.g.*, about 50 L to about 100 L, about 100 L to about 150 L, or about 150 L to about 250 L, *e.g.*, about 50 L, about 100 L, about 150 L, about 200 L, or about 250 L. In some embodiments, compound (A1) is produced in a fermentation volume of about 1,000 L to about 20,000 L, *e.g.*, about 1,000 L to about 10,000 L or about 10,000 L to about 20,000 L, *e.g.*, about 1,000 L, about 2,000 L, about 5,000 L, about 7,500 L, about 10,000 L, about 15,000 L, or about 20,000 L.

Disruption mutants

Alternatively, or in combination, in some embodiments, the *C. vaccinii* mutant has an increased isolate yield of compound (A1) compared to the wild type *C. vaccinii* when cultured under identical conditions. For example, the level or activity of another natural product produced by *C. vaccinii* can be reduced, *e.g.*, the BGC of such natural product can be disrupted. Without wishing to be bound by theory, it is believed that in addition to compound (A1) and its analogs, *C. vaccinii* produces at least two additional natural products classes, which interfere to a certain extent with the purification of compound (A1). These belong to the violacein-class and a class of cyclic lipodepsipeptides, with compound J being the most abundant representative. In some embodiments, the isolate yield of compound (A1) can be increased when the complexity of the byproducts that interferes with the purification of compound (A1) is decreased.

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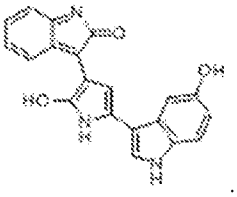
In some embodiments, the BGC of violacein is altered, *e.g.*, disrupted, or one or more genes in the violacein-BGC knocked out. In some embodiments, the level or activity of violacein is reduced, *e.g.*, eliminated.

Violacein is a purple pigment of bis-indole structure that is common to most *Chromobacteria* and even accountable for the naming of this genus. Violacein has a chemical structure of:

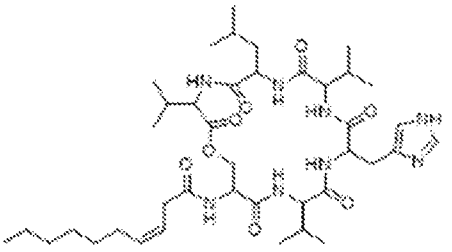


Its biosynthesis has been elucidated and the respective BGC is described, *e.g.*, in August *et al.* (2000) J Mol Microbiol Biotechnol.; 2(4):513-519. The high amounts of violacein, present in the fermentation broth, are troublesome during the extraction process due to precipitation at every evaporation step and the time and effort required for subsequent solubilization. Therefore, a size-exclusion chromatography can be introduced as first purification step, to remove violacein and avoid further precipitation.

In some embodiments, the BGC of compound J is altered, *e.g.*, disrupted, or one or more genes in the compound J-BGC knocked out. For example, the production one or more (*e.g.*, two, three, four, or more) compounds from the compound J-BGC (*e.g.*, any one, two, three, or all of compound J, compound F5, compound F3, or compound D) is reduced, *e.g.*, eliminated. In some embodiments, the production of compound J, compound F5, compound F3, and compound D is reduced, *e.g.*, eliminated.

In some embodiments, the level or activity of Compound J is reduced, *e.g.*, eliminated. In some embodiments, the BGC of Compound J is altered, *e.g.*, disrupted, or one or more genes in the Compound J-BGC knocked out.

Compound J has a chemical structure of:



A retrobiosynthetic analysis of Compound J (cyclic hexadepsipeptide of biosynthetic sequence: N-Acyl-Ser-Val-His-Val-Leu-Val (SEQ ID NO: 318)) allowed for identification of its BGC from the sequenced genome of *C. vaccinii*. Upon size-exclusion chromatography members of the Compound J class co-elute in the Compound (A1)-rich fractions and cause upon solubilization for further purification by preparative HPLC a very high viscosity (almost gel-like) hampering the

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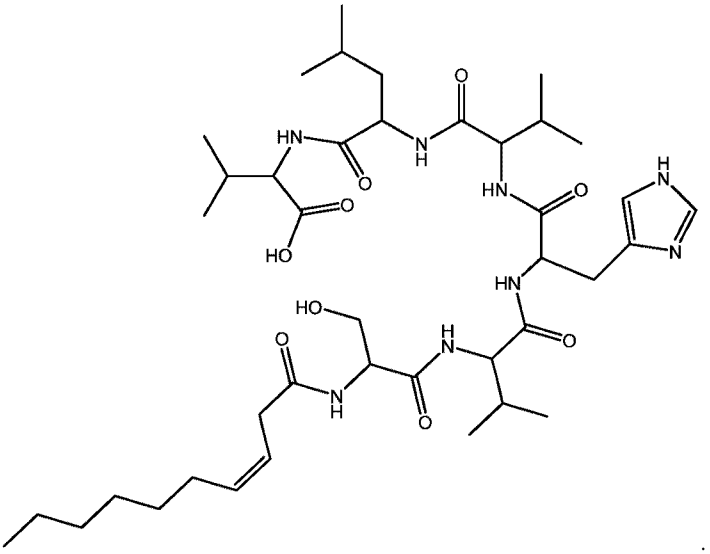
injection onto the column. Consequently, the fractions typically need further dilution and the compound amounts to be processed per run are limited.

In some embodiments, the level or activity of Compound F5 is reduced, *e.g.*, eliminated.

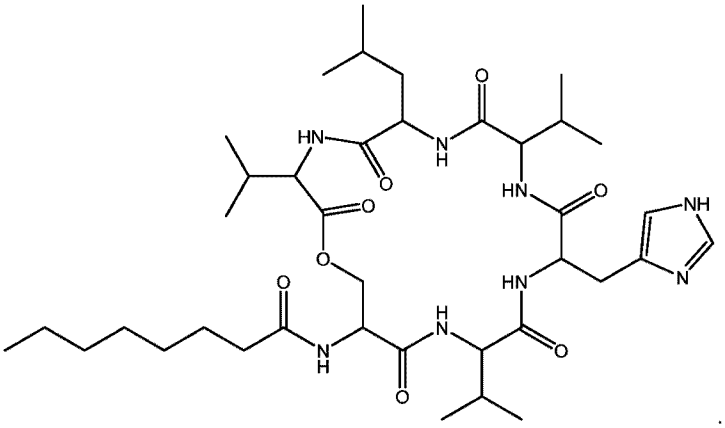
Compound F5 has a chemical structure of:



Compound F5 is a linear lipopeptide of the sequence *N*-(Z)-dec-3-enoyl-^{1}Ser-^{2}Val-^{3}His-^{4}Val-^{5}Leu-^{6}Val (SEQ ID NO: 321). In comparison to compound J, the lactone between the ^{1}Ser hydroxyl group and the C-terminal carboxyl group of ^{6}Val has been hydrolyzed.

In some embodiments, the level or activity of Compound F3 is reduced, *e.g.*, eliminated.

Compound F3 has a chemical structure of:



Compound F3 is a cyclic lipodepsipeptide of the sequence *N*-octanoyl-^{1}Ser-^{2}Val-^{3}His-^{4}Val-^{5}Leu-^{6}Val (SEQ ID NO: 322). The hexapeptide lactone ring is formed between the ^{1}Ser hydroxyl group and the C-terminal carboxyl group of ^{6}Val.

In some embodiments, the level or activity of Compound D is reduced, *e.g.*, eliminated.

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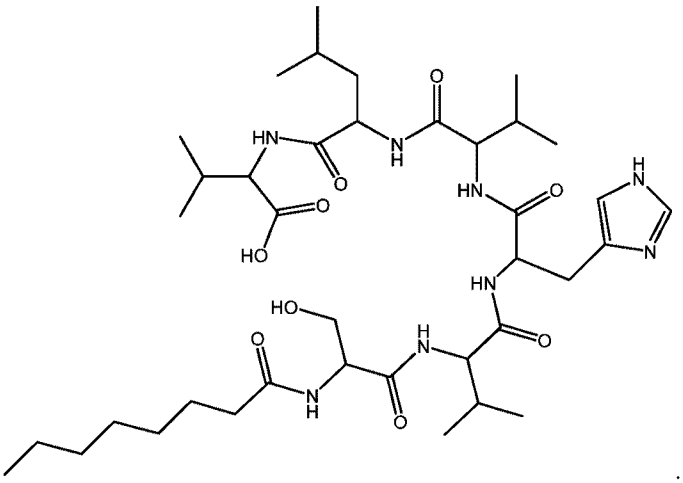
Compound D has a chemical structure of:



Compound D is a linear lipopeptide of the sequence *N*-octanyl-[1]Ser-[2]Val-[3]His-[4]Val-[5]Leu-[6]Val (SEQ ID NO: 323). In comparison to compound F3 the lactone between the [1]Ser hydroxyl group and the C-terminal carboxyl group of [6]Val has been hydrolyzed.

In some embodiments, the *C. vaccinii* disruption mutant has two or more (*e.g.*, three or four) disruption mutations. For example, both violacein-BGC and compound J-BGC can be altered, *e.g.*, disrupted.

In some embodiments, the isolated yield of compound (A1) of the *C. vaccinii* disruption mutant is increased by at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, or about 90%, or at least about 1, about 2, about 3, about 4, or about 5-fold, compared to the isolated yield of a reference *C. vaccinii*, *e.g.*, a *C. vaccinii* that does not have the disruption mutation or a wild type *C. vaccinii*, when cultured under identical conditions. In some embodiments, the isolated yield of compound (A1) of the *C. vaccinii* disruption mutant is at least about 60%, *e.g.*, about 65% to about 70%. In some embodiments, the isolated yield of Compound (A1) of the wild type *C. vaccinii* is about 35% to about 45%, *e.g.*, about 40%.

In some embodiments, the titer of compound (A1) of the *C. vaccinii* disruption mutant differs by no more than about 50%, about 40%, about 35%, about 30%, about 25%, about 20%, about 15%, or about 10%, compared to the titer of a reference *C. vaccinii*, *e.g.*, a *C. vaccinii* that does not have the disruption mutation or a wild type *C. vaccinii*, when cultured under identical conditions. In some embodiments, the titer of compound (A1) of the *C. vaccinii* disruption mutant is at least about 200 mg/L, about 250 mg/L, about 300 mg/L, about 350 mg/L, about 400 mg/L, about 450 mg/L, about 500 mg/L, about 550 mg/L, about 600 mg/L, about 650 mg/L, about 700 mg/L, about 750 mg/L, about 800 mg/L, about 850 mg/L, about 900 mg/L, about 950 mg/L, about 1000 mg/L, about 1100 mg/L, about 1200 mg/L, about 1300 mg/L, about 1400 mg/L, about 1500 mg/L, about 1600 mg/L, about 1700 mg/L, about 1800 mg/L, about 1900 mg/L, or about 2000 mg/L. In some embodiments, the titer of compound (A1) of the *C. vaccinii* disruption mutant is at least about 800 mg/L, *e.g.*, about

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800 mg/L to about 850 mg/L. In some embodiment, the titer of compound (A1) of the *C. vaccinii* disruption mutant is at least about 600 mg/L, *e.g.*, about 600 mg/L to about 650 mg/L, *e.g.*, when cultured in an animal component-free medium.

The *C. vaccinii* disruption mutants described herein can be used to produce compound (A1) in a small scale (*e.g.*, a research scale) or in a large scale (*e.g.*, a production scale). In some embodiments, compound (A1) is produced in a fermenter or a bioreactor. In some embodiments, compound (A1) is produced in a fermentation volume of about 50 L to about 250 L, *e.g.*, about 50 L to about 100 L, about 100 L to about 150 L, or about 150 L to about 250 L, *e.g.*, about 50 L, about 100 L, about 150 L, about 200 L, or about 250 L. In some embodiments, compound (A1) is produced in a fermentation volume of about 1,000 L to about 20,000 L, *e.g.*, about 1,000 L to about 10,000 L or about 10,000 L to about 20,000 L, *e.g.*, about 1,000 L, about 2,000 L, about 5,000 L, about 7,500 L, about 10,000 L, about 15,000 L, or about 20,000 L.

Biosynthesis of Compound (A1)

The depsipeptide, compound (A1), is described in Fujioka *et al.* (1988) J. Org. Chem.; 53 (12) 2820-2825. It was isolated from a methanol extract of the whole plant of *Ardisia crenata*. Almost two decades later, it was discovered that compound (A1) is in fact produced by a strictly obligate bacterial endosymbiont, *Candidatus Burkholderia crenata*, of the plant *Ardisia crenata* (Carlier *et al.* (2016) Environ Microbiol.;18(8):2507-22; GenBank accession number LGTG01000643.1 for the DNA sequence of the biosynthetic gene cluster (BGC) encoding the biosynthetic genes responsible for biosynthesis of compound (A1)). Compound (A1) biosynthesis by heterologous expression in *E. coli*. is described, *e.g.*, in Crüsemann *et al.* (2018) Angew. Chem. Int. Ed.; 57, 836 –840.

As described in Example 1 herein, translated amino acid sequences of the compound (A1)-BGC from *B. crenata* were used as query sequence in a genome mining effort on bacterial genomes from sequence databases and bacterial genomes from sequencing efforts performed with PacBio sequencing technology. A cluster with very high homology in translated amino acid sequence and identical prediction of protein functions was discovered in the sequenced genome of *C. vaccinii*. With the knowledge that *C. vaccinii* harbors the compound (A1)-BGC it was possible to retrieve meaningful BLAST hits from its published draft genome (Vöing *et al.* (2015) Genome Announc 3, e00477–15) for *frsC* and *frsH*, but not for the full length genes *frsA*, *frsD*, *frsE*, *frsF*, *frsG*. A similar approach led to the identification of *C. vaccinii* as producer of A1 by Hermes *et al* (2021). However, the cluster was spread over six contigs and extensive PCR-based gap closure had to be performed to obtain the sequence of the entire compound (A1)-BGC (GenBank accession number: MT876545). The sequence generated in Example 1 herein demonstrates the advantage of PacBio sequencing concerning highly identical repetitive sequence stretches. The complete compound (A1)-BGC could be identified including the up- and downstream regions encoding an additional large NRPS and several transposable elements (GenBank accession number: BankIt2437961 BSeq#1 MW732719).

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The gene organization on transposable element containing BGCs of compound (A1) (*frsA-H*) and compound J (*dis1-4*) in *C. vaccinii* (76974 bp) is shown in **FIG. 1**. The organization of genome region containing compound (A1)-BGC and NRPS A is further shown in **Table 2-1**.

The biosynthesis of Compound (A1), including, but not limited to, the complete compound (A1)-BGC, is also disclosed, *e.g.*, in Pistorius et al. Genetic Engineering of *Chromobacterium Vaccinii* DSM 25150 for Improved Production of FR900359. ChemRxiv. Cambridge: Cambridge Open Engage; 2021, which is incorporated by reference in its entirety.

Cell Culture and Fermentation

The microorganisms described herein can be cultured under conditions that allow for the production of compound (A1). In general, conditions that may be optimized include the type and amount of carbon source, the type and amount of nitrogen source, the carbon-to-nitrogen ratio, the oxygen level, growth temperature, pH, length of the biomass production phase, length of target product accumulation phase, and time of cell harvest.

Culture media generally contain a suitable carbon source. Carbon sources may include, but are not limited to, monosaccharides (*e.g.*, glucose, fructose, xylose), disaccharides (*e.g.*, lactose, sucrose), oligosaccharides, polysaccharides (*e.g.*, starch, cellulose, hemicellulose, other lignocellulosic materials or mixtures thereof), sugar alcohols (*e.g.*, glycerol), and renewable feedstocks (*e.g.*, cheese whey permeate, corn steep liquor, sugar beet molasses, barley malt). Carbon sources also can be selected from one or more of the following non-limiting examples: linear or branched alkanes (*e.g.*, hexane), linear or branched alcohols (*e.g.*, hexanol), fatty acids (*e.g.*, about 10 carbons to about 22 carbons), esters of fatty acids, monoglycerides, diglycerides, triglycerides, phospholipids and various commercial sources of fatty acids including vegetable oils (*e.g.*, soybean oil) and animal fats. A carbon source may include one-carbon sources (*e.g.*, carbon dioxide, methanol, formaldehyde, formate and carbon-containing amines) from which metabolic conversion into key biochemical intermediates can occur. It is expected that the source of carbon utilized may encompass a wide variety of carbon-containing sources and will only be limited by the choice of the engineered microorganism(s). Non-limiting examples of acceptable growth media include Luria broth (LB) agar, King's medium B (KMB) agar, tryptic soy medium, yeast extract mannitol medium (YEM), glycerol yeast extract (GYEA), yeast extract-peptone-glycerol (YPG), peptone yeast extract glucose (PYG) MacConkey agar, or malt extract agar, or in flasks containing suitable liquid media such as, tryptic soy broth, LB broth, YEM broth, KMB broth, GYEA broth. YPG broth, PYG broth, etc.

Nitrogen may be supplied from an inorganic (*e.g.*, $(NH_4)_2SO_4$) or organic source (*e.g.*, urea or glutamate). In addition to appropriate carbon and nitrogen sources, culture media also can contain suitable minerals, salts, cofactors, buffers, vitamins, metal ions (*e.g.*, Mn^{2+}, Co^{2+}, Zn^{2+}, Mg^{2+}) and other components suitable for culture of microorganisms. Other chemicals may be added to the culture as necessary. Non-limiting example of such chemicals include, tryptone peptone, calcium

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carbonate, propionic acid, sodium propionate, L-isoleucine, L-valine, L-phenylalanine, L-phenylbutyric acid, L-phenyllactic acid, sodium chloride, glycerol, Bacto soytone, Bacto peptone, Bacto soytone peptone, Bacto yeast extract, Soy peptone F, Soy peptone papaic digest, veggie peptone, veggie yeast extract, broadbean peptone, casein peptone, HEPES, ammonium sulfate, magnesium sulfate, soyamine, Amicase®, Tubermine®, soy protein, yeast extract without salt, Pharmamedia®, potato starch, L-phenyllactate, and L-phenylpyruvate.

Microorganisms sometimes are cultured in complex media (*e.g.*, yeast extract-peptone-dextrose broth (YPD)). Culture media in some embodiments are common commercially prepared media, *e.g.*, LB media. Other defined or synthetic growth media are known in the arts and may also be used.

Microorganisms may be cultured on or in solid, semi-solid or liquid media. In some embodiments, media is drained from cells adhering to a plate. In certain embodiments, a liquid-cell mixture is centrifuged at a speed sufficient to pellet the cells but not disrupt the cells and allow extraction of the media, as known in the art. The cells may then be resuspended in fresh media. The product of interest may be purified from culture media according to methods known in the art. In certain embodiments, target product is extracted from the cultured microorganisms. The microorganism cells may be concentrated through centrifugation at speed sufficient to shear the cell membranes. In some embodiments, the cells may be physically disrupted (*e.g.*, shear force, sonication) or chemically disrupted (*e.g.*, contacted with detergent or other lysing agent). The phases may be separated by centrifugation or other method known in the art and target product may be isolated according to known methods.

Fermentation conditions can include any culture conditions suitable for maintaining a microorganism (*e.g.*, in a static or proliferative state). For example, fermentation conditions can include several parameters, including without limitation, temperature, oxygen content, nutrient content (*e.g.*, glucose content), pH, agitation level (*e.g.*, rotations per minute), gas flow rate (*e.g.*, air, oxygen, nitrogen gas), redox potential, cell density (*e.g.*, optical density), cell viability and the like. A change in fermentation conditions (*e.g.*, switching fermentation conditions) is an alteration, modification or shift of one or more fermentation parameters. For example, one can change fermentation conditions by increasing or decreasing temperature, increasing or decreasing pH (*e.g.*, adding or removing an acid, a base or carbon dioxide), increasing or decreasing oxygen content (*e.g.*, introducing air, oxygen, carbon dioxide, nitrogen), increasing or decreasing air pressure (*e.g.*, by introducing air, oxygen, carbon dioxide, nitrogen), increasing or decreasing agitation, and/or adding or removing a nutrient (*e.g.*, one or more sugars or sources of sugar, biomass, vitamin and the like), increasing or decreasing the ratio of culture and flask volume, or combinations of the foregoing. Examples of fermentation conditions are described herein. Aerobic conditions often comprise greater than about 50% dissolved oxygen (*e.g.*, about 52%, about 54%, about 56%, about 58%, about 60%, about 62%, about 64%, about 66%, about 68%, about 70%, about 72%, about 74%, about 76%, about

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78%, about 80%, about 82%, about 84%, about 86%, about 88%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98% or about 99%, or greater than any one of the foregoing). Anaerobic conditions often comprise less than about 50% dissolved oxygen (*e.g.*, about 1%, about 2%, about 4%, about 6%, about 8%, about 10%, about 12%, about 14%, about 16%, about 18%, about 20%, about 22%, about 24%, about 26%, about 28%, about 30%, about 32%, about 34%, about 36%, about 38%, about 40%, about 42%, about 44%, about 46%, about 48%, or less than any one of the foregoing).

A variety of fermentation processes may be applied for the production of a product of interest. In some embodiments, the production of a target product from a recombinant microbial host is conducted using a batch, fed-batch or continuous fermentation process, for example.

A batch fermentation process often is a closed system where the media composition is fixed at the beginning of the process and not subject to further additions beyond those required for maintenance of pH and oxygen level during the process. At the beginning of the culturing process the media is inoculated with the desired organism and growth or metabolic activity is permitted to occur without adding additional sources (*e.g.*, carbon and nitrogen sources) to the medium. In batch processes the metabolite and biomass compositions of the system change constantly up to the time the culture is terminated. In a typical batch process, cells proceed through a static lag phase to a high-growth log phase and finally to a stationary phase, wherein the growth rate is diminished or halted. Left untreated, cells in the stationary phase will eventually die.

A variation of the standard batch process is the fed-batch process, where the carbon source is continually added to the fermenter over the course of the fermentation process. Fed-batch processes are useful when catabolite repression is apt to inhibit the metabolism of the cells or where it is desirable to have limited amounts of carbon source in the media at any one time. Measurement of the carbon source concentration in fed-batch systems may be estimated on the basis of the changes of measurable factors such as pH, dissolved oxygen and the partial pressure of waste gases (*e.g.*, CO.sub.2). Batch and fed-batch culturing methods are known in the art. Examples of such methods may be found in Thomas D. Brock in Biotechnology: A Textbook of Industrial Microbiology, 2.sup.nd ed., (1989) Sinauer Associates Sunderland, Mass. and Deshpande, Mukund V., Appl. Biochem. Biotechnol., 36:227 (1992).

In continuous fermentation process a defined media often is continuously added to a bioreactor while an equal amount of culture volume is removed simultaneously for product recovery. Continuous cultures generally maintain cells in the log phase of growth at a constant cell density. Continuous or semi-continuous culture methods permit the modulation of one factor or any number of factors that affect cell growth or end product concentration. For example, an approach may limit the carbon source and allow all other parameters to moderate metabolism. In some systems, a number of factors affecting growth may be altered continuously while the cell concentration, measured by media turbidity, is kept constant. Continuous systems often maintain steady state growth and thus the cell

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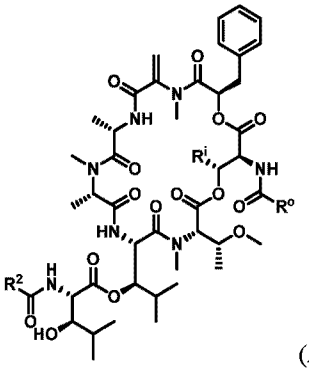
growth rate often is balanced against cell loss due to media being drawn off the culture. Methods of modulating nutrients and growth factors for continuous culture processes, as well as techniques for maximizing the rate of product formation, are known and a variety of methods are detailed by Brock, supra.

The product of interest may be provided within cultured microbes containing target product, and cultured microbes may be supplied fresh or frozen in a liquid media or dried. Fresh or frozen microbes may be contained in appropriate moisture-proof containers that may also be temperature controlled as necessary. Product of interest sometimes is provided in culture medium that is substantially cell-free. In some embodiments, the product of interest is provided in substantially pure form (*e.g.*, 50% pure or greater, 60% pure or greater, 70% pure or greater, 80% pure or greater, 90% pure or greater, 95% pure or greater, 99% pure or greater or 99.5% pure or greater). In some embodiments, the product of interest may be modified into any one of a number of downstream products.

Drug Moiety (D)

In some embodiments, the drug moiety (D) of the antibody drug conjugate of the disclosure is a GNAQ inhibitor, a GNA11 inhibitor, or an inhibitor of GNAQ and GNA11 (GNAQ/GNA11 inhibitor). In some embodiments, the drug moiety (D) of the antibody drug conjugate of the disclosure is a GNAQ inhibitor. In some embodiments, the drug moiety (D) of the antibody drug conjugate of the disclosure is a GNA11 inhibitor. In some embodiments, the drug moiety (D) of the antibody drug conjugate of the disclosure is an inhibitor of GNAQ and GNA11 (GNAQ/GNA11 inhibitor).

In some embodiments, the drug moiety (D) of the antibody drug conjugate of the disclosure is a compound having the structure of Formula (A)



(A),

wherein Ro is methyl or ethyl, Ri is methyl or i-propyl, and R2 is methyl or ethyl.

In some embodiments, the drug moiety (D) of the antibody drug conjugate of the disclosure is a compound (A1) having the following structure,

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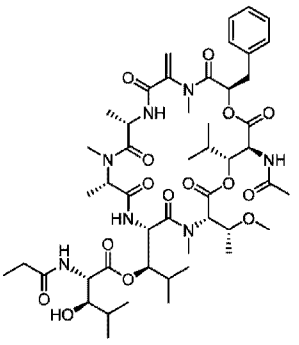
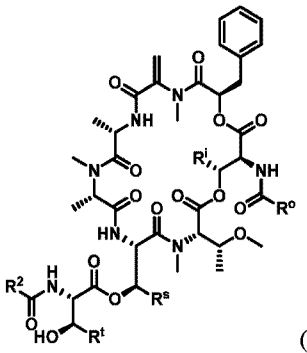


(A1).

Synthesis of Compound (A1) is also described in Example 3 of International Application Publication No. WO 2020/128612, which is incorporated by reference in its entirety.

The metabolic stability and PK properties of Compound (A1) are also described in Examples 8 and 9, respectively, of International Application Publication No. WO 2020/128612, which is incorporated by reference in its entirety.

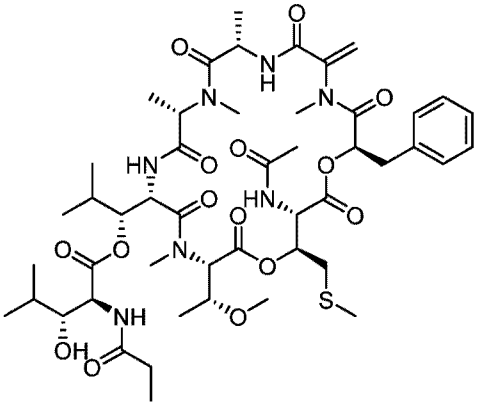
In some embodiments, the drug moiety (D) of the antibody drug conjugate of the disclosure is a compound having the structure of Formula (Ab)



(Ab)

wherein R^o is methyl or ethyl, R^i, R^s, and R^t are each independently methyl, i-propyl, or methylthiomethyl, and R^2 is methyl or ethyl.

In some embodiments, the drug moiety (D) of the antibody drug conjugate of the disclosure is a compound (Ab1) having the following structure,



(Ab1).

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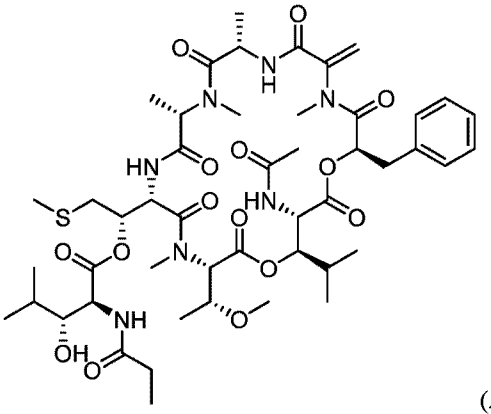
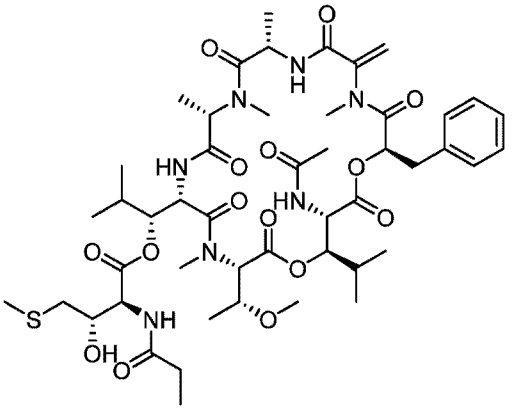
In some embodiments, the drug moiety (D) of the antibody drug conjugate of the disclosure is a compound (Ab2) having the following structure,



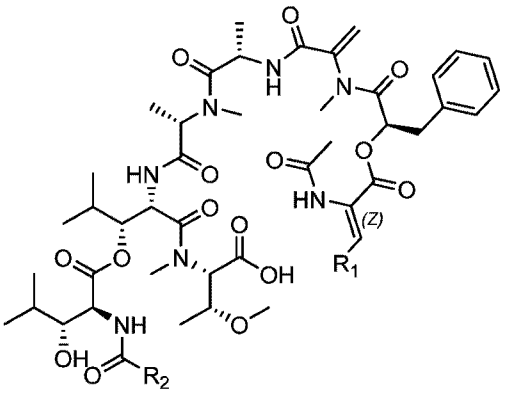
(Ab2).

In some embodiments, the drug moiety (D) of the antibody drug conjugate of the disclosure is a compound (Ab3) having the following structure,



(Ab3).

In some embodiments, the drug moiety (D) of the antibody drug conjugate of the disclosure is a compound having the structure of Formula (Ac)



(Ac)

wherein R^1 is methyl or isopropyl, and R^2 is methyl or ethyl.

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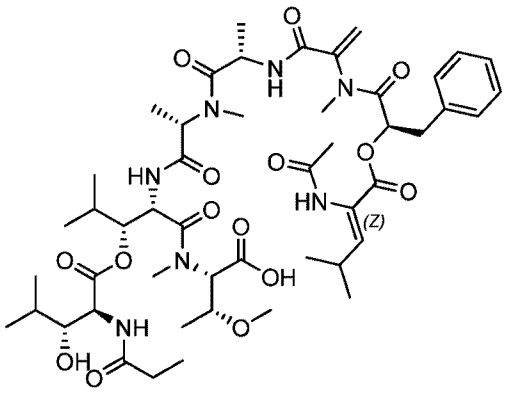
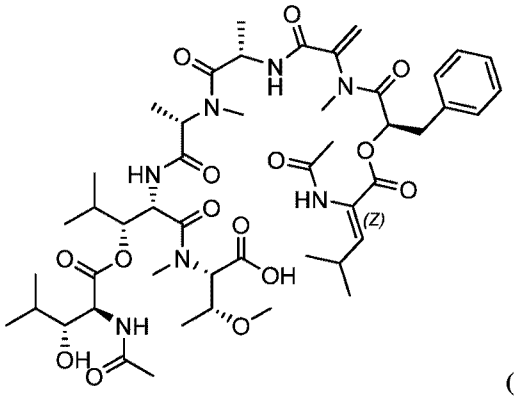
In some embodiments, the drug moiety (D) of the antibody drug conjugate of the disclosure is a compound (Ac1) having the following structure,



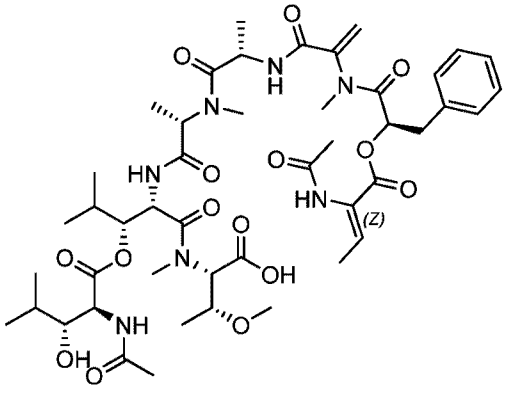
(Ac1).

In some embodiments, the drug moiety (D) of the antibody drug conjugate of the disclosure is a compound (Ac2) having the following structure,



(Ac2).

In some embodiments, the drug moiety (D) of the antibody drug conjugate of the disclosure is a compound (Ac3) having the following structure,



(Ac3).

In some embodiments, the drug moiety (D) of the antibody drug conjugate of the disclosure is a compound (Ac4) having the following structure,

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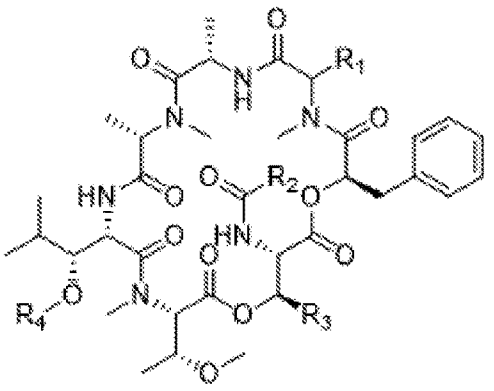

(Ac4)

wherein R_1 is a methyl or a =CH2, R_2 is a methyl or an ethyl, R_3 is methyl, ethyl, or isopropyl, and R_4 is a H, *N*-Ac-Hle, or *N*-Pr-Hle.

In some embodiments, the drug moiety (D) of the antibody drug conjugate of the disclosure is any of compounds (A1) and 2-15, *e.g.*, as disclosed in Example 3.

Linker-Drug Moiety (L_A-$(D)_n$)

In some embodiments, the Linker-Drug moiety, ((L_A-$(D)_n$))), of the Antibody Drug Conjugate of the disclosure comprises one or more Drug moieties covalently attached to a linker (L_A), wherein the one or more Drug moieties are each independently selected from a GNAQ inhibitor, a GNA11 inhibitor or an inhibitor of GNAQ and GNA11 (GNAQ/GNA11 inhibitor).

In some embodiments, the Linker-Drug moiety, ((L_A-$(D)_n$))), of the Antibody Drug Conjugate of the disclosure comprises one or more Drug moieties covalently attached to a linker (L_A), wherein the one or more Drug moieties are each independently selected from a GNAQ inhibitor.

In some embodiments, the Linker-Drug moiety, ((L_A-$(D)_n$))), of the Antibody Drug Conjugate of the disclosure comprises one or more Drug moieties attached to a linker (L_A), wherein the one or more Drug moieties are each independently selected from a GNA11 inhibitor.

In some embodiments, the Linker-Drug moiety, ((L_A-$(D)_n$))), of the Antibody Drug Conjugate of the disclosure comprises one or more Drug moieties covalently attached to a linker (L_A), wherein the one or more Drug moieties are each independently selected from an inhibitor of GNAQ and GNA11 (GNAQ/GNA11 inhibitor).

In some embodiments, the Linker-Drug moiety, ((L_A-$(D)_n$))), of the Antibody Drug Conjugate of the disclosure comprises one or more Drug moieties covalently attached to a linker (L_A), wherein the linker (L_A) is a cleavable linker and the one or more Drug moieties are each independently selected from a GNAQ inhibitor, a GNA11 inhibitor or an inhibitor of GNAQ and GNA11 (GNAQ/GNA11 inhibitor).

In some embodiments, the Linker-Drug moiety, ((L_A-$(D)_n$))), of the Antibody Drug Conjugate of the disclosure comprises one or more Drug moieties covalently attached to a linker (L_A), wherein

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the linker (L_A) is a cleavable linker and the one or more Drug moieties are each independently selected from a GNAQ inhibitor.

In some embodiments, the Linker-Drug moiety, $((L_A\text{-}(D)_n))$, of the Antibody Drug Conjugate of the disclosure comprises one or more Drug moieties covalently attached to a linker (L_A), wherein the linker (L_A) is a cleavable linker and the one or more Drug moieties are each independently selected from a GNA11 inhibitor.

In some embodiments, the Linker-Drug moiety, $((L_A\text{-}(D)_n))$, of the Antibody Drug Conjugate of the disclosure comprises one or more Drug moieties covalently attached to a linker (L_A), wherein the linker (L_A) is a cleavable linker and the one or more Drug moieties are each independently selected from an inhibitor of GNAQ and GNA11 (GNAQ/GNA11 inhibitor).

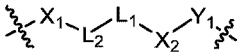
In some embodiments, the Linker-Drug moiety, $((L_A\text{-}(D)_n))$, of the Antibody Drug Conjugate of the disclosure comprises one or more Drug moieties covalently attached to a linker (L_A), wherein the linker (L_A) is a non-cleavable linker and the one or more Drug moieties are each independently selected from a GNAQ inhibitor, a GNA11 inhibitor or an inhibitor of GNAQ and GNA11 (GNAQ/GNA11 inhibitor).

In some embodiments, the Linker-Drug moiety, $((L_A\text{-}(D)_n))$, of the Antibody Drug Conjugate of the disclosure comprises one or more Drug moieties covalently attached to a linker (L_A), wherein the linker (L_A) is a non-cleavable linker and the one or more Drug moieties are each independently selected from a GNAQ inhibitor.

In some embodiments, the Linker-Drug moiety, $((L_A\text{-}(D)_n))$, of the Antibody Drug Conjugate of the disclosure comprises one or more Drug moieties covalently attached to a linker (L_A), wherein the linker (L_A) is a non-cleavable linker and the one or more Drug moieties are each independently selected from a GNA11 inhibitor.

In some embodiments, the Linker-Drug moiety, $((L_A\text{-}(D)_n))$, of the Antibody Drug Conjugate of the disclosure comprises one or more Drug moieties covalently attached to a linker (L_A), wherein the linker (L_A) is a non-cleavable linker and the one or more Drug moieties are each independently selected from an inhibitor of GNAQ and GNA11 (GNAQ/GNA11 inhibitor).

In some embodiments, the linker (L_A) of the Linker-Drug moiety, $((L_A\text{-}(D)_n))$, of the Antibody Drug Conjugate of the disclosure has the following formula:



wherein:

X_1 is a bivalent coupling group;

X_2 is a self-immolative spacer;

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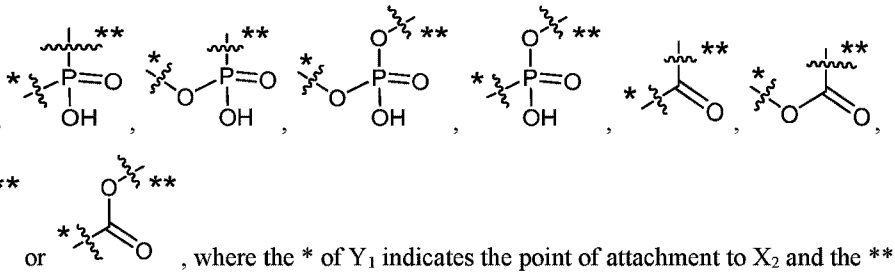
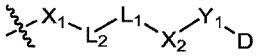
Y_1 is  , where the * of Y_1 indicates the point of attachment to X_2 and the ** of Y_1 indicates the other point of attachment;

L_1 is a bivalent peptide linker, and

L_2 is a bond or a linker.

In some embodiments, the Linker-Drug moiety, $((L_A\text{-}(D)_n))$, of the Antibody Drug Conjugate of the disclosure has the following formula:

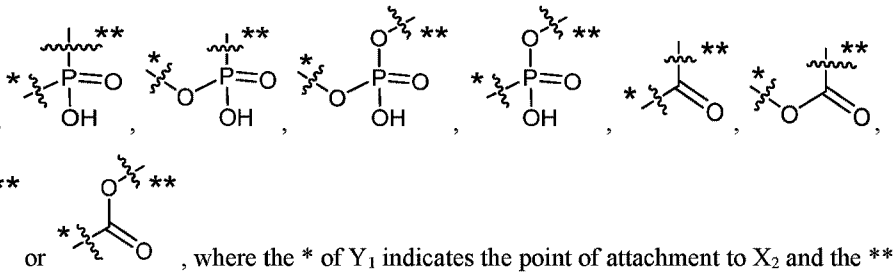


wherein:

D is a GNAQ inhibitor, a GNA11 inhibitor or an inhibitor of GNAQ and GNA11;

X_1 is a bivalent coupling group;

X_2 is a self-immolative spacer;

Y_1 is  , where the * of Y_1 indicates the point of attachment to X_2 and the ** of Y_1 indicates the point of attachment to D;

L_1 is a bivalent peptide linker, and

L_2 is a bond or a linker.

Linker-Drug Compounds (L_B-$(D)_n$)

In some embodiments, the Linker-Drug of the disclosure is a compound having the structure of Formula (B), or stereoisomers or pharmaceutically acceptable salts thereof,

$$R^8\text{-}L_B\text{-}(D)_n \qquad (B)$$

wherein:

D is a GNAQ inhibitor, a GNA11 inhibitor or an inhibitor of GNAQ and GNA11;

R^8 is a reactive group;

L_B is a cleavable linker or non-cleavable linker, and

n is 1, 2, 3 or 4.

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In some embodiments, the Linker-Drug of the disclosure having the structure of Formula (B), or stereoisomers or pharmaceutically acceptable salts thereof, wherein

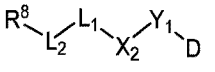
D is a GNAQ inhibitor, a GNA11 inhibitor or an inhibitor of GNAQ and GNA11;

R^8 is a reactive group;

L_B is a cleavable linker comprising one or more linker components selected from a self-immolative spacer, a phosphate group, a carbonate group and a bivalent peptide linker, and

n is 1, 2, 3 or 4.

In some embodiments, the Linker-Drug of the disclosure is a compound having the structure of Formula (B-1), or stereoisomers or pharmaceutically acceptable salts thereof,



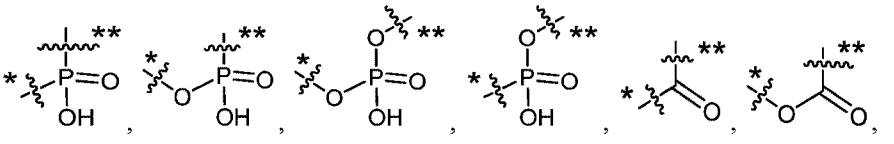
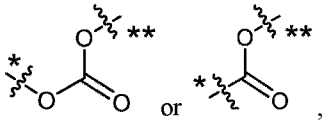
(B-1)

wherein:

D is a GNAQ inhibitor, a GNA11 inhibitor or an inhibitor of GNAQ and GNA11;

R^8 is a reactive group;

X_2 is a self-immolative spacer;

Y_1 is  or  , where the * of Y_1 indicates the point of attachment to X_2 and the ** of Y_1 indicates the point of attachment to D;

L_1 is a bivalent peptide linker, and

L_2 is a bond or a linker.

Certain embodiments and examples of the Linker-Drug compounds of the disclosure are provided in the following listing of additional, enumerated embodiments. It will be recognized that features specified in each embodiment may be combined with other specified features to provide further embodiments of the present disclosure.

Embodiment 1. The compound of Formula (B) or Formula (B-1), or stereoisomers or a pharmaceutically acceptable salt thereof, wherein D is a GNAQ inhibitor.

Embodiment 2. The compound of Formula (B) or Formula (B-1), or stereoisomers or a pharmaceutically acceptable salt thereof, wherein D is a GNA11 inhibitor.

Embodiment 3. The compound of Formula (B) or Formula (B-1), or stereoisomers or a pharmaceutically acceptable salt thereof, wherein D is an inhibitor of GNAQ and GNA11.

Embodiment 4. The compound of Formula (B) or Formula (B-1), or stereoisomers or a pharmaceutically acceptable salt thereof, wherein D is

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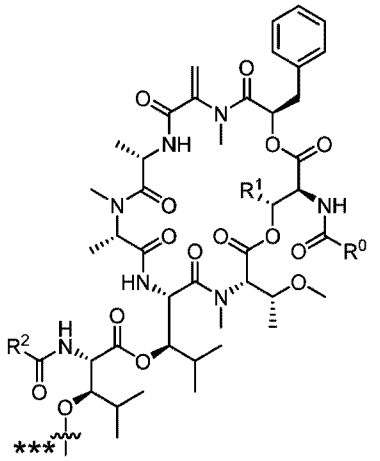
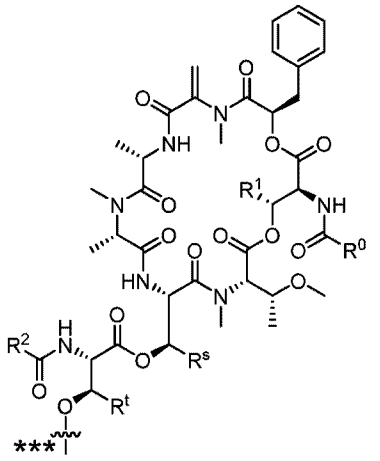


,

wherein R^0 is methyl or ethyl, R^1 is methyl or isopropyl, R^2 is methyl or ethyl, and the *** indicates the point of attachment to L_B or Y_1.

Embodiment 4a. The compound of Formula (Bb) or Formula (Bb-1), or stereoisomers or a pharmaceutically acceptable salt thereof, wherein D is



,

wherein R^0 is methyl or ethyl, R^1, R^s, R^t are each independently methyl, isopropyl, or methylthiomethyl, R^2 is methyl or ethyl, and the *** indicates the point of attachment to L_B or Y_1.

Embodiment 4b. The compound of Formula (Bc) or Formula (Bc-1), or stereoisomers or a pharmaceutically acceptable salt thereof, wherein D is

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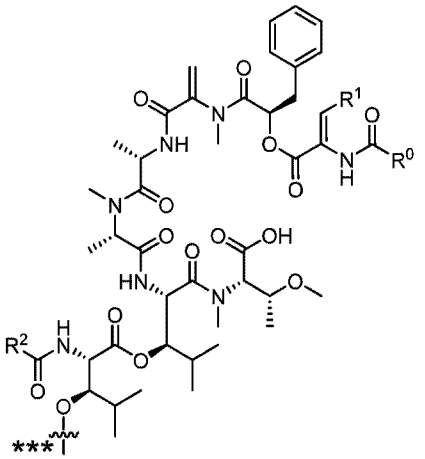
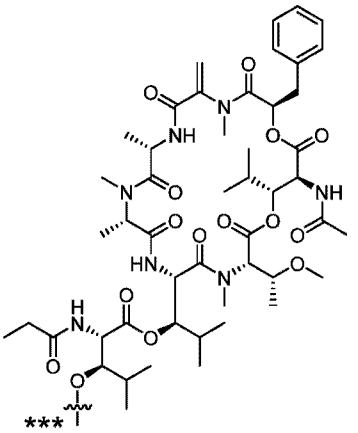


,

wherein R^0 is methyl or ethyl, R^1 is methyl or isopropyl, R^2 is methyl or ethyl, and the *** indicates the point of attachment to L_B or Y_1.

Embodiment 5. The compound of Formula (B) or Formula (B-1), or stereoisomers or a pharmaceutically acceptable salt thereof, wherein D is



,

where the *** indicates the point of attachment to L_B or Y_1.

Embodiment 6. The compound of Formula (B) or Formula (B-1), or stereoisomers or a pharmaceutically acceptable salt thereof, having the structure of Formula (B-2), or a pharmaceutically acceptable salt thereof:

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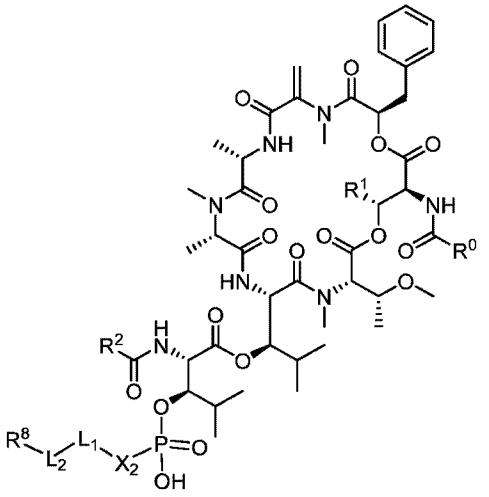


(B-2),

wherein:

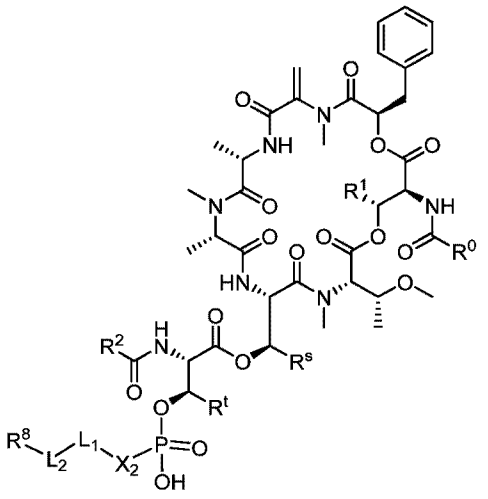
R^0 is methyl or ethyl,;

R^1 is methyl or isopropyl;

R^2 is methyl or ethyl, and

X_2, L_1, L_2 and R^8 are as define in compounds of Formula (B-1) above.

Embodiment 6a. The compound of Formula (Bb) or Formula (Bb-1), or stereoisomers or a pharmaceutically acceptable salt thereof, having the structure of Formula (Bb-2), or a pharmaceutically acceptable salt thereof:



(Bb-2),

wherein:

R^0 is methyl or ethyl;

R^1, R^s, and R^t are each independently methyl, methylthiomethyl or isopropyl;

R^2 is methyl or ethyl, and

X_2, L_1, L_2 and R^8 are as define in compounds of Formula (Bb-1) above.

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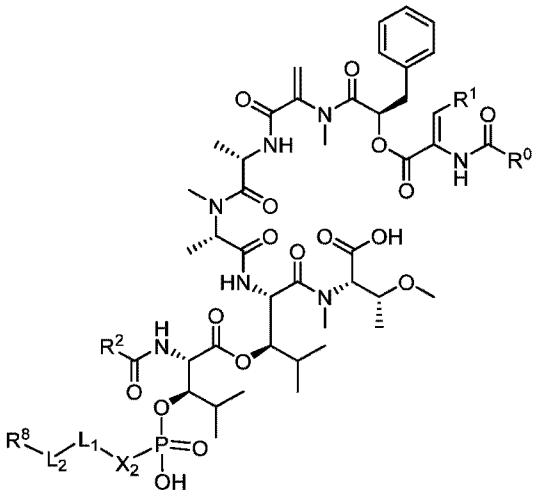
Embodiment 6b. The compound of Formula (Bc) or Formula (Bc-1), or stereoisomers or a pharmaceutically acceptable salt thereof, having the structure of Formula (Bc-2), or a pharmaceutically acceptable salt thereof:



(Bc-2),

wherein:

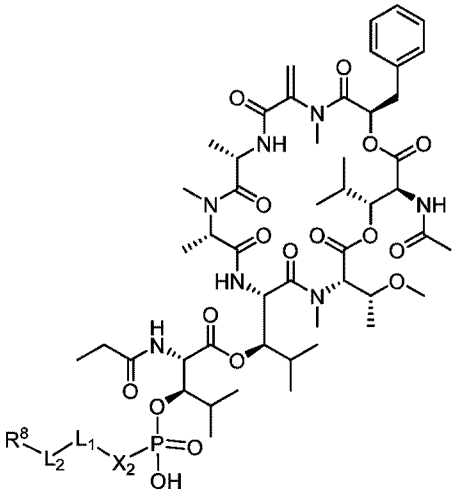
R^0 is methyl or ethyl,;

R^1 is methyl or isopropyl;

R^2 is methyl or ethyl, and

X_2, L_1, L_2 and R^8 are as define in compounds of Formula (Bc-1) above.

Embodiment 7. The compound of Formula (B), Formula (B-1) or Formula (B-2), or stereoisomers or a pharmaceutically acceptable salt thereof, having the structure of Formula (B-2a), or a pharmaceutically acceptable salt thereof:



(B-2a),

wherein:

X_2, L_1, L_2 and R^8 are as define in compounds of Formula (B-1) above.

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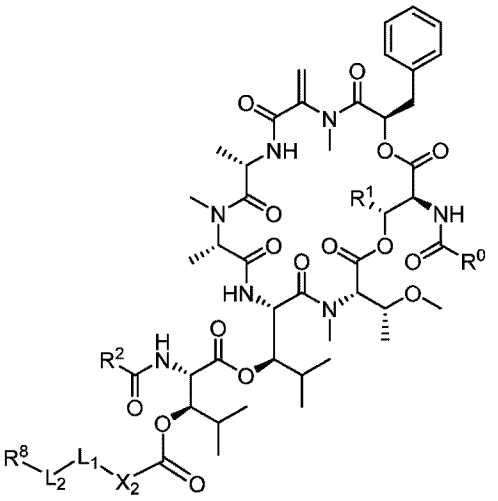
Embodiment 8. The compound of Formula (B) or Formula (B-1), or stereoisomers or a pharmaceutically acceptable salt thereof, having the structure of Formula (B-3), or a pharmaceutically acceptable salt thereof:



(B-3),

wherein:

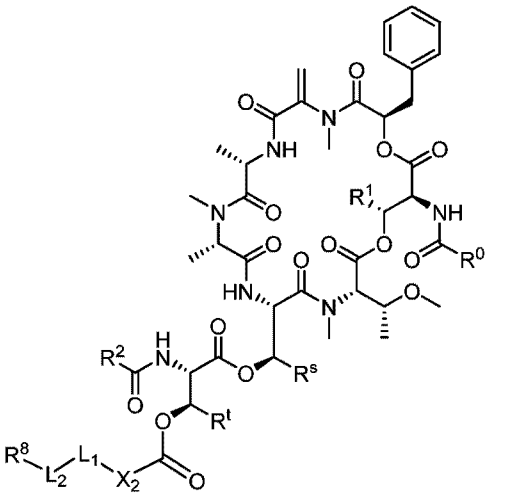
R^0 is methyl or ethyl;

R^1 is methyl or isopropyl;

R^2 is methyl or ethyl, and

X_2, L_1, L_2 and R^8 are as define in compounds of Formula (B-1) above.

Embodiment 8a. The compound of Formula (Bb) or Formula (Bb-1), or stereoisomers or a pharmaceutically acceptable salt thereof, having the structure of Formula (Bb-3), or a pharmaceutically acceptable salt thereof:



(Bb-3),

wherein:

R^0 is methyl or ethyl;

R^1, R^s, and R^t are each independently methyl, methylthiomethyl, or isopropyl;

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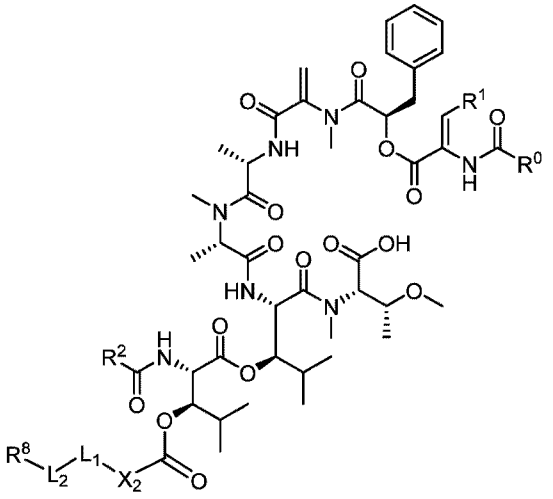
R^2 is methyl or ethyl, and

X_2, L_1, L_2 and R^8 are as define in compounds of Formula (Bb-1) above.

Embodiment 8b. The compound of Formula (Bc) or Formula (Bc-1), or stereoisomers or a pharmaceutically acceptable salt thereof, having the structure of Formula (Bc-3), or a pharmaceutically acceptable salt thereof:



(Bc-3),

wherein:

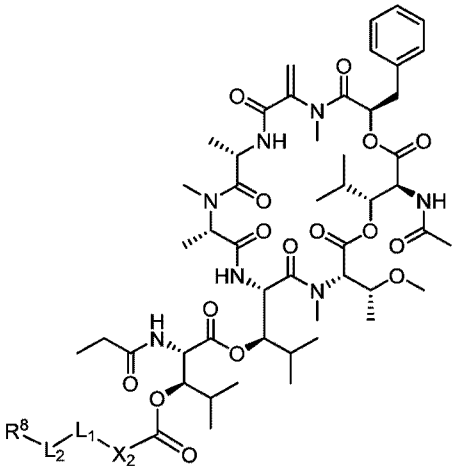
R^0 is methyl or ethyl;

R^1 is methyl or isopropyl;

R^2 is methyl or ethyl, and

X_2, L_1, L_2 and R^8 are as define in compounds of Formula (Bc-1) above.

Embodiment 9. The compound of Formula (B), Formula (B-1) or Formula (B-3), or stereoisomers or a pharmaceutically acceptable salt thereof, having the structure of Formula (B-3a), or a pharmaceutically acceptable salt thereof:



(B-3a),

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wherein:

X_2, L_1, L_2 and R^8 are as define in compounds of Formula (B-1) above.

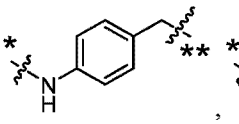
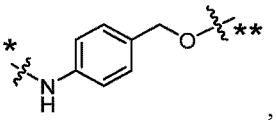
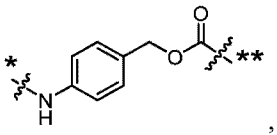
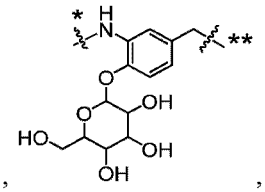
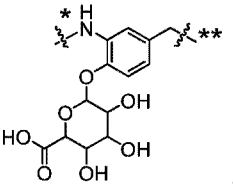
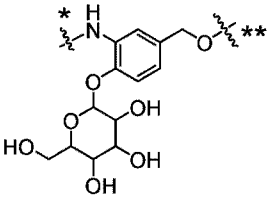
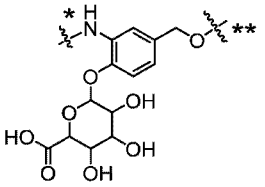
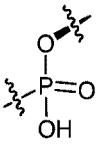
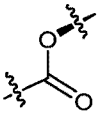
Embodiment 10. The compound of Formula (B-2) of Embodiment 6, Formula (B-3) of Embodiment 8, or a pharmaceutically acceptable salt thereof:

wherein:

R^0 is methyl or ethyl;

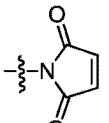
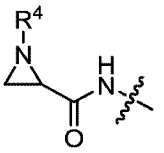
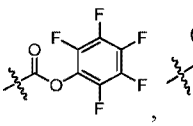
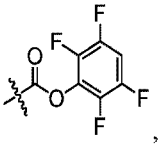
R^1 is methyl or isopropyl;

R^2 is methyl or ethyl;

X_2 is a self-immolative spacer selected from  ,  ,  ,  ,  ,  , and  , where the * of X_2 indicates the point of attachment to L_1 and the ** of X_2 indicates the point of attachment to the  group or the point of attachment to  group;

L_1 is a bivalent peptide linker comprising 2 to 4 amino acid residues;

L_2 is a linker,

R^8 is selected from  , $-N_3$, $-ONH_2$, $-NR^4C(=O)CH=CH_2$, SH, $-SSR^{13}$, $-S(=O)_2(CH=CH_2)$, $-NR^4S(=O)_2(CH=CH_2)$, $-NR^4C(=O)CH_2Br$, $-NR^4C(=O)CH_2I$, $-NHC(=O)CH_2Br$, $-NHC(=O)CH_2I$, $-C(=O)NHNH_2$,  , $-CO_2H$, $-NH_2$,  ,  ,

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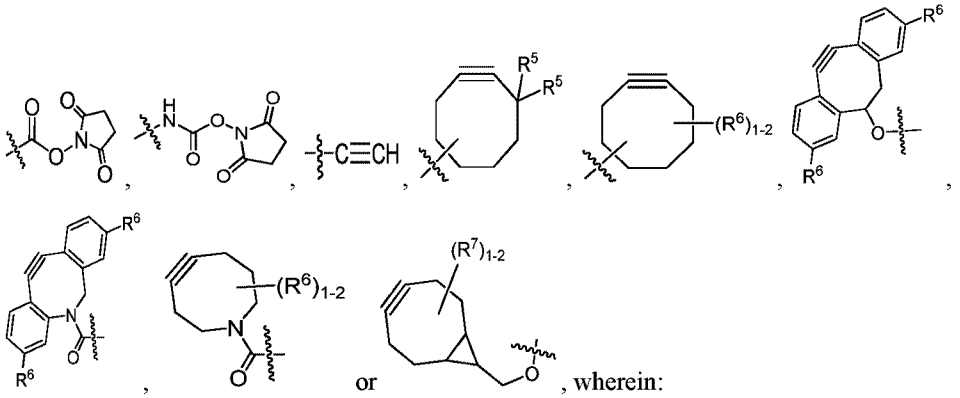


, wherein:

each R^4 is independently selected from H and C_1-C_6alkyl;

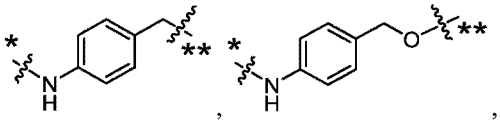
each R^5 is independently selected from H, C_1-C_6alkyl, F, Cl, and –OH;

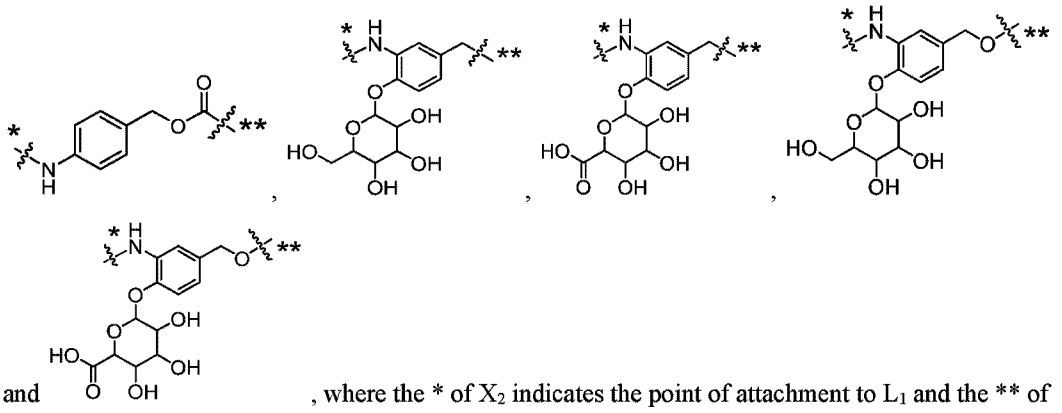
each R^6 is independently selected from H, C_1-C_6alkyl, F, Cl, $-NH_2$, $-OCH_3$, $-OCH_2CH_3$, $-N(CH_3)_2$, -CN, $-NO_2$ and –OH,

and

each R^7 is independently selected from H, C_{1-6}alkyl, fluoro, benzyloxy substituted with –C(=O)OH, benzyl substituted with –C(=O)OH, C_{1-4}alkoxy substituted with –C(=O)OH and C_{1-4}alkyl substituted with –C(=O)OH.

Embodiment 11. The compound of any one of Embodiments 1 to 10, wherein X_2 is a self-immolative spacer selected from





and

, where the * of X_2 indicates the point of attachment to L_1 and the ** of

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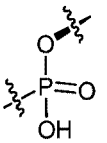
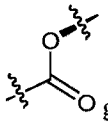
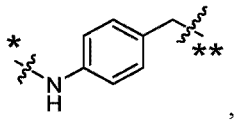
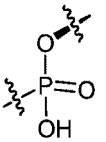
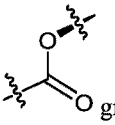
X_2 indicates the point of attachment to Y_1, the point of attachment to the  group or the point of attachment to  group.

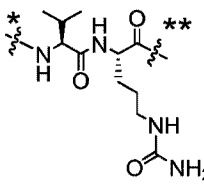
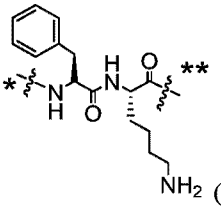
Embodiment 12. The compound of any one of Embodiments 1 to 11, wherein X_2 is  , where the * of X_2 indicates the point of attachment to L_1 and the ** of X_2 indicates the point of attachment to Y_1, the point of attachment to the  group or the point of attachment to  group.

Embodiment 13. The compound of any one of Embodiments 1 to 12, wherein L_1 is a bivalent peptide linker comprising 2 to 4 amino acid residues.

Embodiment 14. The compound of any one of Embodiments 1 to 12, wherein L_1 is a is a bivalent peptide linker comprising an amino acid residue selected from valine, citrulline, lysine, isoleucine, phenylalanine, methionine, asparagine, proline, alanine, leucine, tryptophan, and tyrosine.

Embodiment 15. The compound of any one of Embodiments 1 to 12, wherein L_1 is a bivalent peptide linker comprising at least one valine (Val) or citrulline (Cit) residue.

Embodiment 16. The compound of any one of Embodiments 1 to 12, wherein L_1 is a bivalent dipeptide linker selected from ValCit, PheLys, ValAla and ValLys.

Embodiment 17. The compound of any one of Embodiments 1 to 12, wherein L_1 is a bivalent dipeptide linker selected from  (ValCit),  (PheLys),

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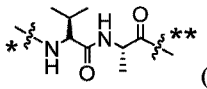
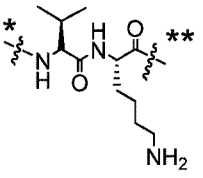
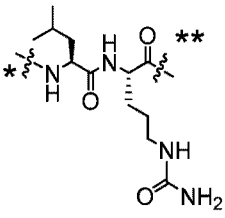
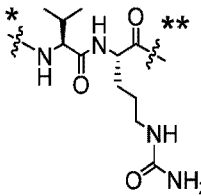

(ValAla), 
(ValLys) and 
(LeuCit), where the * of L_1 indicates the attachment point to L_2 and the ** of L_1 indicates the attachment point to X_2.

Embodiment 18. The compound of any one of Embodiments 1 to 12, wherein L_1 is ValCit.

Embodiment 19. The compound of any one of Embodiments 1 to 12, wherein

L_1 is 
(ValCit), where the * of L_1 indicates the attachment point to L_2 and the ** of L_1 indicates the attachment point to X_2.

Embodiment 20. The compound of any one of Embodiments 1 to 19, wherein L_2 is a linker.

Embodiment 21. The compound of any one of Embodiments 1 to 19, wherein L_2 is a linker selected from:

-*C(=O)((CH$_2$)$_m$O)$_p$(CH$_2$)$_m$**-, -*C(=O)(CH$_2$)$_m$**-, -*C(=O)(CH$_2$)$_n$NHC(=O)(CH$_2$)$_m$**-, -*C(=O)(CH$_2$)$_m$NHC(=O)((CH$_2$)$_m$O)$_p$(CH$_2$)$_m$**-, -* ((CH$_2$)$_m$O)$_p$(CH$_2$)$_m$**-, -* ((CH$_2$)$_m$O)$_p$(CH$_2$)$_m$**-, -(CH$_2$)$_m$-, -*(CH$_2$)$_m$NHC(=O)(CH$_2$)$_m$**-, -* (CH$_2$)$_m$NHC(=O)(CH$_2$)$_m$C(=O)NH(CH$_2$)$_m$**-, -* ((CH$_2$)$_m$O)$_p$(CH$_2$)$_m$NHC(=O)(CH$_2$)$_m$**-, -* *((CH$_2$)$_m$O)$_p$CH$_2$)$_m$C(=O)NH(CH$_2$)$_m$**-, -* (CH$_2$)$_m$C(R$_3$)$_2$**-, and -* (CH$_2$)$_m$C(R$_3$)$_2$SS(CH$_2$)$_m$NHC(=O)(CH$_2$)$_m$**-, where the * of L_2 indicates the attachment point to L_1 and the ** of L_2 indicates the point of attachment to R_8;

and wherein:

each R_3 is independently selected from H and C_1-C_6alkyl;

each m is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, and

each p is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14.

Embodiment 22. The compound of any one of Embodiments 1 to 19, wherein L_2 is -*C(=O)((CH$_2$)$_m$O)$_p$(CH$_2$)$_m$**- or -*C(=O)(CH$_2$)$_m$**-, where the * of L_2 indicates the point of attachment to L_1 and the ** of L_2 indicates the point of attachment to R_8,

and wherein each m is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 and p is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14.

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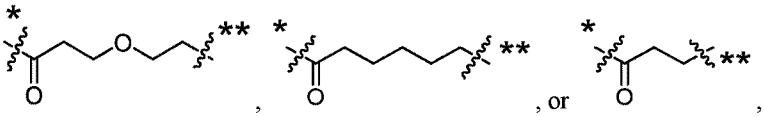
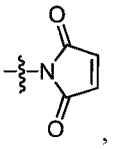
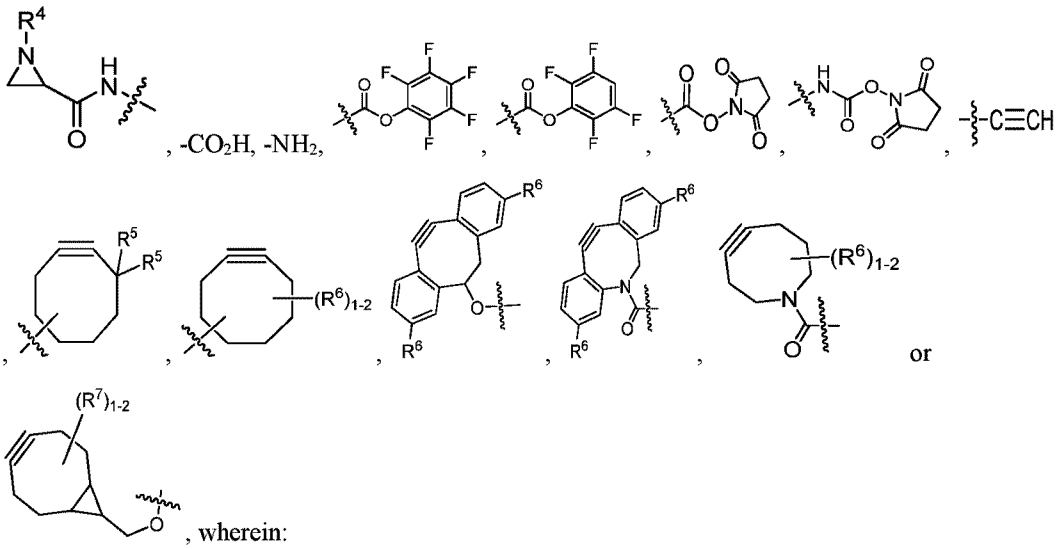
Embodiment 23. The compound of any one of Embodiments 1 to 19, wherein L_2 is



, where the * of L_2 indicates the point of attachment to L_1 and the ** of L_2 indicates the point of attachment to R_8.

Embodiment 24. The compound of any one of Embodiments 1 to 23, wherein R^8 is  ,

$-N_3$, $-ONH_2$, $-NR^4C(=O)CH=CH_2$, SH, $-SSR^{13}$, $-S(=O)_2(CH=CH_2)$, $-NR^4S(=O)_2(CH=CH_2)$, $-NR^4C(=O)CH_2Br$, $-NR^4C(=O)CH_2I$, $-NHC(=O)CH_2Br$, $-NHC(=O)CH_2I$, $-C(=O)NHNH_2$,



, wherein:

each R^4 is independently selected from H and C_1-C_6alkyl;

each R^5 is independently selected from H, C_1-C_6alkyl, F, Cl, and –OH;

each R^6 is independently selected from H, C_1-C_6alkyl, F, Cl, $-NH_2$, $-OCH_3$, $-OCH_2CH_3$, $-N(CH_3)_2$, -CN, $-NO_2$ and –OH,

and

each R^7 is independently selected from H, C_{1-6}alkyl, fluoro, benzyloxy substituted with –C(=O)OH, benzyl substituted with –C(=O)OH, C_{1-4}alkoxy substituted with –C(=O)OH and C_{1-4}alkyl substituted with –C(=O)OH.

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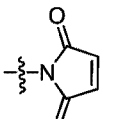
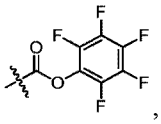
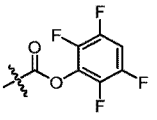
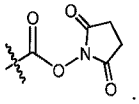
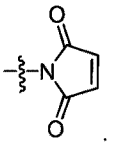
Embodiment 25. The compound of any one of Embodiments 1 to 23, wherein R^8 is  , -ONH_2,  ,  or  .

Embodiment 26. The compound of any one of Embodiments 1 to 23, wherein R^8 is  .

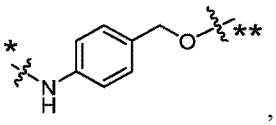
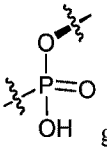
Embodiment 27. The compound of Formula (B-2) of Embodiment 6, or a pharmaceutically acceptable salt thereof:

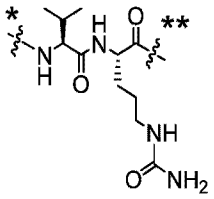
wherein:

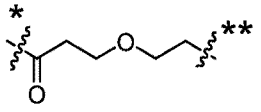
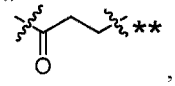
R^0 is methyl or ethyl;

R^1 is methyl or isopropyl;

R^2 is methyl or ethyl;

X_2 is  , where the * of X_2 indicates the point of attachment to L_1 and the ** of X_2 indicates the point of attachment to the  group;

L_1 is  (ValCit), where the * of L_1 indicates the attachment point to L_2 and the ** of L_1 indicates the attachment point to X_2;

L_2 is  or  , where the * of L_2 indicates the point of attachment to L_1 and the ** of L_2 indicates the point of attachment to R_8,

and

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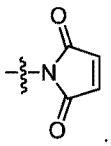
R^8 is  .

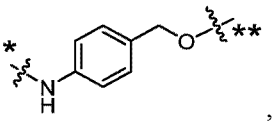
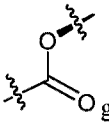
Embodiment 28. The compound of Formula (B-3) of Embodiment 8, or a pharmaceutically acceptable salt thereof:

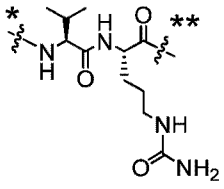
wherein:

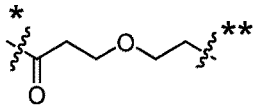
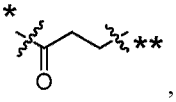
R^0 is methyl or ethyl;

R^1 is methyl or isopropyl;

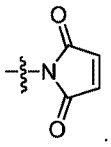
R^2 is methyl or ethyl;

X_2 is  , where the * of X_2 indicates the point of attachment to L_1 and the ** of X_2 indicates the point of attachment to the  group;

L_1 is  (ValCit), where the * of L_1 indicates the attachment point to L_2 and the ** of L_1 indicates the attachment point to X_2;

L_2 is  or  , where the * of L_2 indicates the point of attachment to L_1 and the ** of L_2 indicates the point of attachment to R_8,

and

R^8 is  .

Embodiment 29. The compound of Formula (B), Formula (B-1) or Formula (B-2), wherein the compound is

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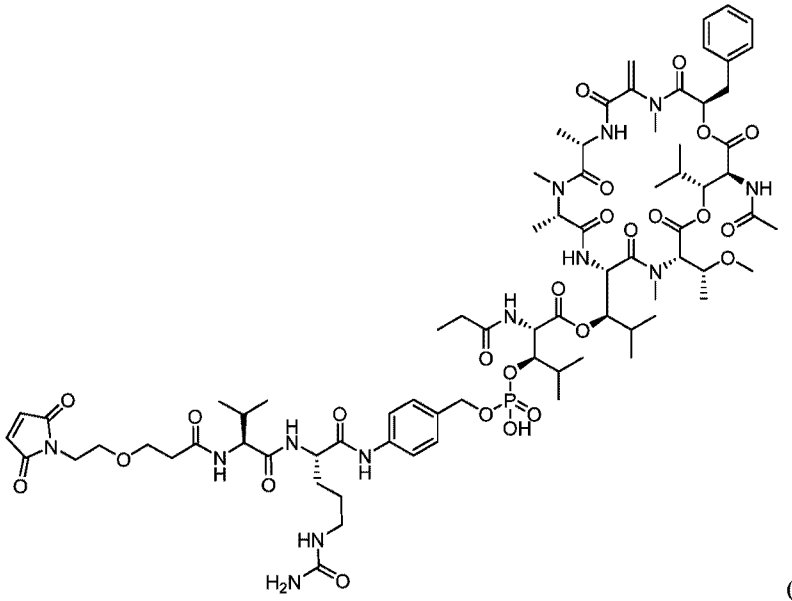
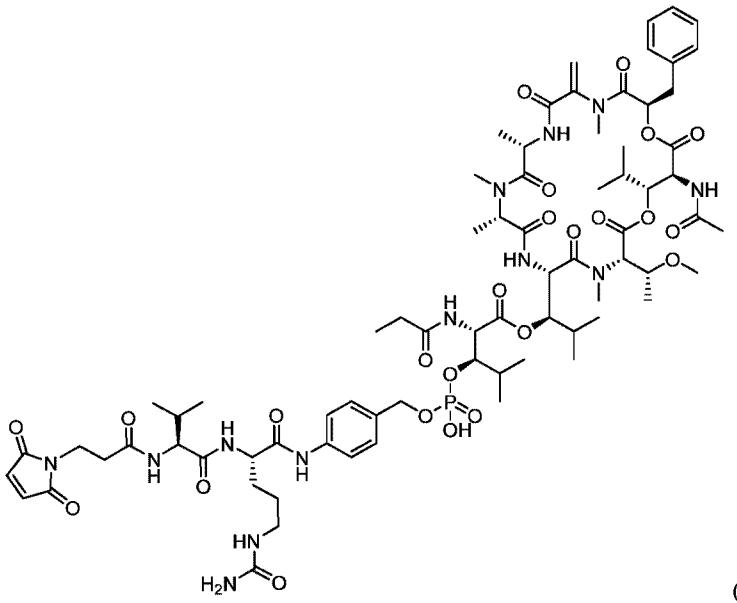


(B1).

Embodiment 30. The compound of Formula (B), Formula (B-1) or Formula (B-2), wherein the compound is



(B4).

Embodiment 31. The compound of Formula (B), Formula (B-1) or Formula (B-3), wherein the compound is

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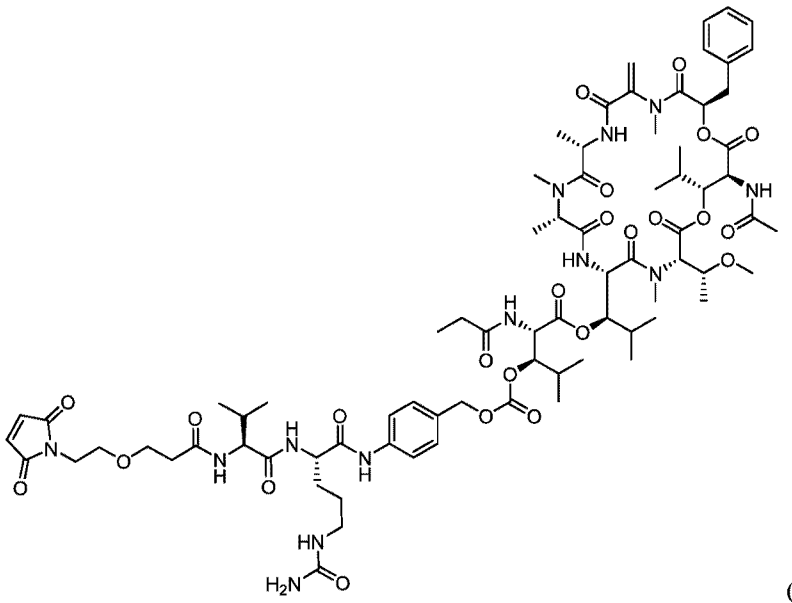
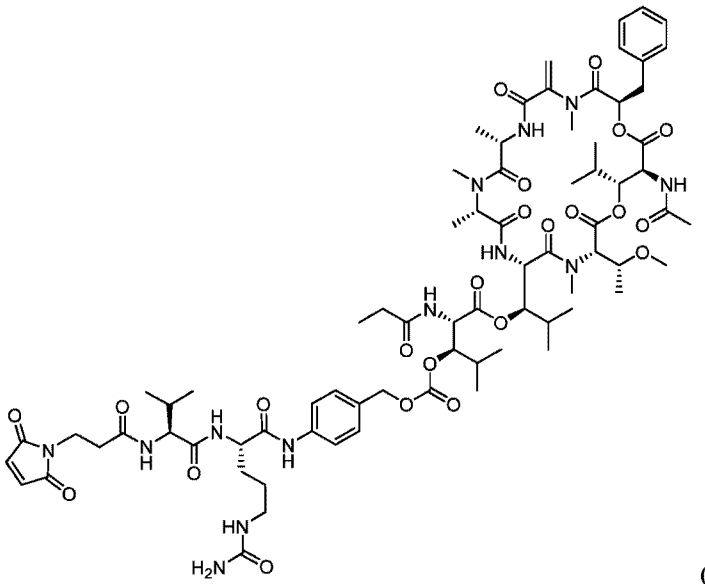


(B7).

Embodiment 32. The compound of Formula (B), Formula (B-1) or Formula (B-3), wherein the compound is



(B10).

Synthesis of exemplary Linker-Drug Compounds are also described in Example 1 of International Application Publication No. WO 2020/128612, which is incorporated by reference in its entirety.

Antibody Drug Conjugates

In some embodiments, the antibody drug conjugate of the disclosure is a conjugate of Formula (C):

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$$Ab\text{-}(L_A\text{-}(D)_n)_y \qquad (C)$$

wherein:

D is a drug moiety;

Ab is an antibody or antigen binding fragment thereof that binds to human PMEL17 protein;

L_A is a linker;

n is 1, 2, 3 or 4, and

y is 1, 2, 3 or 4,

where the Linker-Drug moiety $(L_A\text{-}(D)_n)$ is covalently attached to the antibody or antigen binding fragment thereof.

In some embodiments, the Antibody Drug Conjugate of the disclosure having the structure of Formula (C), wherein:

D is a GNAQ inhibitor, a GNA11 inhibitor or an inhibitor of GNAQ and GNA11;

Ab is an antibody or antigen binding fragment thereof that binds to human PMEL17 protein;

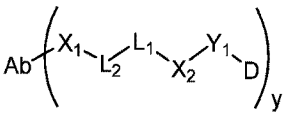
L_A is a cleavable linker comprising one or more linker components selected from a self-immolative spacer, a phosphate group, a carbonate group and a bivalent peptide linker;

n is 1, 2, 3 or 4, and

y is 1, 2, 3 or 4,

where the Linker-Drug moiety $(L_A\text{-}(D)_n)$ is covalently attached to the antibody or antigen binding fragment thereof.

In some embodiments, the Antibody Drug Conjugate of Formula (C) is a conjugate of Formula (C-1):



(C-1)

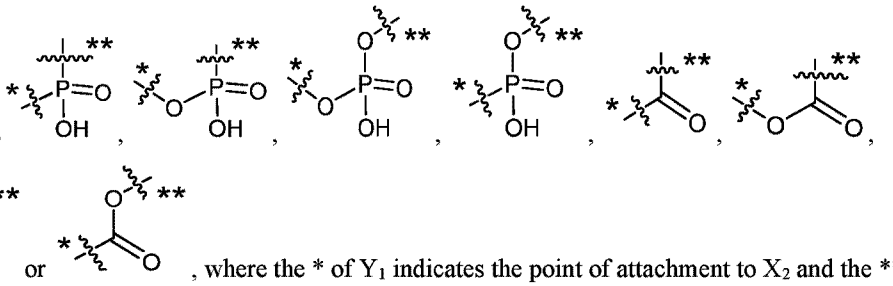
wherein:

D is a GNAQ inhibitor, a GNA11 inhibitor or an inhibitor of GNAQ and GNA11;

Ab is an antibody or antigen binding fragment thereof that binds to human PMEL17 protein;

X_1 is a bivalent coupling group;

X_2 is a self-immolative spacer;

Y_1 is  , where the * of Y_1 indicates the point of attachment to X_2 and the ** of Y_1 indicates the point of attachment to D;

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L_1 is a bivalent peptide linker;

L_2 is a bond or a linker, and

y is 1, 2, 3 or 4.

In the conjugates of Formula (C), one or more Linker-Drug moiety (L_B-$(D)_n$) can be covalently attached to the antibody or antigen binding fragment thereof, Ab, thereby covalently attaching one or more drug moieties, D, to the antibody or antigen binding fragment thereof, Ab, through linker, L_A. L_A is any chemical moiety that is capable of linking the antibody or antigen binding fragment thereof, Ab, to one or more drug moieties, D. The conjugates of Formula (C), wherein one or more drug moieties, D, are covalently linked to an antibody or antigen binding fragment thereof, Ab, can be formed using a bifunctional or multifunctional linker reagent having one or more reactive functional groups that are the same or different. One of the reactive functional groups of the bifunctional or multifunctional linker reagent is used to react with a group on the antibody or antigen binding fragment thereof, Ab, by way of example, a thiol or an amine (*e.g.* a cysteine, an N-terminus or amino acid side chain such as lysine) to form a covalent linkage with one end of the linker L_A. Such reactive functional groups of the bifunctional or multifunctional linker reagent include, but are not limited to, a maleimide, a thiol and an NHS ester. The other reactive functional group or groups of the bifunctional or multifunctional linker reagent are used to covalently attached one or more drug moieties, D, to linker L_A.

In some embodiments, L_A is a cleavable linker. In some embodiments, L_A is a non-cleavable linker. In some embodiments, L_A is an acid-labile linker, photo-labile linker, peptidase cleavable linker, esterase cleavable linker, glycosidase cleavable linker, phosphodiesterase cleavable linker, a disulfide bond reducible linker, a hydrophilic linker, or a dicarboxylic acid-based linker.

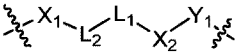
In some embodiments, L_A is a cleavable linker comprising one or more linker components selected from a self-immolative spacer, a phosphate group, a carbonate group and a bivalent peptide linker.

In some embodiments, L_A is a cleavable linker comprising one or more linker components selected from a self-immolative spacer, a phosphate group, a carbonate group, a bivalent peptide linker and a bivalent coupling group.

In some embodiments, L_A is a cleavable linker comprising one or more linker components selected from a self-immolative spacer, a phosphate group and a bivalent peptide linker.

In some embodiments, L_A is a cleavable linker comprising one or more linker components selected from a self-immolative spacer, a phosphate group, a bivalent peptide linker and a bivalent coupling group.

In some embodiments, the linker (L_A) has the following formula:



wherein:

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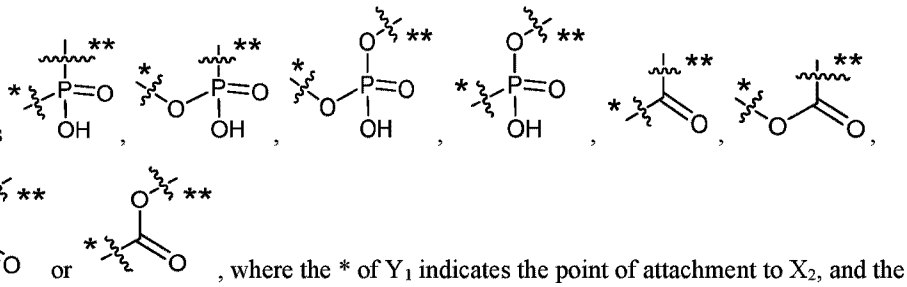
X_1 is a bivalent coupling group;

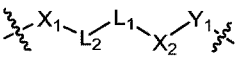
X_2 is a self-immolative spacer;

Y_1 is  , where the * of Y_1 indicates the point of attachment to X_2, and the ** of Y_1 indicates the other point of attachment;

L_1 is a bivalent peptide linker, and

L_2 is a bond or a linker.

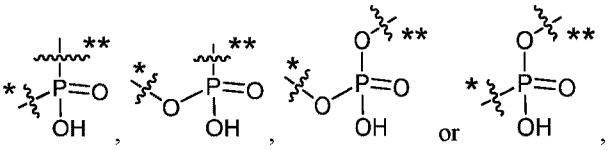
In some embodiments, the linker (L_A) has the following formula:



wherein:

X_1 is a bivalent coupling group;

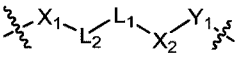
X_2 is a self-immolative spacer;

Y_1 is  , where the * of Y_1 indicates the point of attachment to X_2;

L_1 is a bivalent peptide linker, and

L_2 is a bond or a linker.

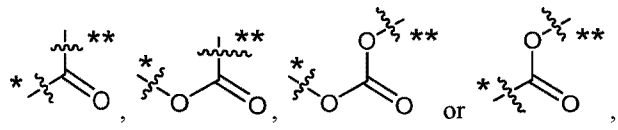
In some embodiments, the linker (L_A) has the following formula:



wherein:

X_1 is a bivalent coupling group;

X_2 is a self-immolative spacer;

Y_1 is  , where the * of Y_1 indicates the point of attachment to X_2;

L_1 is a bivalent peptide linker, and

L_2 is a bond or a linker.

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While the drug to antibody ratio has an exact integer value for a specific conjugate molecule (*e.g.*, the product of n and y in Formula (C), it is understood that the value will often be an average value when used to describe a sample containing many molecules, due to some degree of heterogeneity, typically associated with the conjugation step. The average loading for a sample of a conjugate is referred to herein as the drug to antibody ratio, or "DAR." In some embodiments, the DAR is between about 1 and about 5, and typically is about 1, 2, 3, or 4. In some embodiments, at least 50% of a sample by weight is compound having the average DAR plus or minus 2, and preferably at least 50% of the sample is a conjugate that contains the average DAR plus or minus 1. Other embodiments include conjugates wherein the DAR is about 2. In some embodiments, a DAR of 'about y' means the measured value for DAR is within 20% of the product of n and y in Formula (I). In some embodiments, a DAR of 'about n' means the measured value for DAR is within 20% of n in Formula (II).

In some embodiments, the average molar ratio of the drug to the antibody in the conjugates of Formula (C) (*i.e.*, average value of the product of n and y, also known as drug to antibody ratio (DAR)) is about 1 to about 10, about 1 to about 6 (*e.g.*, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, or 6.0), about 1 to about 5, about 1.5 to about 4.5, or about 2 to about 4.

In some embodiments provided by the disclosure, the conjugate has substantially high purity and has one or more of the following features: (a) greater than about 90% (*e.g.*, greater than or equal to about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%), preferably greater than about 95%, of conjugate species are monomeric, (b) unconjugated linker level in the conjugate preparation is less than about 10% (*e.g.*, less than or equal to about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, about 2%, about 1%, or about 0%) (relative to total linker), (c) less than 10% of conjugate species are crosslinked (*e.g.*, less than or equal to about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, about 2%, about 1%, or about 0%), (d) free drug (ADP-induced platelet aggregation inhibitor, *e.g.*, a GNAQ inhibitor, a GNA11 inhibitor, or a GNAQ and a GNA11 inhibitor) level in the conjugate preparation is less than about 2% (*e.g.*, less than or equal to about 1.5%, about 1.4%, about 1.3%, about 1.2%, about 1.1%, about 1.0%, about 0.9%, about 0.8%, about 0.7%, about 0.6%, about 0.5%, about 0.4%, about 0.3%, about 0.2%, about 0.1%, or about 0%) (mol/mol relative to total drug).

Certain embodiments and examples of the Antibody Drug Conjugates of the disclosure are provided in the following listing of additional, enumerated embodiments. It will be recognized that features specified in each embodiment may be combined with other specified features to provide further embodiments of the present disclosure.

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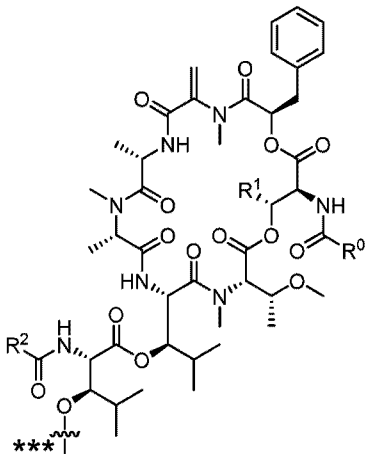
Embodiment 33. The conjugate of Formula (C) or Formula (C-1), wherein D is a GNAQ inhibitor.

Embodiment 34. The conjugate of Formula (C) or Formula (C-1), wherein D is a GNA11 inhibitor.

Embodiment 35. The conjugate of Formula (C) or Formula (C-1), wherein D is an inhibitor of GNAQ and GNA11.

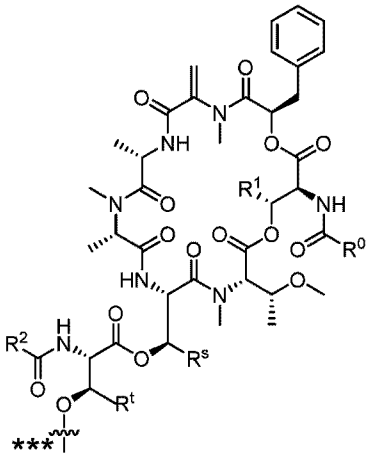
Embodiment 36. The conjugate of Formula (C) or Formula (C-1), wherein D is



,

wherein R^0 is methyl or ethyl, R_1 is methyl or isopropyl, R_2 is methyl or ethyl, and the *** indicates the point of attachment to L_A or Y_1.

Embodiment 36a. The conjugate of Formula (Cb) or Formula (Cb-1), wherein D is



,

wherein R^0 is methyl or ethyl, R^1, R^s, and R^t are each independently methyl, methylthiomethyl, or isopropyl, R_2 is methyl or ethyl, and the *** indicates the point of attachment to L_A or Y_1.

Embodiment 36b. The conjugate of Formula (Cc) or Formula (Cc-1), wherein D is

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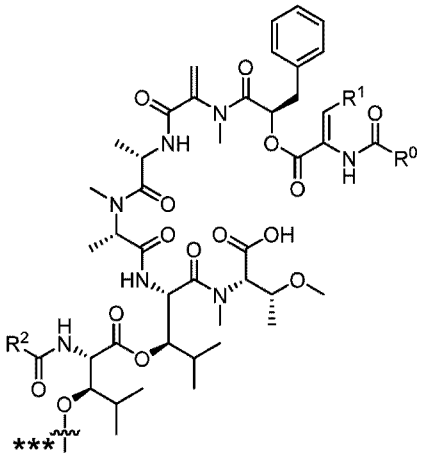
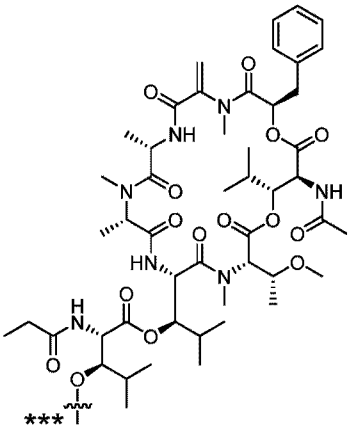


,

wherein R^0 is methyl or ethyl, R_1 is methyl or isopropyl, R_2 is methyl or ethyl, and the *** indicates the point of attachment to L_A or Y_1.

Embodiment 37. The conjugate of Formula (C) or Formula (C-1), wherein D is



,

where the *** indicates the point of attachment to L_A or Y_1.

Embodiment 38. The conjugate of Formula (C) or Formula (C-1), having the structure of Formula (C-2):

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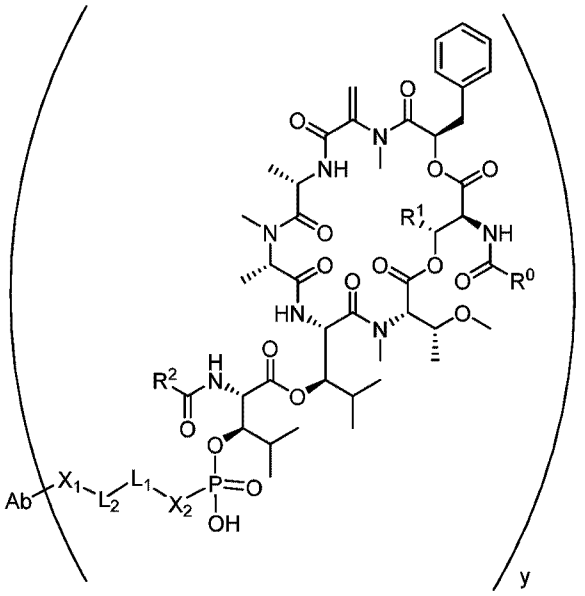

(C-2),

wherein:

R^0 is methyl or ethyl;

R^1 is methyl or isopropyl;

R^2 is methyl or ethyl;

Ab is an antibody or antigen binding fragment thereof that binds to human PMEL17 protein;

X_1 is a bivalent coupling group;

X_2 is a self-immolative spacer;

L_1 is a bivalent peptide linker;

L_2 is a bond or a linker, and

y is 1, 2, 3 or 4.

Embodiment 38a. The conjugate of Formula (Cb) or Formula (Cb-1), having the structure of Formula (Cb-2):

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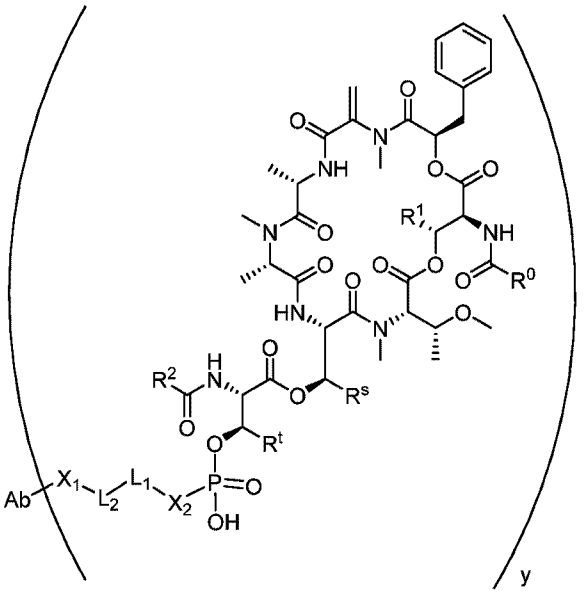

(Cb-2),

wherein:

R^0 is methyl or ethyl;

R^1, R^s, and R^t are each independently methyl, methylthiomethyl, or isopropyl;

R^2 is methyl or ethyl;

Ab is an antibody or antigen binding fragment thereof that binds to human PMEL17 protein;

X_1 is a bivalent coupling group;

X_2 is a self-immolative spacer;

L_1 is a bivalent peptide linker;

L_2 is a bond or a linker, and

y is 1, 2, 3 or 4.

Embodiment 38b. The conjugate of Formula (Cc) or Formula (Cc-1), having the structure of Formula (Cc-2):

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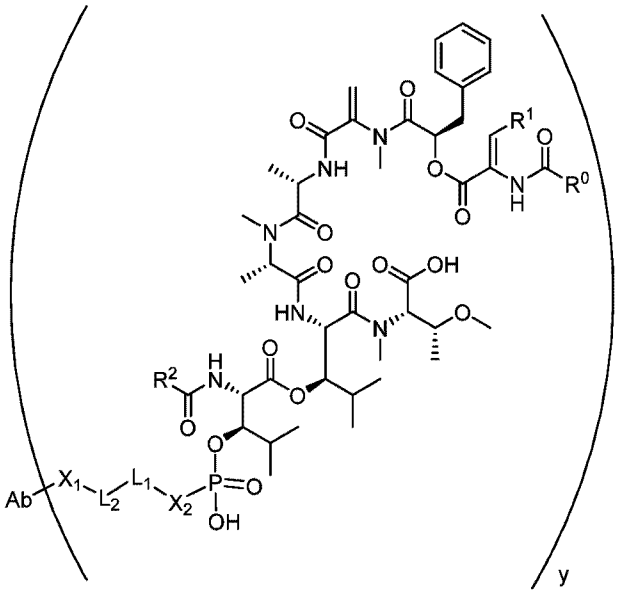

(Cc-2),

wherein:

R^0 is methyl or ethyl;

R^1 is methyl or isopropyl;

R^2 is methyl or ethyl;

Ab is an antibody or antigen binding fragment thereof that binds to human PMEL17 protein;

X_1 is a bivalent coupling group;

X_2 is a self-immolative spacer;

L_1 is a bivalent peptide linker;

L_2 is a bond or a linker, and

y is 1, 2, 3 or 4.

Embodiment 39. The conjugate of Formula (C) or Formula (C-1), having the structure of Formula (C-2a):

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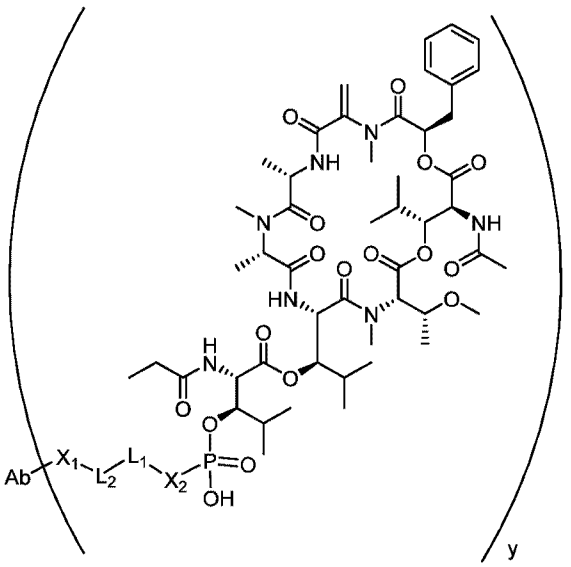


(C-2a),

wherein:

Ab is an antibody or antigen binding fragment thereof that binds to human PMEL17 protein;

X_1 is a bivalent coupling group;

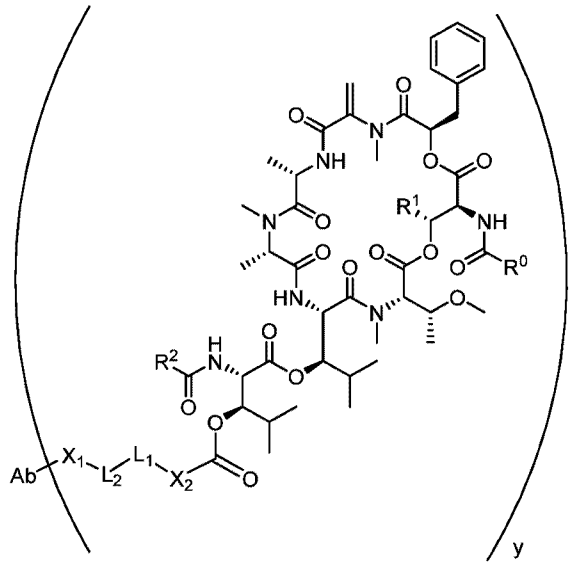
X_2 is a self-immolative spacer;

L_1 is a bivalent peptide linker;

L_2 is a bond or a linker, and

y is 1, 2, 3 or 4.

Embodiment 40. The conjugate of Formula (C) or Formula (C-1), having the structure of Formula (C-3):



(C-3),

wherein:

R^0 is methyl or ethyl;

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R^1 is methyl or isopropyl;

R^2 is methyl or ethyl;

Ab is an antibody or antigen binding fragment thereof that binds to human PMEL17 protein;

X_1 is a bivalent coupling group;

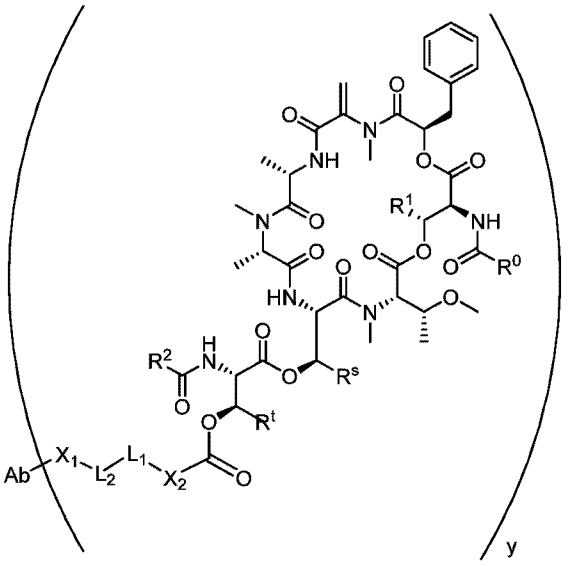
X_2 is a self-immolative spacer;

L_1 is a bivalent peptide linker;

L_2 is a bond or a linker, and

y is 1, 2, 3 or 4.

Embodiment 40a. The conjugate of Formula (Cb) or Formula (Cb-1), having the structure of Formula (Cb-3):



(Cb-3),

wherein:

R^0 is methyl or ethyl;

R^1, R^s, and R^t are each independently methyl, methylthiomethyl, or isopropyl;

R^2 is methyl or ethyl;

Ab is an antibody or antigen binding fragment thereof that binds to human PMEL17 protein;

X_1 is a bivalent coupling group;

X_2 is a self-immolative spacer;

L_1 is a bivalent peptide linker;

L_2 is a bond or a linker, and

y is 1, 2, 3 or 4.

Embodiment 40b. The conjugate of Formula (Cc) or Formula (Cc-1), having the structure of Formula (Cc-3):

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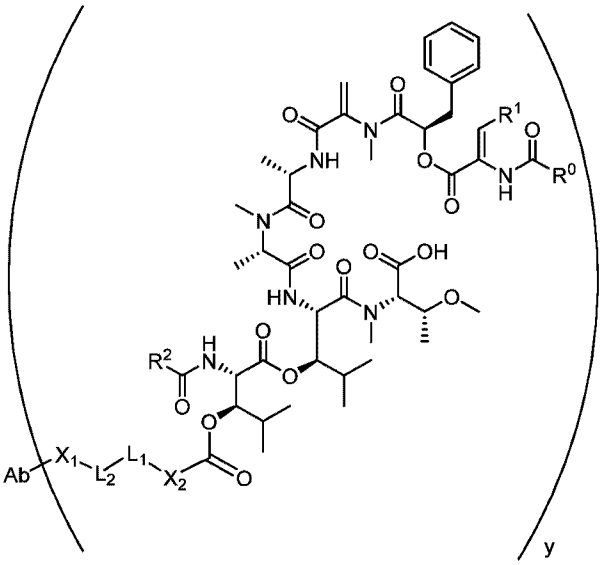

(Cc-3),

wherein:

R^0 is methyl or ethyl;

R^1 is methyl or isopropyl;

R^2 is methyl or ethyl;

Ab is an antibody or antigen binding fragment thereof that binds to human PMEL17 protein;

X_1 is a bivalent coupling group;

X_2 is a self-immolative spacer;

L_1 is a bivalent peptide linker;

L_2 is a bond or a linker, and

y is 1, 2, 3 or 4.

Embodiment 41. The conjugate of Formula (C) or Formula (C-1), having the structure of Formula (C-3a):

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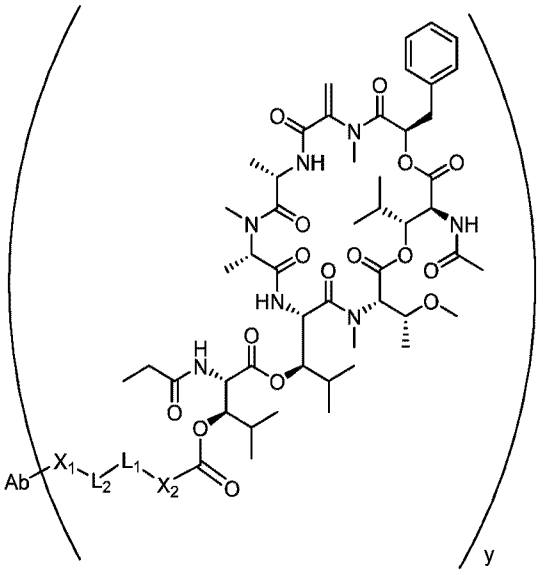


(C-3a),

wherein:

Ab is an antibody or antigen binding fragment thereof that binds to human PMEL17 protein;

X_1 is a bivalent coupling group;

X_2 is a self-immolative spacer;

L_1 is a bivalent peptide linker;

L_2 is a bond or a linker, and

y is 1, 2, 3 or 4.

Embodiment 42. The conjugate of Formula (C-2) of Embodiment 54 or the conjugate of Formula (C-3) of Embodiment 58:

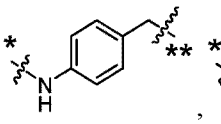
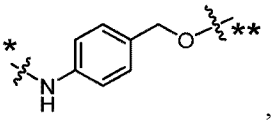
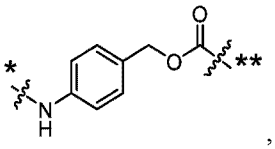
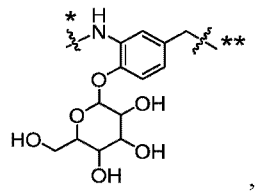
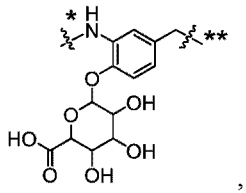
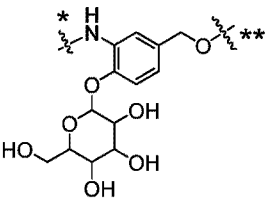
wherein:

R^0 is methyl or ethyl;

R^1 is methyl or isopropyl;

R^2 is methyl or ethyl;

Ab is an antibody or antigen binding fragment thereof that binds to human PMEL17 protein;

X_2 is a self-immolative spacer selected from  ,  ,  ,  ,  ,  .

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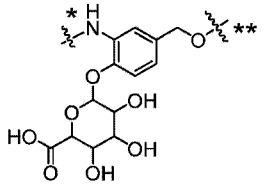
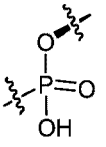
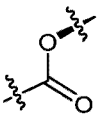
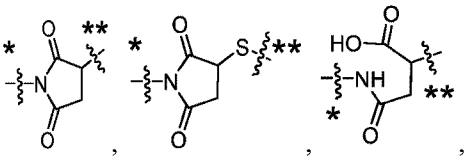
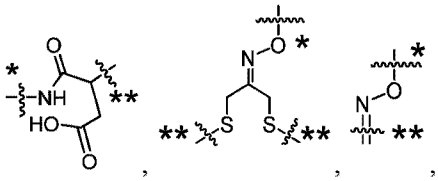
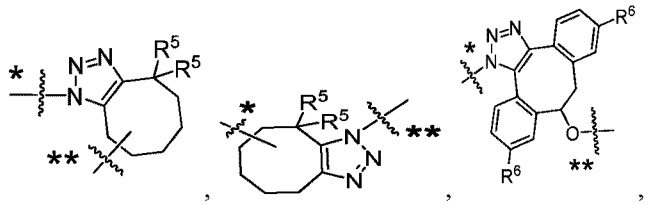
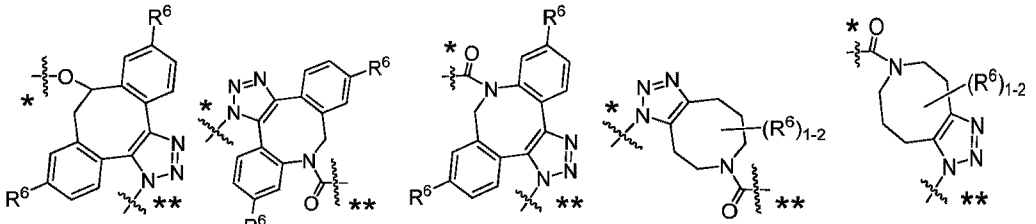


and , where the * of X_2 indicates the point of attachment to L_1 and the ** of X_2 indicates the point of attachment to the  group or the point of attachment to  group;

L_1 is a bivalent peptide linker comprising 2 to 4 amino acid residues;

L_2 is a linker;

X_1 is a bivalent coupling group selected from  ,  , -*$NR^4C(=O)CH_2$**-, -*NHC(=O)CH_2**-, -*$S(=O)_2CH_2CH_2$**-, -* $(CH_2)_2S(=O)_2CH_2CH_2$**-, -*$NR^4S(=O)_2CH_2CH_2$**-, -*$NR^4C(=O)CH_2CH_2$**-, -NH-, -C(=O)-, -*NHC(=O) **-, -*$CH_2NHCH_2CH_2$**-, -*$NHCH_2CH_2$**-, -S-,  ,  , **-, where the * of X_1 indicates the point of attachment to L_2 and the ** of X_1 indicates the point of attachment to Ab;

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and wherein:

each R^4 is independently selected from H and C_1-C_6alkyl;

each R^5 is independently selected from H, C_1-C_6alkyl, F, Cl, and –OH;

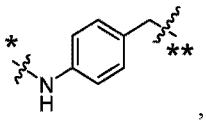
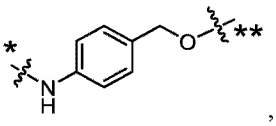
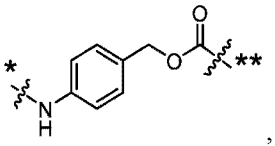
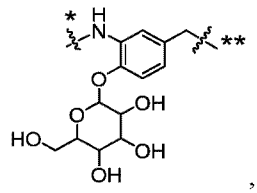
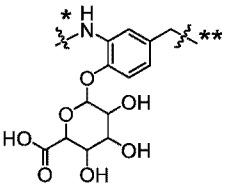
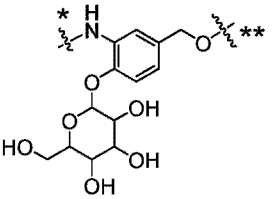
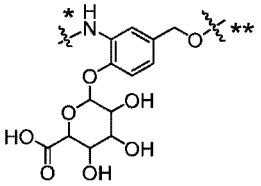
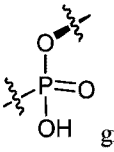
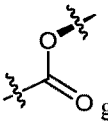
each R^6 is independently selected from H, C_1-C_6alkyl, F, Cl, -NH_2, -OCH_3, -OCH_2CH_3, -$N(CH_3)_2$, -CN, -NO_2 and –OH,

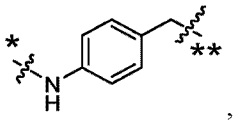
and

each R^7 is independently selected from H, C_{1-6}alkyl, fluoro, benzyloxy substituted with –C(=O)OH, benzyl substituted with –C(=O)OH, C_{1-4}alkoxy substituted with –C(=O)OH and C_{1-4}alkyl substituted with –C(=O)OH,

and

y is 1, 2, 3 or 4.

Embodiment 43. The conjugate of any one of Embodiments 38 to 42, wherein X_2 is a self-immolative spacer selected from  ,  ,  ,  ,  ,  , and  , where the * of X_2 indicates the point of attachment to L_1 and the ** of X_2 indicates the point of attachment to Y_1 or the point of attachment to the  group or the point of attachment to  group.

Embodiment 44. The conjugate of any one of Embodiments 38 to 43, wherein X_2 is  , where the * of X_2 indicates the point of attachment to L_1 and the ** of X_2

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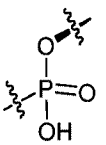
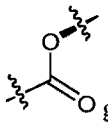
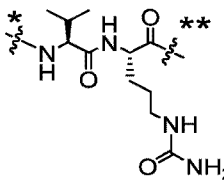
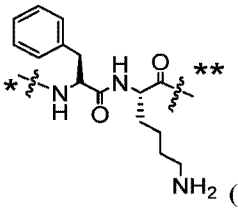
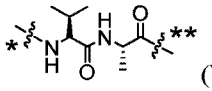
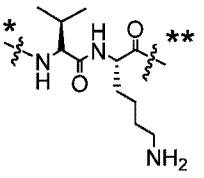
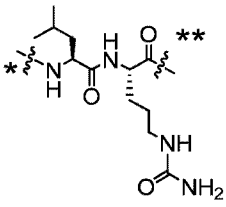
indicates the point of attachment to Y_1 or the point of attachment to the  group or the point of attachment to  group.

Embodiment 45. The conjugate of any one of Embodiments 38 to 44, wherein L_1 is a bivalent peptide linker comprising 2 to 4 amino acid residues.

Embodiment 46. The conjugate of any one of Embodiments 38 to 45, wherein L_1 is abivalent peptide linker comprising an amino acid residue selected from valine, citrulline, lysine, isoleucine, phenylalanine, methionine, asparagine, proline, alanine, leucine, tryptophan, and tyrosine.

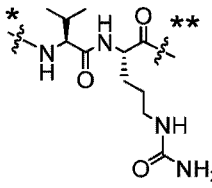
Embodiment 47. The conjugate of any one of Embodiments 38-44, wherein L_1 is a bivalent peptide linker comprising at least one valine (Val) or citrulline (Cit) residue.

Embodiment 48. The conjugate of any one of Embodiments 38 to 44, wherein L_1 is a bivalent dipeptide linker selected from ValCit, PheLys, ValAla and ValLys.

Embodiment 49. The conjugate of any one of Embodiments 38 to 44, wherein L_1 is a bivalent dipeptide linker selected from  (ValCit),  (PheLys),  (ValAla),  (ValLys) and  (LeuCit), where the * of L_1 indicates the attachment point to L_2 and the ** of L_1 indicates the attachment point to X_2.

Embodiment 50. The conjugate of any one of Embodiments 38 to 44, wherein L_1 is ValCit.

Embodiment 51. The conjugate of any one of Embodiments 38 to 44, wherein

L_1 is  (ValCit), where the * of L_1 indicates the attachment point to L_2 and the ** of L_1 indicates the attachment point to X_2.

Embodiment 52. The conjugate of any one of Embodiments 38 to 51, wherein L_2 is a linker.

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Embodiment 53. The conjugate of any one of Embodiments 38 to 51, wherein L_2 is a linker selected from:

-*C(=O)((CH$_2$)$_m$O)$_p$(CH$_2$)$_m$**-, -*C(=O)(CH$_2$)$_m$**-, -*C(=O)(CH$_2$)$_n$NHC(=O)(CH$_2$)$_m$**-, -*C(=O)(CH$_2$)$_m$NHC(=O)((CH$_2$)$_m$O)$_p$(CH$_2$)$_m$**-, -* ((CH$_2$)$_m$O)$_p$(CH$_2$)$_m$**-, -* ((CH$_2$)$_m$O)$_p$(CH$_2$)$_m$**-, -(CH$_2$)$_m$-, -*(CH$_2$)$_m$NHC(=O)(CH$_2$)$_m$**-, -* (CH$_2$)$_m$NHC(=O)(CH$_2$)$_m$C(=O)NH(CH$_2$)$_m$**-, -* ((CH$_2$)$_m$O)$_p$(CH$_2$)$_m$NHC(=O)(CH$_2$)$_m$**-, -* *((CH$_2$)$_m$O)$_p$CH$_2$)$_m$C(=O)NH(CH$_2$)$_m$**-, -* (CH$_2$)$_m$C(R$_3$)$_2$**-, and -* (CH$_2$)$_m$C(R$_3$)$_2$SS(CH$_2$)$_m$NHC(=O)(CH$_2$)$_m$**-, where the * of L_2 indicates the attachment point to L_1 and the ** of L_2 indicates the point of attachment to X_1;

and wherein:

each R_3 is independently selected from H and C_1-C_6alkyl;

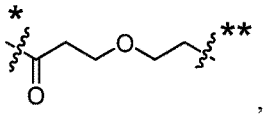
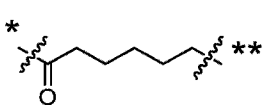
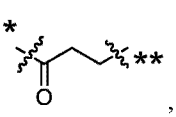
each m is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, and

each p is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14.

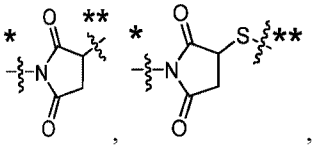
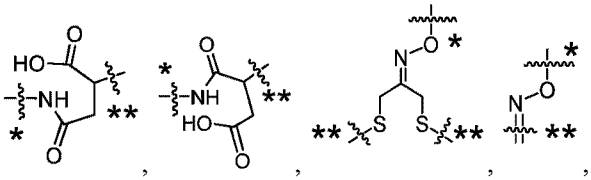
Embodiment 54. The conjugate of any one of Embodiments 38 to 51, wherein L_2 is -*C(=O)((CH$_2$)$_m$O)$_p$(CH$_2$)$_m$**- or -*C(=O)(CH$_2$)$_m$**-, where the * of L_2 indicates the point of attachment to L_1 and the ** of L_2 indicates the point of attachment to X_1,

and wherein each m is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 and p is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14.

Embodiment 55. The conjugate of any one of Embodiments 38 to 51, wherein L_2 is


, 
or 
, where the * of L_2 indicates the point of attachment to L_1 and the ** of L_2 indicates the point of attachment to X_1.

Embodiment 56. The conjugate of any one of Embodiments 38 to 55,

wherein X_1 is a bivalent coupling group selected from 
, 
, -*NR^{4}C(=O)CH$_2$**-, -*NHC(=O)CH$_2$**-, -*S(=O)$_2$CH$_2$CH$_2$**-, -* (CH$_2$)$_2$S(=O)$_2$CH$_2$CH$_2$**-, -*NR^{4}S(=O)$_2$CH$_2$CH$_2$**-, -*NR^{4}C(=O)CH$_2$CH$_2$**-, -NH-, -C(=O)-, -*NHC(=O) **-, -

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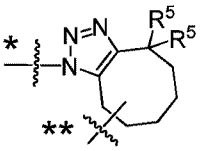
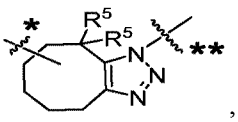
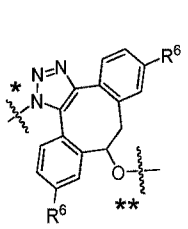
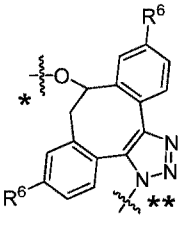
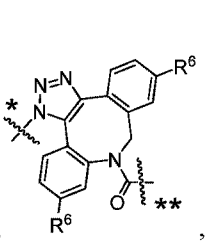
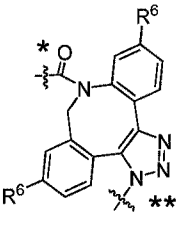
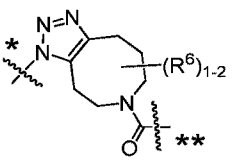
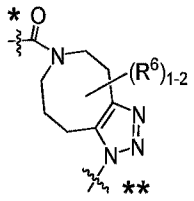
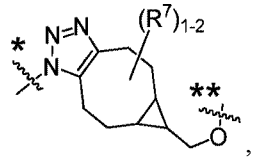
*$CH_2NHCH_2CH_2$**-, -*$NHCH_2CH_2$**-, -S-,  ,  ,  ,  ,  ,  ,  ,  ,  , **-, where the * of X_1 indicates the point of attachment to L_2 and the ** of X_1 indicates the point of attachment to Ab;

and wherein:

each R^4 is independently selected from H and C_1-C_6alkyl;

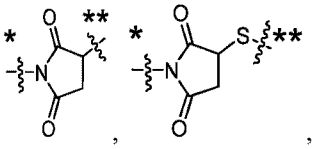
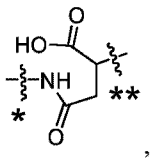
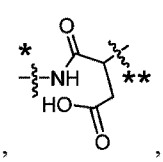
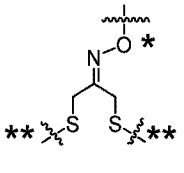
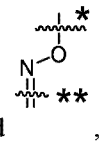
each R^5 is independently selected from H, C_1-C_6alkyl, F, Cl, and –OH;

each R^6 is independently selected from H, C_1-C_6alkyl, F, Cl, $-NH_2$, $-OCH_3$, $-OCH_2CH_3$, $-N(CH_3)_2$, -CN, $-NO_2$ and –OH,

and

each R^7 is independently selected from H, C_{1-6}alkyl, fluoro, benzyloxy substituted with –C(=O)OH, benzyl substituted with –C(=O)OH, C_{1-4}alkoxy substituted with –C(=O)OH and C_{1-4}alkyl substituted with –C(=O)OH.

Embodiment 57. The conjugate of any one of Embodiments 38 to 55,

wherein X_1 is a bivalent coupling group selected from  ,  ,  ,  and  , where the * of X_1 indicates the point of attachment to L_2 and the ** of X_1 indicates the point of attachment to Ab.

Embodiment 58. The conjugate of any one of Embodiments 38 to 55,

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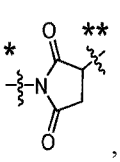
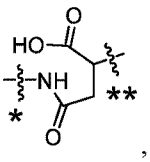
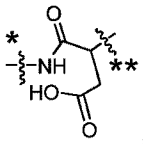
wherein X_1 is a bivalent coupling group selected from  ,  , and  , where the * of X_1 indicates the point of attachment to L_2 and the ** of X_1 indicates the point of attachment to Ab.

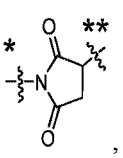
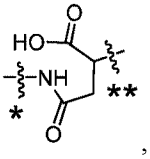
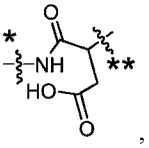
Embodiment 59. The conjugate of Formula (C-2) of Embodiment 38 wherein:

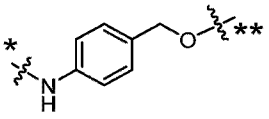
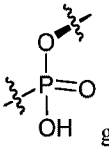
Ab is an antibody or antigen binding fragment thereof that binds to human PMEL17 protein;

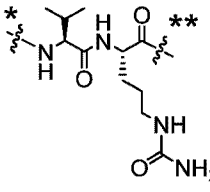
R^0 is methyl or ethyl;

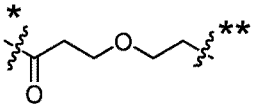
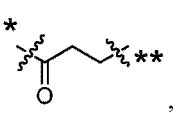
R^1 is methyl or isopropyl;

R^2 is methyl or ethyl;

X_1 is a bivalent coupling group selected from  ,  , and  , where the * of X_1 indicates the point of attachment to L_2 and the ** of X_1 indicates the point of attachment to Ab

X_2 is  , where the * of X_2 indicates the point of attachment to L_1 and the ** of X_2 indicates the point of attachment to the  group;

L_1 is  (ValCit), where the * of L_1 indicates the attachment point to L_2 and the ** of L_1 indicates the attachment point to X_2;

L_2 is  or  , where the * of L_2 indicates the point of attachment to L_1 and the ** of L_2 indicates the point of attachment to R_8,

and

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y is 1, 2, 3 or 4.

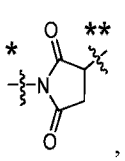
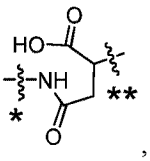
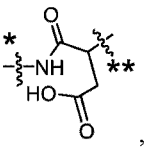
Embodiment 60. The conjugate of Formula (C-3) of Embodiment 40 wherein:

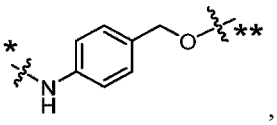
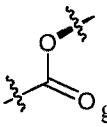
Ab is an antibody or antigen binding fragment thereof that binds to human PMEL17 protein;

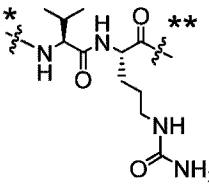
R^0 is methyl or ethyl;

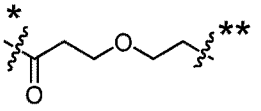
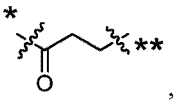
R^1 is methyl or isopropyl;

R^2 is methyl or ethyl;

X_1 is a bivalent coupling group selected from  ,  , and  , where the * of X_1 indicates the point of attachment to L_2 and the ** of X_1 indicates the point of attachment to Ab

X_2 is  , where the * of X_2 indicates the point of attachment to L_1 and the ** of X_2 indicates the point of attachment to the  group;

L_1 is  (ValCit), where the * of L_1 indicates the attachment point to L_2 and the ** of L_1 indicates the attachment point to X_2;

L_2 is  or  , where the * of L_2 indicates the point of attachment to L_1 and the ** of L_2 indicates the point of attachment to R_8,

and

y is 1, 2, 3 or 4.

Embodiment 61. The conjugate of Formula (C), Formula (C-1) or Formula (C-2) having the structure:

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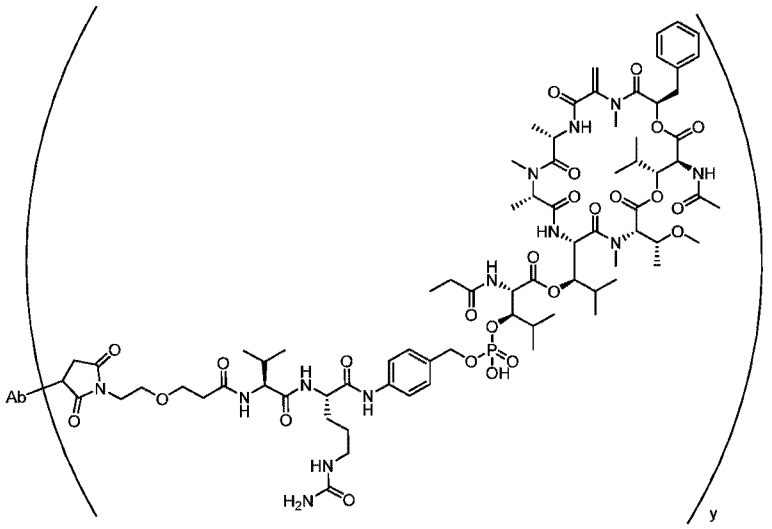
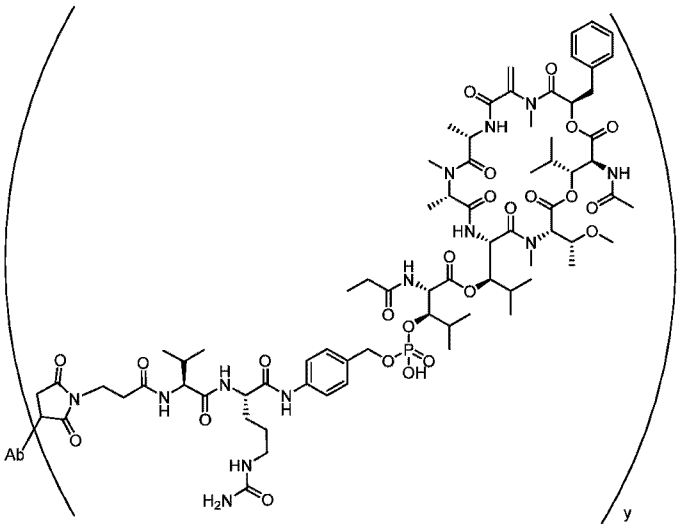


wherein:

y is 2 or 4 and

Ab is an antibody or antigen binding fragment thereof that binds to human PMEL17 protein.

Embodiment 62. The conjugate of Formula (C), Formula (C-1) or Formula (C-2) having the structure:



wherein:

y is 2 or 4 and

Ab is an antibody or antigen binding fragment thereof that binds to human PMEL17 protein.

Embodiment 63. The conjugate of Formula (C), Formula (C-1) or Formula (C-3) having the structure:

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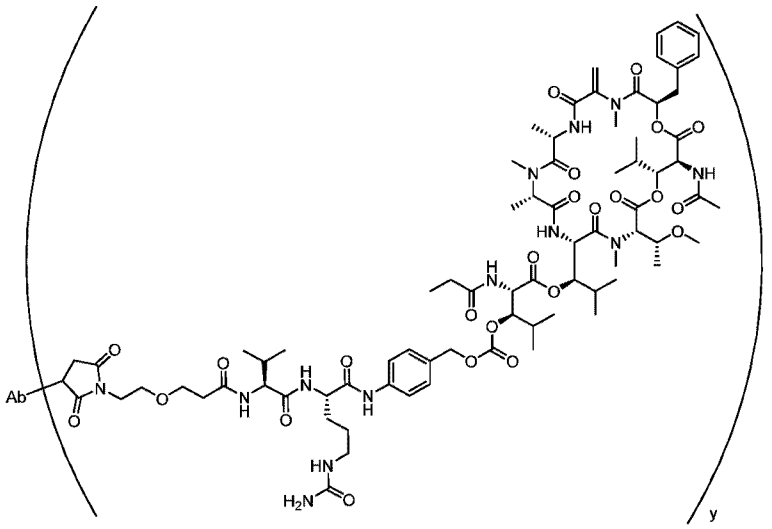
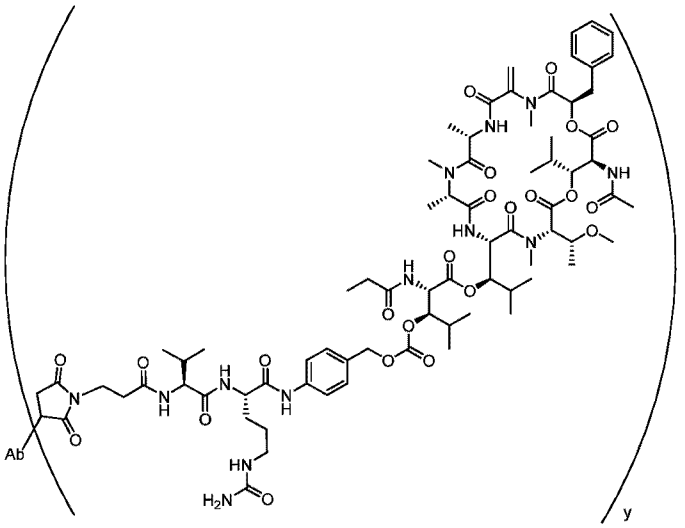


wherein:

y is 2 or 4 and

Ab is an antibody or antigen binding fragment thereof that binds to human PMEL17 protein.

Embodiment 64. The conjugate of Formula (C), Formula (C-1) or Formula (C-3) having the structure:



wherein:

y is 2 or 4 and

Ab is an antibody or antigen binding fragment thereof that binds to human PMEL17 protein.

Embodiment 65. The conjugate of compound (E) having the structure:

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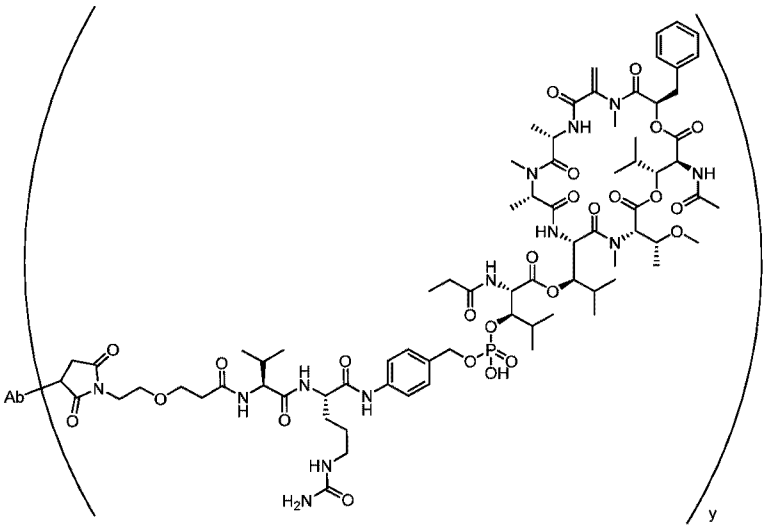

wherein:

y is 2; and

Ab is an antibody or antigen binding fragment thereof that binds to human PMEL17 protein.

Further, the antibodies, antibody fragments (*e.g.*, antigen binding fragments) or functional equivalents of the present disclosure may be conjugated to a drug moiety that modifies a given biological response. Drug moieties are not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein, peptide, or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, cholera toxin, or diphtheria toxin, a protein such as tumor necrosis factor, α-interferon, β-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, a cytokine, an apoptotic agent, an anti-angiogenic agent, or, a biological response modifier such as, for example, a lymphokine.

In one embodiment, the antibodies, antibody fragments (*e.g.*, antigen binding fragments) or functional equivalents of the present disclosure are conjugated to a drug moiety, such as a cytotoxin, a drug (*e.g.*, an immunosuppressant) or a radiotoxin. Examples of cytotoxins include but are not limited to, taxanes (*see, e.g.*, International (PCT) Patent Application Nos. WO 01/38318 and PCT/US03/02675), DNA-alkylating agents (*e.g.*, CC-1065 analogs), anthracyclines, tubulysin analogs, duocarmycin analogs, auristatin E, auristatin F, maytansinoids, pyrrolobenzodiazipines (PBDs), and cytotoxic agents comprising a reactive polyethylene glycol moiety (*see, e.g.*, Sasse *et al.*, J. Antibiot. (Tokyo), 53, 879-85 (2000), Suzawa *et al.*, Bioorg. Med. Chem., 8, 2175-84 (2000), Ichimura *et al.*, J. Antibiot. (Tokyo), 44, 1045-53 (1991), Francisco *et al.*, Blood (2003) (electronic publication prior to print publication), U.S. Pat. Nos. 5,475,092, 6,340,701, 6,372,738, and 6,436,931, U.S. Patent Application Publication No. 2001/0036923 A1, Pending U.S. patent application Ser. Nos. 10/024,290 and 10/116,053, and International (PCT) Patent Application No. WO 01/49698), taxon,

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cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, t. colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents also include, for example, anti-metabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), ablating agents (*e.g.*, mechlorethamine, thiotepa chlorambucil, meiphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin, anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine). (See *e.g.*, Seattle Genetics US20090304721).

Other examples of cytotoxins that can be conjugated to the antibodies, antibody fragments (antigen binding fragments) or functional equivalents of the disclosure include duocarmycins, calicheamicins, maytansines and auristatins, and derivatives thereof.

Various types of cytotoxins, linkers and methods for conjugating therapeutic agents to antibodies are known in the art, *see, e.g.*, Saito *et al.*, (2003) Adv. Drug Deliv. Rev. 55:199-215; Trail *et al.*, (2003) Cancer Immunol. Immunother. 52:328-337; Payne, (2003) Cancer Cell 3:207-212; Allen, (2002) Nat. Rev. Cancer 2:750-763; Pastan and Kreitman, (2002) Curr. Opin. Investig. Drugs 3:1089-1091; Senter and Springer, (2001) Adv. Drug Deliv. Rev. 53:247-264.

The antibodies, antibody fragments (*e.g.*, antigen binding fragments) or functional equivalents of the present disclosure can also be conjugated to a radioactive isotope to generate cytotoxic radiopharmaceuticals, referred to as radioimmunoconjugates. Examples of radioactive isotopes that can be conjugated to antibodies for use diagnostically or therapeutically include, but are not limited to, iodine-131, indium-111, yttrium-90, and lutetium-177. Methods for preparing radioimmunoconjugates are established in the art. Examples of radioimmunoconjugates are commercially available, including Zevalin™ (DEC Pharmaceuticals) and Bexxar™ (Corixa Pharmaceuticals), and similar methods can be used to prepare radioimmunoconjugates using the antibodies of the disclosure. In certain embodiments, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA) which can be attached to the antibody via a linker molecule. Such linker molecules are commonly known in the art and described in Denardo *et al.*, (1998) Clin Cancer Res. 4(10):2483-90; Peterson *et al.*, (1999) Bioconjug. Chem. 10(4):553-7; and Zimmerman *et al.*, (1999) Nucl. Med. Biol. 26(8):943-50, each incorporated by reference in their entireties.

The antibodies, antibody fragments (*e.g.*, antigen binding fragments) or functional equivalents of the present disclosure can also conjugated to a heterologous protein or polypeptide (or fragment thereof, preferably to a polypeptide of at least about 10, at least about 20, at least about 30, at least about 40, at least about 50, at least about 60, at least about 70, at least about 80, at least about 90 or

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at least about 100 amino acids) to generate fusion proteins. In particular, the disclosure provides fusion proteins comprising an antibody fragment (*e.g.*, antigen binding fragment) described herein (*e.g.*, a Fab fragment, Fd fragment, Fv fragment, $F(ab)_2$ fragment, a VH domain, a VH CDR, a VL domain or a VL CDR) and a heterologous protein, polypeptide, or peptide.

Additional fusion proteins may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to alter the activities of antibodies of the disclosure or fragments thereof (*e.g.*, antibodies or fragments thereof with higher affinities and lower dissociation rates). *See, generally*, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458; Patten *et al.*, (1997) Curr. Opinion Biotechnol. 8:724-33; Harayama, (1998) Trends Biotechnol. 16(2):76-82; Hansson *et al.*, (1999) J. Mol. Biol. 287:265-76; and Lorenzo and Blasco, (1998) Biotechniques 24(2):308- 313 (each of these patents and publications are hereby incorporated by reference in its entirety). Antibodies or fragments thereof, or the encoded antibodies or fragments thereof, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. A polynucleotide encoding an antibody or fragment thereof that specifically binds to an antigen may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

Moreover, the antibodies, antibody fragments (*e.g.*, antigen binding fragments) or functional equivalents of the present disclosure can be conjugated to marker sequences, such as a peptide, to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide (SEQ ID NO: 267), such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz *et al.*, (1989) Proc. Natl. Acad. Sci. USA 86:821-824, for instance, hexa-histidine (SEQ ID NO: 267) provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the hemagglutinin ("HA") tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson *et al.*, (1984) Cell 37:767), and the "FLAG" tag (A. Einhauer *et al.*, J. Biochem. Biophys. Methods 49: 455–465, 2001). According to the present disclosure, antibodies or antigen binding fragments can also be conjugated to tumor-penetrating peptides in order to enhance their efficacy.

In other embodiments, the antibodies, antibody fragments (*e.g.*, antigen binding fragments) or functional equivalents of the present disclosure are conjugated to a diagnostic or detectable agent. Such immunoconjugates can be useful for monitoring or prognosing the onset, development, progression and/or severity of a disease or disorder as part of a clinical testing procedure, such as determining the efficacy of a particular therapy. Such diagnosis and detection can be accomplished by coupling the antibody to detectable substances including, but not limited to, various enzymes, such as, but not limited to, horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; prosthetic groups, such as, but not limited to, streptavidin/biotin and

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avidin/biotin; fluorescent materials, such as, but not limited to, Alexa Fluor 350, Alexa Fluor 405, Alexa Fluor 430, Alexa Fluor 488, Alexa Fluor 500, Alexa Fluor 514, Alexa Fluor 532, Alexa Fluor 546, Alexa Fluor 555, Alexa Fluor 568, Alexa Fluor 594, Alexa Fluor 610, Alexa Fluor 633, Alexa Fluor 647, Alexa Fluor 660, Alexa Fluor 680, Alexa Fluor 700, Alexa Fluor 750, umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; luminescent materials, such as, but not limited to, luminol; bioluminescent materials, such as but not limited to, luciferase, luciferin, and aequorin; radioactive materials, such as, but not limited to, iodine (^{131}I, ^{125}I, ^{123}I, and ^{121}I,), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{115}In, ^{113}In, ^{112}In, and ^{111}In,), technetium (^{99}Tc), thallium (^{201}Ti), gallium (^{68}Ga, ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm, ^{177}Lu, ^{159}Gd, ^{149}Pm, ^{140}La, ^{175}Yb, ^{166}Ho, ^{90}Y, ^{47}Sc, ^{186}Re, ^{188}Re, ^{142}Pr, ^{105}Rh, ^{97}Ru, ^{68}Ge, ^{57}Co, ^{65}Zn, ^{85}Sr, ^{32}P, ^{153}Gd, ^{169}Yb, ^{51}Cr, ^{54}Mn, ^{75}Se, ^{64}Cu, ^{113}Sn, and ^{117}Sn; and positron emitting metals using various positron emission tomographies, and non-radioactive paramagnetic metal ions.

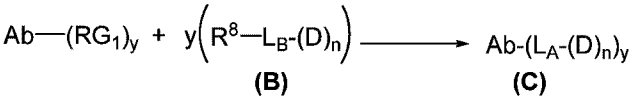
The antibodies, antibody fragments (*e.g.*, antigen binding fragments) or functional equivalents of the disclosure may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

Conjugation and Preparation of ADCs

Processes for Making Antibody Drug conjugates of Formula (C), Formula (C-1) and Formula (C-2)

A general reaction scheme for the formation of conjugates of Formula (C) is shown in Scheme 1 below:

Scheme 1



where: RG_1 is a reactive group on the antibody or antigen binding fragment thereof, Ab, by way of example only a thiol, amine, or ketone, which reacts with a compatible reactive group, R^8, attached to the linker-drug compound thereby covalently linking antibody or antigen binding fragment thereof, Ab, to one or more linker-drug moieties. Non-limiting examples of such reactions of RG_1 and R^8 groups are a maleimide (R^8) reacting with a thiol (RG_1) to give a succinimide ring, or a hydroxylamine (R^8) reacting with a ketone (RG_1) to give an oxime.

In one embodiment, D is a GNAQ inhibitor, a GNA11 inhibitor, or an inhibitor of GNAQ and GNA11 (GNAQ/GNA11 inhibitor), L_a is a linker further comprising a bivalent coupling group formed when RG_1 and R^8 react, n is 1, 2, 3 or 4, and y is 1, 2, 3 or 4.

A general reaction scheme for the formation of conjugates of Formula (C-1) is shown in Scheme 2 below:

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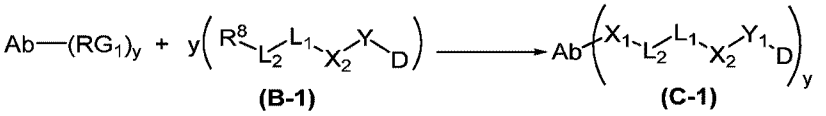
Scheme 2



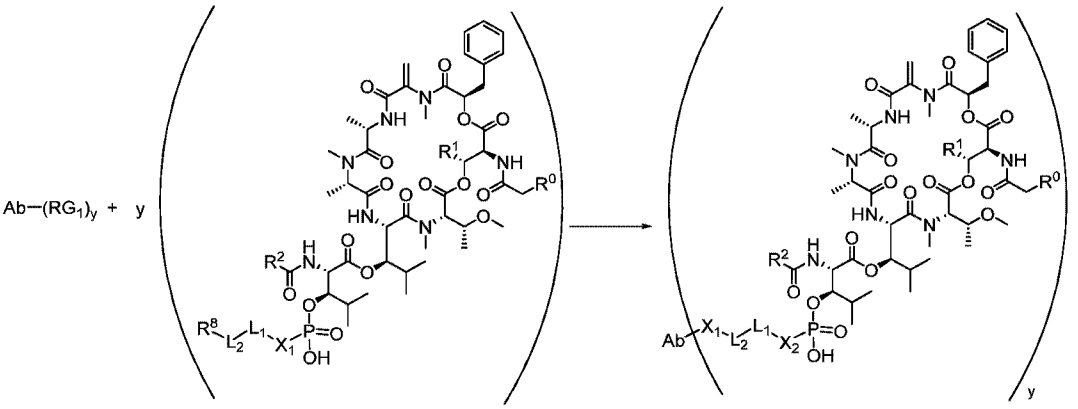
(B-1) (C-1)

where: RG_1 is a reactive group on the antibody or antigen binding fragment thereof, Ab, by way of example only a thiol, amine, or ketone, which reacts with a compatible reactive group, R^8, attached to the linker-drug moiety thereby covalently linking antibody or antigen binding fragment thereof, Ab, to one or more linker-drug moieties. Non-limiting examples of such reactions of RG_1 and R^8 groups are a maleimide (R^8) reacting with a thiol (RG_1) to give a succinimide ring, or a hydroxylamine (R^8) reacting with a ketone (RG_1) to give an oxime.

In one embodiment, D is a GNAQ inhibitor, a GNA11 inhibitor, or an inhibitor of GNAQ and GNA11 (GNAQ/GNA11 inhibitor), X_1 is a bivalent coupling group formed when RG_1 and R^8 react (*e.g.*, a succinimide ring or an oxime), Y_1 is a phosphate group, X_2 is a self-immolative spacer, L_1 is a bivalent peptide linker, L_2 is a bond or a linker, and y is 1, 2, 3 or 4.

A general reaction scheme for the formation of conjugates of Formula (C-2) is shown in Scheme 3 below:

Scheme 3



(B-2) (C-2)

where: RG_1 is a reactive group on the antibody or antigen binding fragment thereof, Ab, by way of example only a thiol, amine, or ketone, which reacts with a compatible reactive group, R^8, attached to the linker-drug moiety thereby covalently linking antibody or antigen binding fragment thereof, Ab, to one or more linker-drug moieties. Non-limiting examples of such reactions of RG_1 and R^8 groups are a maleimide (R^8) reacting with a thiol (RG_1) to give a succinimide ring, or a hydroxylamine (R^8) reacting with a ketone (RG_1) to give an oxime.

Here R^0 is methyl, R^1 is isopropyl, R^2 is ethyl, X_1 is a bivalent coupling group formed when RG_1 and R^8 react (*e.g.* a succinimide ring or an oxime), X_2 is a self-immolative spacer, L_1 is a bivalent peptide linker, L_2 is a bond or a linker, and y is 1, 2, 3 or 4.

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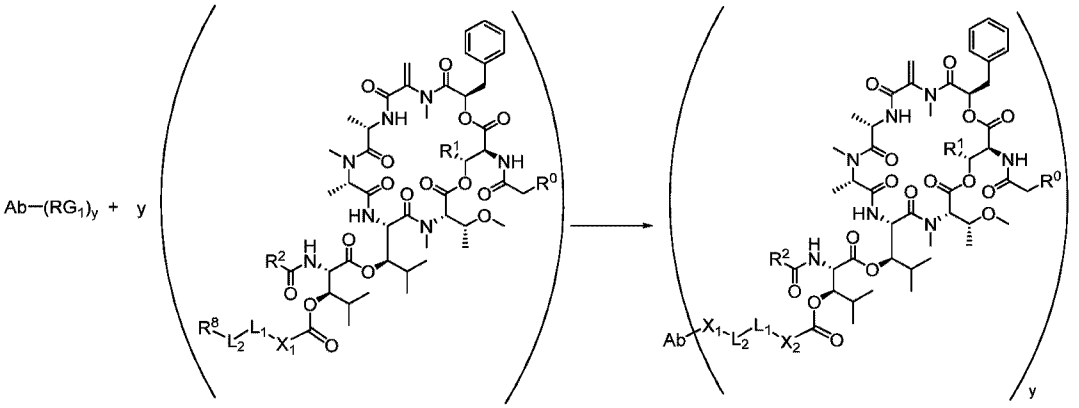
A general reaction scheme for the formation of conjugates of Formula (C-2) is shown in Scheme 4 below:

Scheme 4



where: RG_1 is a reactive group on the antibody or antigen binding fragment thereof, Ab, by way of example only a thiol, amine or ketone, which reacts with a compatible reactive group, R^8, attached to the linker-drug moiety thereby covalently linking antibody or antigen binding fragment thereof, Ab, to one or more linker-drug moieties. Non-limiting examples of such reactions of RG_1 and R^8 groups are a maleimide (R^8) reacting with a thiol (RG_1) to give a succinimide ring, or a hydroxylamine (R^8) reacting with a ketone (RG_1) to give an oxime.

Here R^0 is methyl, R^1 is isopropyl, R^2 is ethyl, X_1 is a bivalent coupling group formed when RG_1 and R^8 react (*e.g.*, a succinimide ring or an oxime), X_2 is a self-immolative spacer, L_1 is a bivalent peptide linker, L_2 is a bond or a linker, and y is 1, 2, 3 or 4.

Process for Conjugation to Engineered Cysteine Antibody Residues

Conjugates of the disclosure can be prepared using cysteine residues engineered into an antibody by, for example, site-directed mutagenesis. Such site-specific conjugates are homogenous and have improved properties (Junutula JR, Raab H, Clark S, Bhakta S, Leipold DD, Weir S, Chen Y, Simpson M, Tsai SP, Dennis MS, Lu Y, Meng YG, Ng C, Yang J, Lee CC, Duenas E, Gorrell J, Katta V, Kim A, McDorman K, Flagella K, Venook R, Ross S, Spencer SD, Lee Wong W, Lowman HB, Vandlen R, Sliwkowski MX, Scheller RH, Polakis P, Mallet W. (2008) Nature Biotechnology 26:925-932.)

Because engineered cysteines in antibodies expressed in mammalian cells are modified by adducts (disulfides) such as glutathione (GSH) and/or cysteine during their biosynthesis (Chen *et al.* 2009), the engineered cysteine residues in the product as initially expressed are unreactive to thiol reactive reagents such as maleimido or bromo-or iodo-acetamide groups. To conjugate payload to an engineered cysteine after expression, glutathione or cysteine adducts need to be removed by reducing

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these disulfide adducts, which generally entails also reducing native disulfides in the expressed protein. Deprotection of adducted engineered cysteines can be accomplished by first exposing antibody to a reducing agent, *e.g.*, dithiothreitol (DTT), TCEP, or reduced cysteine, followed by a procedure that allows for re-oxidation of all native disulfide bonds of an antibody to restore and/or stabilize the functional antibody structure.

Several methods can be employed to reduce and re-oxidize antibodies with engineered cysteine residues for preparation of antibody drug conjugates. Attempts to follow re-oxidation protocols previously described in the literature using high concentration of $CuSO_4$ resulted in protein precipitation (Junutula JR, Raab H, Clark S, Bhakta S, Leipold DD, Weir S, Chen Y, Simpson M, Tsai SP, Dennis MS, Lu Y, Meng YG, Ng C, Yang J, Lee CC, Duenas E, Gorrell J, Katta V, Kim A, McDorman K, Flagella K, Venook R, Ross S, Spencer SD, Lee Wong W, Lowman HB, Vandlen R, Sliwkowski MX, Scheller RH, Polakis P, Mallet W. (2008) Nature Biotechnology 26:925). We have successfully prepared and obtained antibody drug conjugates with several different methods for reduction and antibody re-oxidation.

The following is a method to reduce and re-oxidize antibodies with engineered cysteine residues for preparation of antibody drug conjugates: Freshly prepared DTT is added to purified Cys mutant antibodies to a final concentration of 10 mM. After incubation with DTT at room temperature for 1 hour, mixture is dialyzed at 4°C against PBS for three days with daily buffer exchange to remove DTT and re-oxidize native disulfide bonds of the antibody. An alternative method is to remove reducing reagents through a desalting column such as Sephadex G-25, equilibrated with PBS. Once protein is fully reduced, 1 mM oxidized ascorbate (dehydro-ascorbic acid) is optionally added to desalted samples and re-oxidation incubations are carried out for 20-24 hours.

In another exemplary method, deprotection of engineered Cys residues is accomplished by adding fully reduced cysteine at 20 mM concentration to antibodies bound to protein A-Sepharose resin. Reduction of the Cys adducts is achieved by incubation for approximately 30-60 minutes at room temperature, then reductant is rapidly removed by washing resin with 50 beds of PBS. Re-oxidation of the reduced antibody is achieved by incubating washed slurry at room temperature with or without addition of 50-2000 nM $CuCl_2$ as an accelerant. With the exception of use of copper sulfate, examples herein use each of the protocols described herein with similar results. Reoxidation restores intra-chain disulfides, while dialysis, desalting or protein A chromatography removes reducing agent as well as cysteines and glutathiones initially connected to engineered cysteine(s) of the antibody. HPLC reverse phase chromatography is typically used to monitor the reoxidation process: Antibodies are loaded onto a PLRP-S column (4000 Å, 50 mm x 2.1 mm, Agilent) heated to 80° C and eluted using a linear gradient of 30-45% CH3CN in water containing 0.1% TFA at 1.5 mL/min. and peak detection at 215, 254, and 280 nm.

After re-oxidation, the antibody is conjugated to a linker-drug compound of, by way of example, compounds of Formula (B), Formula (B-1), Formula (B-2) or Formula (B-3) (see schemes

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1-4). By way of example, a compound of Formula (B), Formula (B-1), Formula (B-2) or Formula (B-3) is added to re-oxidized Cys mutant antibody at 5-10 molar equivalents relative to antibody in PBS buffer (pH 7.2). Incubations are carried out for 1-2 hours. The conjugation process is monitored by reverse-phase HPLC, which is able to separate conjugated antibodies from non-conjugated ones. Conjugation reaction mixtures are analyzed on a PRLP-S column (4000 Å, 50 mm x 2.1 mm, Agilent) heated to 80°C and elution of the column are carried out by a linear gradient of 30-60% acetonitrile in water containing 0.1% TFA at a flow rate of 1.5 ml/min. Elution of proteins from the column is monitored at 280 nm, 254 nm and 215 nm.

Alternatively, for antibodies bound to a Protein A resin, once the antibody is re-oxidized, the resin is washed with 10 column volumes PBS and the resin is then resuspended in equal volume PBS and an 8x excess of a compound of Formula (B), Formula (B-1), Formula (B-2) or Formula (B-3) (in DMSO) is added and incubated at room temperature for 2 hours. The resin is then washed with 50 column volumes of PBS and the resulting antibody drug conjugate is eluted from the Protein A resin, neutralized with 1/10 volume 1 M Tris pH 9.0 and buffer exchanged into appropriate buffer to perform preparative size exclusion chromatography (if needed).

Immunoconjugates are also characterized in terms of average loading of a drug moiety to antibody binding moiety, generally referred to as drug-to-antibody ratio (DAR). The DAR value is extrapolated, for example, from LC-MS data for reduced and deglycosylated samples. LC/MS allows quantitation of the average number of molecules of payload (drug moiety) attached to an antibody in an ADC. HPLC separates an antibody into light and heavy chains, and also separates heavy chain (HC) and light chain (LC) according to the number of Linker-Payload groups per chain. Mass spectral data enables identification of the component species in the mixture, *e.g.*, LC, LC+1, LC+2, HC, HC+1, HC+2, etc. From average loading of LC and HC chains, the average DAR can be calculated for an ADC. The DAR for a given immunoconjugate sample represents the average number of drug (payload) molecules attached to a tetrameric antibody containing two light chains and two heavy chains.

Throughout the text of this application, should there be a discrepancy between the text of the specification and the sequence listing, the text of the specification shall prevail.

Table 2. Examples of Anti-PMEL17 Antibodies

G1 E152C_S375C_wt LC		
SEQ ID NO: 1	HCDR1 (Combined)	GGTFSDYAIT
SEQ ID NO: 2	HCDR2 (Combined)	GIIPIFGTANYAQKFQG
SEQ ID NO: 3	HCDR3 (Combined)	EGGLLTDISYSRYWFAY
SEQ ID NO: 4	HCDR1 (Kabat)	DYAIT
SEQ ID NO: 2	HCDR2 (Kabat)	GIIPIFGTANYAQKFQG
SEQ ID NO: 3	HCDR3 (Kabat)	EGGLLTDISYSRYWFAY

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SEQ ID NO: 5	HCDR1 (Chothia)	GGTFSDY
SEQ ID NO: 6	HCDR2 (Chothia)	IPIFGT
SEQ ID NO: 3	HCDR3 (Chothia)	EGGLLTDISYSRYWFAY
SEQ ID NO: 7	HCDR1 (IMGT)	GGTFSDYA
SEQ ID NO: 8	HCDR2 (IMGT)	IIPIFGTA
SEQ ID NO: 9	HCDR3 (IMGT)	AREGGLLTDISYSRYWFAY
SEQ ID NO: 10	VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSDYAITWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYCAREGGLLTDISYSRYWFA YWGQGTLVTVSS
SEQ ID NO: 11	DNA VH	CAGGTGCAATTGGTGCAGAGCGGTGCCGAAGTGAAAAA ACCGGGCAGCAGCGTGAAAGTTAGCTGCAAAGCATCCG GAGGGACGTTTTCTGACTACGCTATCACTTGGGTGCGC CAGGCCCCGGGCCAGGGCCTCGAGTGGATGGGCGGTAT CATCCCGATCTTCGGCACTGCGAACTACGCCCAGAAAT TTCAGGGCCGGGTGACCATTACCGCCGATGAAAGCACC AGCACCGCCTATATGGAACTGAGCAGCCTGCGCAGCGA AGATACGGCCGTGTATTATTGCGCGCGTGAAGGTGGTC TGCTGACTGACATCTCTTACTCTCGTTACTGGTTCGCT TACTGGGGCCAAGGCACCCTGGTGACTGTTAGCTCA
SEQ ID NO: 12	Heavy Chain	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSDYAITWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYCAREGGLLTDISYSRYWFA YWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPCPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKS CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG FYPCDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 13	DNA Heavy Chain	CAGGTGCAATTGGTGCAGAGCGGTGCCGAAGTGAAAAA ACCGGGCAGCAGCGTGAAAGTTAGCTGCAAAGCATCCG GAGGGACGTTTTCTGACTACGCTATCACTTGGGTGCGC CAGGCCCCGGGCCAGGGCCTCGAGTGGATGGGCGGTAT CATCCCGATCTTCGGCACTGCGAACTACGCCCAGAAAT TTCAGGGCCGGGTGACCATTACCGCCGATGAAAGCACC AGCACCGCCTATATGGAACTGAGCAGCCTGCGCAGCGA AGATACGGCCGTGTATTATTGCGCGCGTGAAGGTGGTC TGCTGACTGACATCTCTTACTCTCGTTACTGGTTCGCT TACTGGGGCCAAGGCACCCTGGTGACTGTTAGCTCAGC TAGCACCAAGGGCCCAAGTGTGTTTCCCCTGGCCCCCA GCAGCAAGTCTACTTCCGGCGGAACTGCTGCCCTGGGT TGCCTGGTGAAGGACTACTTCCCCTGTCCCGTGACAGT GTCCTGGAACTCTGGGGCTCTGACTTCCGGCGTGCACA CCTTCCCCGCCGTGCTGCAGAGCAGCGGCCTGTACAGC CTGAGCAGCGTGGTGACAGTGCCCTCCAGCTCTCTGGG AACCCAGACCTATATCTGCAACGTGAACCACAAGCCCA GCAACACCAAGGTGGACAAGAGAGTGGAGCCCAAGAGC TGCGACAAGACCCACACCTGCCCCCCCTGCCCAGCTCC AGAACTGCTGGGAGGGCCTTCCGTGTTCCTGTTCCCCC CCAAGCCCAAGGACACCCTGATGATCAGCAGGACCCCC GAGGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGA

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		CCCAGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGG AGGTGCACAACGCCAAGACCAAGCCCAGAGAGGAGCAG TACAACAGCACCTACAGGGTGGTGTCCGTGCTGACCGT GCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGT GCAAAGTCTCCAACAAGGCCCTGCCAGCCCCAATCGAA AAGACAATCAGCAAGGCCAAGGGCCAGCCACGGGAGCC CCAGGTGTACACCCTGCCCCCCAGCCGGGAGGAGATGA CCAAGAACCAGGTGTCCCTGACCTGTCTGGTGAAGGGC TTCTACCCCTGTGATATCGCCGTGGAGTGGGAGAGCAA CGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCAG TGCTGGACAGCGACGGCAGCTTCTTCCTGTACAGCAAG CTGACCGTGGACAAGTCCAGGTGGCAGCAGGGCAACGT GTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACC ACTACACCCAGAAGTCCCTGAGCCTGAGCCCCGGCAAG
SEQ ID NO: 14	LCDR1 (Combined)	SGDALRDKFVY
SEQ ID NO: 15	LCDR2 (Combined)	DDNNRPS
SEQ ID NO: 16	LCDR3 (Combined)	QSWDHSYSLVV
SEQ ID NO: 14	LCDR1 (Kabat)	SGDALRDKFVY
SEQ ID NO: 15	LCDR2 (Kabat)	DDNNRPS
SEQ ID NO: 16	LCDR3 (Kabat)	QSWDHSYSLVV
SEQ ID NO: 17	LCDR1 (Chothia)	DALRDKF
SEQ ID NO: 18	LCDR2 (Chothia)	DDN
SEQ ID NO: 19	LCDR3 (Chothia)	WDHSYSLV
SEQ ID NO: 20	LCDR1 (IMGT)	ALRDKF
SEQ ID NO: 18	LCDR2 (IMGT)	DDN
SEQ ID NO: 16	LCDR3 (IMGT)	QSWDHSYSLVV
SEQ ID NO: 21	VL	DIELTQPPSVSVSPGQTASITCSGDALRDKFVYWYQQK PGQAPVLVIYDDNNRPSGIPERFSGSNSGNTATLTISG TQAEDEADYYCQSWDHSYSLVVFGGGTKLTVL
SEQ ID NO: 22	DNA VL	GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGTGAG CCCGGGCCAGACCGCGAGCATTACCTGTAGCGGCGATG CTCTGCGTGACAAATTCGTTTACTGGTACCAGCAGAAA CCGGGCCAGGCGCCGGTGCTGGTGATCTACGACGACAA CAACCGTCCGAGCGGCATCCCGGAACGTTTTAGCGGAT CCAACAGCGGCAACACCGCGACCCTGACCATTAGCGGC ACCCAGGCGGAAGACGAAGCGGATTATTACTGCCAGTC TTGGGACCATTCTTACTCTCTGGTTGTGTTTGGCGGCG GCACGAAGTTAACTGTCCTG
SEQ ID NO: 23	Light Chain	DIELTQPPSVSVSPGQTASITCSGDALRDKFVYWYQQK PGQAPVLVIYDDNNRPSGIPERFSGSNSGNTATLTISG TQAEDEADYYCQSWDHSYSLVVFGGGTKLTVLGQPKAA PSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKA DSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSH RSYSCQVTHEGSTVEKTVAPTECS
SEQ ID NO: 24	DNA Light Chain	GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGTGAG CCCGGGCCAGACCGCGAGCATTACCTGTAGCGGCGATG CTCTGCGTGACAAATTCGTTTACTGGTACCAGCAGAAA CCGGGCCAGGCGCCGGTGCTGGTGATCTACGACGACAA CAACCGTCCGAGCGGCATCCCGGAACGTTTTAGCGGAT CCAACAGCGGCAACACCGCGACCCTGACCATTAGCGGC ACCCAGGCGGAAGACGAAGCGGATTATTACTGCCAGTC TTGGGACCATTCTTACTCTCTGGTTGTGTTTGGCGGCG

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		GCACGAAGTTAACTGTCCTGGGACAACCTAAGGCCGCT CCCTCCGTGACCCTGTTCCCCCCCAGCTCCGAGGAACT GCAGGCCAACAAGGCCACCCTGGTGTGCCTGATCAGCG ACTTCTACCCTGGCGCCGTGACCGTGGCCTGGAAGGCC GACAGCAGCCCCGTGAAGGCCGGCGTGGAGACAACCAC CCCCAGCAAGCAGAGCAACAACAAGTACGCCGCCAGCA GCTACCTGAGCCTGACCCCCGAGCAGTGGAAGAGCCAC AGAAGCTACAGCTGCCAGGTCACCCACGAGGGCAGCAC CGTGGAGAAAACCGTGGCCCCCACCGAGTGCAGC
G1 E152C_S375C_3J LC		
SEQ ID NO: 1	HCDR1 (Combined)	GGTFSDYAIT
SEQ ID NO: 2	HCDR2 (Combined)	GIIPIFGTANYAQKFQG
SEQ ID NO: 3	HCDR3 (Combined)	EGGLLTDISYSRYWFAY
SEQ ID NO: 4	HCDR1 (Kabat)	DYAIT
SEQ ID NO: 2	HCDR2 (Kabat)	GIIPIFGTANYAQKFQG
SEQ ID NO: 3	HCDR3 (Kabat)	EGGLLTDISYSRYWFAY
SEQ ID NO: 5	HCDR1 (Chothia)	GGTFSDY
SEQ ID NO: 6	HCDR2 (Chothia)	IPIFGT
SEQ ID NO: 3	HCDR3(Chothia)	EGGLLTDISYSRYWFAY
SEQ ID NO: 7	HCDR1 (IMGT)	GGTFSDYA
SEQ ID NO: 8	HCDR2 (IMGT)	IIPIFGTA
SEQ ID NO: 9	HCDR3 (IMGT)	AREGGLLTDISYSRYWFAY
SEQ ID NO: 10	VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSDYAITWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYCAREGGLLTDISYSRYWFA YWGQGTLVTVSS
SEQ ID NO: 11	DNA VH	CAGGTGCAATTGGTGCAGAGCGGTGCCGAAGTGAAAAA ACCGGGCAGCAGCGTGAAAGTTAGCTGCAAAGCATCCG GAGGGACGTTTTCTGACTACGCTATCACTTGGGTGCGC CAGGCCCCGGGCCAGGGCCTCGAGTGGATGGGCGGTAT CATCCCGATCTTCGGCACTGCGAACTACGCCCAGAAAT TTCAGGGCCGGGTGACCATTACCGCCGATGAAAGCACC AGCACCGCCTATATGGAACTGAGCAGCCTGCGCAGCGA AGATACGGCCGTGTATTATTGCGCGCGTGAAGGTGGTC TGCTGACTGACATCTCTTACTCTCGTTACTGGTTCGCT TACTGGGGCCAAGGCACCCTGGTGACTGTTAGCTCA
SEQ ID NO: 12	Heavy Chain	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSDYAITWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYCAREGGLLTDISYSRYWFA YWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPCPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKS CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG FYPCDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 13	DNA Heavy Chain	CAGGTGCAATTGGTGCAGAGCGGTGCCGAAGTGAAAAA ACCGGGCAGCAGCGTGAAAGTTAGCTGCAAAGCATCCG GAGGGACGTTTTCTGACTACGCTATCACTTGGGTGCGC CAGGCCCCGGGCCAGGGCCTCGAGTGGATGGGCGGTAT

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		CATCCCGATCTTCGGCACTGCGAACTACGCCCAGAAAT TTCAGGGCCGGGTGACCATTACCGCCGATGAAAGCACC AGCACCGCCTATATGGAACTGAGCAGCCTGCGCAGCGA AGATACGGCCGTGTATTATTGCGCGCGTGAAGGTGGTC TGCTGACTGACATCTCTTACTCTCGTTACTGGTTCGCT TACTGGGGCCAAGGCACCCTGGTGACTGTTAGCTCAGC TAGCACCAAGGGCCCAAGTGTGTTTCCCCTGGCCCCCA GCAGCAAGTCTACTTCCGGCGGAACTGCTGCCCTGGGT TGCCTGGTGAAGGACTACTTCCCCTGTCCCGTGACAGT GTCCTGGAACTCTGGGGCTCTGACTTCCGGCGTGCACA CCTTCCCCGCCGTGCTGCAGAGCAGCGGCCTGTACAGC CTGAGCAGCGTGGTGACAGTGCCCTCCAGCTCTCTGGG AACCCAGACCTATATCTGCAACGTGAACCACAAGCCCA GCAACACCAAGGTGGACAAGAGAGTGGAGCCCAAGAGC TGCGACAAGACCCACACCTGCCCCCCCTGCCCAGCTCC AGAACTGCTGGGAGGGCCTTCCGTGTTCCTGTTCCCCC CCAAGCCCAAGGACACCCTGATGATCAGCAGGACCCCC GAGGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGA CCCAGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGG AGGTGCACAACGCCAAGACCAAGCCCAGAGAGGAGCAG TACAACAGCACCTACAGGGTGGTGTCCGTGCTGACCGT GCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGT GCAAAGTCTCCAACAAGGCCCTGCCAGCCCCAATCGAA AAGACAATCAGCAAGGCCAAGGGCCAGCCACGGGAGCC CCAGGTGTACACCCTGCCCCCCAGCCGGGAGGAGATGA CCAAGAACCAGGTGTCCCTGACCTGTCTGGTGAAGGGC TTCTACCCCTGTGATATCGCCGTGGAGTGGGAGAGCAA CGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCAG TGCTGGACAGCGACGGCAGCTTCTTCCTGTACAGCAAG CTGACCGTGGACAAGTCCAGGTGGCAGCAGGGCAACGT GTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACC ACTACACCCAGAAGTCCCTGAGCCTGAGCCCCGGCAAG
SEQ ID NO: 14	LCDR1 (Combined)	SGDALRDKFVY
SEQ ID NO: 15	LCDR2 (Combined)	DDNNRPS
SEQ ID NO: 16	LCDR3 (Combined)	QSWDHSYSLVV
SEQ ID NO: 14	LCDR1 (Kabat)	SGDALRDKFVY
SEQ ID NO: 15	LCDR2 (Kabat)	DDNNRPS
SEQ ID NO: 16	LCDR3 (Kabat)	QSWDHSYSLVV
SEQ ID NO: 17	LCDR1 (Chothia)	DALRDKF
SEQ ID NO: 18	LCDR2 (Chothia)	DDN
SEQ ID NO: 19	LCDR3 (Chothia)	WDHSYSLV
SEQ ID NO: 20	LCDR1 (IMGT)	ALRDKF
SEQ ID NO: 18	LCDR2 (IMGT)	DDN
SEQ ID NO: 16	LCDR3 (IMGT)	QSWDHSYSLVV
SEQ ID NO: 25	VL	SYELTQPLSVSVALGQTARITCSGDALRDKFVYWYQQK PGQAPVLVIYDDNNRPSGIPERFSGSNSGNTATLTISR AQAGDEADYYCQSWDHSYSLVVFGGGTKLTVL
SEQ ID NO: 26	DNA VL	AGCTACGAGCTGACCCAGCCGCTGTCGGTGTCAGTCGC CCTGGGACAAACTGCCAGGATCACTTGTTCCGGGGACG CATTGCGGGACAAGTTCGTGTACTGGTACCAGCAGAAG CCGGGTCAAGCCCCAGTGCTCGTGATCTACGACGACAA CAACCGGCCTTCCGGTATCCCCGAACGCTTCTCCGGAT

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		CCAATAGCGGAAACACCGCCACCCTGACCATTTCGAGA GCTCAGGCCGGGGATGAAGCGGACTACTACTGCCAGTC ATGGGATCACTCGTACTCCCTCGTCGTGTTTGGAGGCG GCACGAAGCTTACTGTGCTG
SEQ ID NO: 27	Light Chain	SYELTQPLSVSVALGQTARITCSGDALRDKFVYWYQQK PGQAPVLVIYDDNNRPSGIPERFSGSNSGNTATLTISR AQAGDEADYYCQSWDHSYSLVVFGGGTKLTVLGQPKAA PSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKA DSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSH RSYSCQVTHEGSTVEKTVAPTECS
SEQ ID NO: 28	DNA Light Chain	AGCTACGAGCTGACCCAGCCGCTGTCGGTGTCAGTCGC CCTGGGACAAACTGCCAGGATCACTTGTTCCGGGGACG CATTGCGGGACAAGTTCGTGTACTGGTACCAGCAGAAG CCGGGTCAAGCCCCAGTGCTCGTGATCTACGACGACAA CAACCGGCCTTCCGGTATCCCCGAACGCTTCTCCGGAT CCAATAGCGGAAACACCGCCACCCTGACCATTTCGAGA GCTCAGGCCGGGGATGAAGCGGACTACTACTGCCAGTC ATGGGATCACTCGTACTCCCTCGTCGTGTTTGGAGGCG GCACGAAGCTTACTGTGCTGGGCCAGCCTAAGGCCGCT CCCTCCGTGACCCTGTTCCCCCCCAGCTCCGAGGAACT GCAGGCCAACAAGGCCACCCTGGTGTGCCTGATCAGCG ACTTCTACCCTGGCGCCGTGACCGTGGCCTGGAAGGCC GACAGCAGCCCCGTGAAGGCCGGCGTGGAGACAACCAC CCCCAGCAAGCAGAGCAACAACAAGTACGCCGCCAGCA GCTACCTGAGCCTGACCCCCGAGCAGTGGAAGAGCCAC AGAAGCTACAGCTGCCAGGTCACCCACGAGGGCAGCAC CGTGGAGAAAACCGTGGCCCCCACCGAGTGCAGC
G1 E152C_S375C_3R LC		
SEQ ID NO: 1	HCDR1 (Combined)	GGTFSDYAIT
SEQ ID NO: 2	HCDR2 (Combined)	GIIPIFGTANYAQKFQG
SEQ ID NO: 3	HCDR3 (Combined)	EGGLLTDISYSRYWFAY
SEQ ID NO: 4	HCDR1 (Kabat)	DYAIT
SEQ ID NO: 2	HCDR2 (Kabat)	GIIPIFGTANYAQKFQG
SEQ ID NO: 3	HCDR3 (Kabat)	EGGLLTDISYSRYWFAY
SEQ ID NO: 5	HCDR1 (Chothia)	GGTFSDY
SEQ ID NO: 6	HCDR2 (Chothia)	IPIFGT
SEQ ID NO: 3	HCDR3(Chothia)	EGGLLTDISYSRYWFAY
SEQ ID NO: 7	HCDR1 (IMGT)	GGTFSDYA
SEQ ID NO: 8	HCDR2 (IMGT)	IIPIFGTA
SEQ ID NO: 9	HCDR3 (IMGT)	AREGGLLTDISYSRYWFAY
SEQ ID NO: 10	VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSDYAITWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYCAREGGLLTDISYSRYWFA YWGQGTLVTVSS
SEQ ID NO: 11	DNA VH	CAGGTGCAATTGGTGCAGAGCGGTGCCGAAGTGAAAAA ACCGGGCAGCAGCGTGAAAGTTAGCTGCAAAGCATCCG GAGGGACGTTTTCTGACTACGCTATCACTTGGGTGCGC CAGGCCCCGGGCCAGGGCCTCGAGTGGATGGGCGGTAT CATCCCGATCTTCGGCACTGCGAACTACGCCCAGAAAT TTCAGGGCCGGGTGACCATTACCGCCGATGAAAGCACC AGCACCGCCTATATGGAACTGAGCAGCCTGCGCAGCGA AGATACGGCCGTGTATTATTGCGCGCGTGAAGGTGGTC

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		TGCTGACTGACATCTCTTACTCTCGTTACTGGTTCGCT TACTGGGGCCAAGGCACCCTGGTGACTGTTAGCTCA
SEQ ID NO: 12	Heavy Chain	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSDYAITWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYCAREGGLLTDISYSRYWFA YWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPCPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKS CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG FYPCDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 13	DNA Heavy Chain	CAGGTGCAATTGGTGCAGAGCGGTGCCGAAGTGAAAAA ACCGGGCAGCAGCGTGAAAGTTAGCTGCAAAGCATCCG GAGGGACGTTTTCTGACTACGCTATCACTTGGGTGCGC CAGGCCCCGGGCCAGGGCCTCGAGTGGATGGGCGGTAT CATCCCGATCTTCGGCACTGCGAACTACGCCCAGAAAT TTCAGGGCCGGGTGACCATTACCGCCGATGAAAGCACC AGCACCGCCTATATGGAACTGAGCAGCCTGCGCAGCGA AGATACGGCCGTGTATTATTGCGCGCGTGAAGGTGGTC TGCTGACTGACATCTCTTACTCTCGTTACTGGTTCGCT TACTGGGGCCAAGGCACCCTGGTGACTGTTAGCTCAGC TAGCACCAAGGGCCCAAGTGTGTTTCCCCTGGCCCCCA GCAGCAAGTCTACTTCCGGCGGAACTGCTGCCCTGGGT TGCCTGGTGAAGGACTACTTCCCCTGTCCCGTGACAGT GTCCTGGAACTCTGGGGCTCTGACTTCCGGCGTGCACA CCTTCCCCGCCGTGCTGCAGAGCAGCGGCCTGTACAGC CTGAGCAGCGTGGTGACAGTGCCCTCCAGCTCTCTGGG AACCCAGACCTATATCTGCAACGTGAACCACAAGCCCA GCAACACCAAGGTGGACAAGAGAGTGGAGCCCAAGAGC TGCGACAAGACCCACACCTGCCCCCCCTGCCCAGCTCC AGAACTGCTGGGAGGGCCTTCCGTGTTCCTGTTCCCCC CCAAGCCCAAGGACACCCTGATGATCAGCAGGACCCCC GAGGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGA CCCAGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGG AGGTGCACAACGCCAAGACCAAGCCCAGAGAGGAGCAG TACAACAGCACCTACAGGGTGGTGTCCGTGCTGACCGT GCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGT GCAAAGTCTCCAACAAGGCCCTGCCAGCCCCAATCGAA AAGACAATCAGCAAGGCCAAGGGCCAGCCACGGGAGCC CCAGGTGTACACCCTGCCCCCCAGCCGGGAGGAGATGA CCAAGAACCAGGTGTCCCTGACCTGTCTGGTGAAGGGC TTCTACCCCTGTGATATCGCCGTGGAGTGGGAGAGCAA CGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCAG TGCTGGACAGCGACGGCAGCTTCTTCCTGTACAGCAAG CTGACCGTGGACAAGTCCAGGTGGCAGCAGGGCAACGT GTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACC ACTACACCCAGAAGTCCCTGAGCCTGAGCCCCGGCAAG
SEQ ID NO: 14	LCDR1 (Combined)	SGDALRDKFVY
SEQ ID NO: 15	LCDR2 (Combined)	DDNNRPS
SEQ ID NO: 16	LCDR3 (Combined)	QSWDHSYSLVV
SEQ ID NO: 14	LCDR1 (Kabat)	SGDALRDKFVY

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SEQ ID NO: 15	LCDR2 (Kabat)	DDNNRPS
SEQ ID NO: 16	LCDR3 (Kabat)	QSWDHSYSLVV
SEQ ID NO: 17	LCDR1 (Chothia)	DALRDKF
SEQ ID NO: 18	LCDR2 (Chothia)	DDN
SEQ ID NO: 19	LCDR3 (Chothia)	WDHSYSLV
SEQ ID NO: 20	LCDR1 (IMGT)	ALRDKF
SEQ ID NO: 18	LCDR2 (IMGT)	DDN
SEQ ID NO: 16	LCDR3 (IMGT)	QSWDHSYSLVV
SEQ ID NO: 29	VL	SYELTQPPSVSVSPGQTASITCSGDALRDKFVYWYQQK PGQSPVLVIYDDNNRPSGIPERFSGSNSGNTATLTISG TQAMDEADYYCQSWDHSYSLVVFGGGTKLTVL
SEQ ID NO: 30	DNA VL	TCGTACGAGCTCACTCAACCGCCTTCTGTGTCCGTGTC ACCCGGGCAGACTGCCTCCATTACCTGTTCGGGAGATG CCCTGCGCGACAAGTTTGTGTACTGGTACCAGCAGAAG CCCGGACAGTCGCCAGTGCTCGTCATCTATGACGACAA CAACAGACCTTCCGGTATCCCGGAACGGTTCAGCGGAA GCAATTCCGGCAACACCGCTACCCTGACCATTAGCGGC ACTCAGGCCATGGACGAAGCGGATTACTACTGCCAATC CTGGGACCACTCATACTCCCTTGTGGTGTTCGGTGGCG GAACGAAGCTGACCGTCCTG
SEQ ID NO: 31	Light Chain	SYELTQPPSVSVSPGQTASITCSGDALRDKFVYWYQQK PGQSPVLVIYDDNNRPSGIPERFSGSNSGNTATLTISG TQAMDEADYYCQSWDHSYSLVVFGGGTKLTVLGQPKAA PSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKA DSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSH RSYSCQVTHEGSTVEKTVAPTECS
SEQ ID NO: 32	DNA Light Chain	TCGTACGAGCTCACTCAACCGCCTTCTGTGTCCGTGTC ACCCGGGCAGACTGCCTCCATTACCTGTTCGGGAGATG CCCTGCGCGACAAGTTTGTGTACTGGTACCAGCAGAAG CCCGGACAGTCGCCAGTGCTCGTCATCTATGACGACAA CAACAGACCTTCCGGTATCCCGGAACGGTTCAGCGGAA GCAATTCCGGCAACACCGCTACCCTGACCATTAGCGGC ACTCAGGCCATGGACGAAGCGGATTACTACTGCCAATC CTGGGACCACTCATACTCCCTTGTGGTGTTCGGTGGCG GAACGAAGCTGACCGTCCTGGGCCAGCCTAAGGCCGCT CCCTCCGTGACCCTGTTCCCCCCCAGCTCCGAGGAACT GCAGGCCAACAAGGCCACCCTGGTGTGCCTGATCAGCG ACTTCTACCCTGGCGCCGTGACCGTGGCCTGGAAGGCC GACAGCAGCCCCGTGAAGGCCGGCGTGGAGACAACCAC CCCCAGCAAGCAGAGCAACAACAAGTACGCCGCCAGCA GCTACCTGAGCCTGACCCCCGAGCAGTGGAAGAGCCAC AGAAGCTACAGCTGCCAGGTCACCCACGAGGGCAGCAC CGTGGAGAAAACCGTGGCCCCCACCGAGTGCAGC
G4 E152_S375C		
SEQ ID NO: 33	HCDR1 (Combined)	GGTFSTYAIS
SEQ ID NO: 34	HCDR2 (Combined)	RIIPILGIANYAQKFQG
SEQ ID NO: 35	HCDR3 (Combined)	EVRMIFDY
SEQ ID NO: 36	HCDR1 (Kabat)	TYAIS
SEQ ID NO: 34	HCDR2 (Kabat)	RIIPILGIANYAQKFQG
SEQ ID NO: 35	HCDR3 (Kabat)	EVRMIFDY
SEQ ID NO: 37	HCDR1 (Chothia)	GGTFSTY

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SEQ ID NO: 38	HCDR2 (Chothia)	IPILGI
SEQ ID NO: 35	HCDR3(Chothia)	EVRMIFDY
SEQ ID NO: 39	HCDR1 (IMGT)	GGTFSTYA
SEQ ID NO: 40	HCDR2 (IMGT)	IIPILGIA
SEQ ID NO: 41	HCDR3 (IMGT)	AREVRMIFDY
SEQ ID NO: 42	VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSTYAISWVR QAPGQGLEWMGRIIPILGIANYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYCAREVRMIFDYWGQGTLVT VSS
SEQ ID NO: 43	DNA VH	CAGGTGCAATTGGTGCAGAGCGGTGCCGAAGTGAAAAA ACCGGGCAGCAGCGTGAAAGTTAGCTGCAAAGCATCCG GAGGGACGTTTTCTACTTACGCTATCTCTTGGGTGCGC CAGGCCCCGGGCCAGGGCCTCGAGTGGATGGGCCGTAT CATCCCGATCCTGGGCATCGCGAACTACGCCCAGAAAT TTCAGGGCCGGGTGACCATTACCGCCGATGAAAGCACC AGCACCGCCTATATGGAACTGAGCAGCCTGCGCAGCGA AGATACGGCCGTGTATTATTGCGCGCGTGAAGTTCGTA TGATCTTCGATTACTGGGGCCAAGGCACCCTGGTGACT GTTAGCTCA
SEQ ID NO: 44	Heavy Chain	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSTYAISWVR QAPGQGLEWMGRIIPILGIANYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYCAREVRMIFDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPC PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPCDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 45	DNA Heavy Chain	CAGGTGCAATTGGTGCAGAGCGGTGCCGAAGTGAAAAA ACCGGGCAGCAGCGTGAAAGTTAGCTGCAAAGCATCCG GAGGGACGTTTTCTACTTACGCTATCTCTTGGGTGCGC CAGGCCCCGGGCCAGGGCCTCGAGTGGATGGGCCGTAT CATCCCGATCCTGGGCATCGCGAACTACGCCCAGAAAT TTCAGGGCCGGGTGACCATTACCGCCGATGAAAGCACC AGCACCGCCTATATGGAACTGAGCAGCCTGCGCAGCGA AGATACGGCCGTGTATTATTGCGCGCGTGAAGTTCGTA TGATCTTCGATTACTGGGGCCAAGGCACCCTGGTGACT GTTAGCTCAGCCTCTACGAAAGGCCCAAGCGTATTTCC CCTGGCTCCTTCTAGTAAATCAACCTCAGGTGGTACAG CAGCCCTTGGCTGCCTGGTCAAAGACTATTTCCCCTGT CCGGTGACCGTCTCATGGAACTCAGGTGCTTTGACATC TGGTGTGCATACATTCCCAGCTGTGCTGCAAAGTAGTG GACTGTACAGCCTTTCCAGCGTGGTCACGGTGCCAAGT AGCTCCTTGGGTACTCAGACTTATATCTGCAATGTGAA CCACAAGCCCTCTAACACGAAGGTGGACAAGCGCGTGG AGCCCAAATCTTGCGATAAGACGCATACTTGTCCCCCA TGCCCTGCTCCTGAGCTGTTGGGAGGCCCGTCAGTGTT CTTGTTCCCTCCGAAGCCTAAGGACACTTTGATGATAA GTAGGACACCAGAGGTGACTTGCGTGGTGGTTGATGTG TCCCATGAAGATCCCGAGGTCAAATTTAATTGGTACGT AGATGGTGTCGAAGTTCACAATGCTAAGACTAAGCCAA

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		GGGAAGAGCAGTACAACAGTACATATAGGGTAGTCTCC GTGCTGACAGTCCTCCACCAGGACTGGTTGAACGGCAA GGAATACAAATGTAAGGTGTCAAACAAAGCTCTGCCTG CTCCCATTGAGAAAACAATCTCTAAAGCCAAAGGCCAG CCGAGAGAGCCCCAAGTCTACACTTTGCCCCCGAGCAG GGAGGAAATGACCAAGAATCAGGTGAGTCTGACGTGCC TCGTCAAAGGATTTTATCCATGCGATATTGCAGTTGAA TGGGAGAGCAATGGCCAGCCAGAGAACAACTATAAAAC CACACCACCCGTGCTCGACTCTGATGGCAGCTTCTTCC TCTATAGCAAGCTGACAGTCGATAAATCTCGCTGGCAG CAAGGCAATGTGTTCTCCTGCTCCGTCATGCACGAGGC TTTGCATAACCATTATACTCAAAAATCTCTGTCCCTGT CACCTGGTAAA
SEQ ID NO: 46	LCDR1 (Combined)	RASQSISSYLA
SEQ ID NO: 47	LCDR2 (Combined)	AASSLQS
SEQ ID NO: 48	LCDR3 (Combined)	QQSYDYYT
SEQ ID NO: 46	LCDR1 (Kabat)	RASQSISSYLA
SEQ ID NO: 47	LCDR2 (Kabat)	AASSLQS
SEQ ID NO: 48	LCDR3 (Kabat)	QQSYDYYT
SEQ ID NO: 49	LCDR1 (Chothia)	SQSISSY
SEQ ID NO: 50	LCDR2 (Chothia)	AAS
SEQ ID NO: 51	LCDR3 (Chothia)	SYDYY
SEQ ID NO: 52	LCDR1 (IMGT)	QSISSY
SEQ ID NO: 50	LCDR2 (IMGT)	AAS
SEQ ID NO: 48	LCDR3 (IMGT)	QQSYDYYT
SEQ ID NO: 53	VL	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLAWYQQ KPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCQQSYDYYTFGQGTKVEIK
SEQ ID NO: 54	DNA VL	GATATCCAGATGACCCAGAGCCCGAGCAGCCTGAGCGC CAGCGTGGGCGATCGCGTGACCATTACCTGCAGAGCCA GCCAGTCTATTTCTTCTTACCTGGCTTGGTACCAGCAG AAACCGGGCAAAGCGCCGAAACTATTAATCTACGCTGC TTCTTCTCTGCAAAGCGGCGTGCCGAGCCGCTTTAGCG GCAGCGGATCCGGCACCGATTTCACCCTGACCATTAGC TCTCTGCAACCGGAAGACTTTGCGACCTATTATTGCCA GCAGTCTTACGACTACTACACCTTTGGCCAGGGCACGA AAGTTGAAATTAAA
SEQ ID NO: 55	Light Chain	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLAWYQQ KPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCQQSYDYYTFGQGTKVEIKRTVAAPSV FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA LQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 56	DNA Light Chain	GATATCCAGATGACCCAGAGCCCGAGCAGCCTGAGCGC CAGCGTGGGCGATCGCGTGACCATTACCTGCAGAGCCA GCCAGTCTATTTCTTCTTACCTGGCTTGGTACCAGCAG AAACCGGGCAAAGCGCCGAAACTATTAATCTACGCTGC TTCTTCTCTGCAAAGCGGCGTGCCGAGCCGCTTTAGCG GCAGCGGATCCGGCACCGATTTCACCCTGACCATTAGC TCTCTGCAACCGGAAGACTTTGCGACCTATTATTGCCA GCAGTCTTACGACTACTACACCTTTGGCCAGGGCACGA AAGTTGAAATTAAACGTACGGTGGCAGCTCCGTCTGTT

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		TTCATCTTTCCACCTAGCGACGAGCAACTCAAAAGTGG TACAGCATCCGTGGTTTGTCTGCTGAACAATTTTTACC CCAGGGAGGCTAAGGTCCAGTGGAAAGTCGATAACGCT CTTCAGTCTGGCAACAGTCAGGAGAGCGTCACAGAGCA GGACTCTAAGGATAGCACTTATAGTCTGTCCTCCACGC TGACACTGTCTAAAGCGGATTATGAGAAGCACAAGGTT TACGCCTGTGAGGTAACGCACCAAGGACTCTCCTCCCC AGTTACCAAATCTTTCAACAGAGGAGAATGT
M1 E152C_S375C		
SEQ ID NO: 57	HCDR1 (Combined)	GGTFSDYAIS
SEQ ID NO: 58	HCDR2 (Combined)	GIIPIFGDANYAQKFQG
SEQ ID NO: 59	HCDR3 (Combined)	EGSSYFYMAY
SEQ ID NO: 60	HCDR1 (Kabat)	DYAIS
SEQ ID NO: 58	HCDR2 (Kabat)	GIIPIFGDANYAQKFQG
SEQ ID NO: 59	HCDR3 (Kabat)	EGSSYFYMAY
SEQ ID NO: 5	HCDR1 (Chothia)	GGTFSDY
SEQ ID NO: 61	HCDR2 (Chothia)	IPIFGD
SEQ ID NO: 59	HCDR3(Chothia)	EGSSYFYMAY
SEQ ID NO: 7	HCDR1 (IMGT)	GGTFSDYA
SEQ ID NO: 62	HCDR2 (IMGT)	IIPIFGDA
SEQ ID NO: 63	HCDR3 (IMGT)	AREGSSYFYMAY
SEQ ID NO: 64	VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSDYAISWVR QAPGQGLEWMGGIIPIFGDANYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYCAREGSSYFYMAYWGQGTL VTVSS
SEQ ID NO: 65	DNA VH	CAGGTTCAGCTGGTGCAGTCTGGCGCCGAAGTGAAGAA ACCTGGCAGCAGCGTGAAGGTGTCCTGCAAAGCAAGCG GCGGCACCTTCAGCGATTACGCCATCTCTTGGGTCCGA CAGGCCCCTGGACAAGGCTTGGAATGGATGGGCGGCAT CATCCCCATCTTCGGCGACGCCAATTACGCCCAGAAAT TCCAGGGCAGAGTGACCATCACCGCCGACGAGTCTACA AGCACCGCCTACATGGAACTGAGCAGCCTGAGAAGCGA GGACACCGCCGTGTACTACTGTGCCAGAGAGGGCAGCA GCTACTTCTACATGGCCTATTGGGGCCAGGGCACCCTG GTCACAGTTAGCTCT
SEQ ID NO: 66	Heavy Chain	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSDYAISWVR QAPGQGLEWMGGIIPIFGDANYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYCAREGSSYFYMAYWGQGTL VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF PCPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPCDIA VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 67	DNA Heavy Chain	CAGGTTCAGCTGGTGCAGTCTGGCGCCGAAGTGAAGAA ACCTGGCAGCAGCGTGAAGGTGTCCTGCAAAGCAAGCG GCGGCACCTTCAGCGATTACGCCATCTCTTGGGTCCGA CAGGCCCCTGGACAAGGCTTGGAATGGATGGGCGGCAT CATCCCCATCTTCGGCGACGCCAATTACGCCCAGAAAT

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		TCCAGGGCAGAGTGACCATCACCGCCGACGAGTCTACA AGCACCGCCTACATGGAACTGAGCAGCCTGAGAAGCGA GGACACCGCCGTGTACTACTGTGCCAGAGAGGGCAGCA GCTACTTCTACATGGCCTATTGGGGCCAGGGCACCCTG GTCACAGTTAGCTCTGCTAGCACCAAGGGCCCAAGTGT GTTTCCCCTGGCCCCCAGCAGCAAGTCTACTTCCGGCG GAACTGCTGCCCTGGGTTGCCTGGTGAAGGACTACTTC CCCTGTCCCGTGACAGTGTCCTGGAACTCTGGGGCTCT GACTTCCGGCGTGCACACCTTCCCCGCCGTGCTGCAGA GCAGCGGCCTGTACAGCCTGAGCAGCGTGGTGACAGTG CCCTCCAGCTCTCTGGGAACCCAGACCTATATCTGCAA CGTGAACCACAAGCCCAGCAACACCAAGGTGGACAAGA GAGTGGAGCCCAAGAGCTGCGACAAGACCCACACCTGC CCCCCCTGCCCAGCTCCAGAACTGCTGGGAGGGCCTTC CGTGTTCCTGTTCCCCCCCAAGCCCAAGGACACCCTGA TGATCAGCAGGACCCCCGAGGTGACCTGCGTGGTGGTG GACGTGTCCCACGAGGACCCAGAGGTGAAGTTCAACTG GTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCA AGCCCAGAGAGGAGCAGTACAACAGCACCTACAGGGTG GTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAA CGGCAAAGAATACAAGTGCAAAGTCTCCAACAAGGCCC TGCCAGCCCCAATCGAAAAGACAATCAGCAAGGCCAAG GGCCAGCCACGGGAGCCCCAGGTGTACACCCTGCCCCC CAGCCGGGAGGAGATGACCAAGAACCAGGTGTCCCTGA CCTGTCTGGTGAAGGGCTTCTACCCCTGTGATATCGCC GTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTA CAAGACCACCCCCCCAGTGCTGGACAGCGACGGCAGCT TCTTCCTGTACAGCAAGCTGACCGTGGACAAGTCCAGG TGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCA CGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGA GCCTGAGCCCCGGCAAG
SEQ ID NO: 68	LCDR1 (Combined)	SGDNIGSKLAS
SEQ ID NO: 69	LCDR2 (Combined)	DDSNRPS
SEQ ID NO: 70	LCDR3 (Combined)	AATAGDRWAYV
SEQ ID NO: 68	LCDR1 (Kabat)	SGDNIGSKLAS
SEQ ID NO: 69	LCDR2 (Kabat)	DDSNRPS
SEQ ID NO: 70	LCDR3 (Kabat)	AATAGDRWAYV
SEQ ID NO: 71	LCDR1 (Chothia)	DNIGSKL
SEQ ID NO: 72	LCDR2 (Chothia)	DDS
SEQ ID NO: 73	LCDR3 (Chothia)	TAGDRWAY
SEQ ID NO: 74	LCDR1 (IMGT)	NIGSKL
SEQ ID NO: 72	LCDR2 (IMGT)	DDS
SEQ ID NO: 70	LCDR3 (IMGT)	AATAGDRWAYV
SEQ ID NO: 75	VL	SYELTQPLSVSVALGQTARITCSGDNIGSKLASWYQQK PGQAPVLVIYDDSNRPSGIPERFSGSNSGNTATLTISR AQAGDEADYYCAATAGDRWAYVFGGGTKLTVL
SEQ ID NO: 76	DNA VL	AGCTATGAGCTGACACAGCCTCTGTCCGTGTCTGTGGC TCTGGGACAGACCGCCAGAATCACCTGTAGCGGCGACA ACATCGGCAGCAAGCTGGCCTCTTGGTATCAGCAGAAG CCTGGACAGGCCCCTGTGCTGGTCATCTACGACGACAG CAATAGACCCAGCGGCATCCCCGAGAGATTCAGCGGCA GCAATAGCGGCAATACCGCCACACTGACCATCAGCAGA

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		GCACAGGCTGGCGACGAGGCCGATTACTATTGTGCTGC CACAGCCGGCGACAGATGGGCCTATGTTTTTGGCGGCG GAACAAAGCTGACCGTGCTG
SEQ ID NO: 77	Light Chain	SYELTQPLSVSVALGQTARITCSGDNIGSKLASWYQQK PGQAPVLVIYDDSNRPSGIPERFSGSNSGNTATLTISR AQAGDEADYYCAATAGDRWAYVFGGGTKLTVLGQPKAA PSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKA DSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSH RSYSCQVTHEGSTVEKTVAPTECS
SEQ ID NO: 78	DNA Light Chain	AGCTATGAGCTGACACAGCCTCTGTCCGTGTCTGTGGC TCTGGGACAGACCGCCAGAATCACCTGTAGCGGCGACA ACATCGGCAGCAAGCTGGCCTCTTGGTATCAGCAGAAG CCTGGACAGGCCCCTGTGCTGGTCATCTACGACGACAG CAATAGACCCAGCGGCATCCCCGAGAGATTCAGCGGCA GCAATAGCGGCAATACCGCCACACTGACCATCAGCAGA GCACAGGCTGGCGACGAGGCCGATTACTATTGTGCTGC CACAGCCGGCGACAGATGGGCCTATGTTTTTGGCGGCG GAACAAAGCTGACCGTGCTGGGACAGCCTAAGGCCGCT CCCTCCGTGACCCTGTTCCCCCCCCAGCTCCGAGGAACT GCAGGCCAACAAGGCCACCCTGGTGTGCCTGATCAGCG ACTTCTACCCTGGCGCCGTGACCGTGGCCTGGAAGGCC GACAGCAGCCCCGTGAAGGCCGGCGTGGAGACAACCAC CCCCAGCAAGCAGAGCAACAACAAGTACGCCGCCAGCA GCTACCTGAGCCTGACCCCCGAGCAGTGGAAGAGCCAC AGAAGCTACAGCTGCCAGGTCACCCACGAGGGCAGCAC CGTGGAGAAAACCGTGGCCCCCACCGAGTGCAGC
M2 E152C_S375C		
SEQ ID NO: 79	HCDR1 (Combined)	GFTFSSFGMS
SEQ ID NO: 80	HCDR2 (Combined)	AISYSGSDTYYADSVKG
SEQ ID NO: 81	HCDR3 (Combined)	DVGVMDY
SEQ ID NO: 82	HCDR1 (Kabat)	SFGMS
SEQ ID NO: 80	HCDR2 (Kabat)	AISYSGSDTYYADSVKG
SEQ ID NO: 81	HCDR3 (Kabat)	DVGVMDY
SEQ ID NO: 83	HCDR1 (Chothia)	GFTFSSF
SEQ ID NO: 84	HCDR2 (Chothia)	SYSGSD
SEQ ID NO: 81	HCDR3(Chothia)	DVGVMDY
SEQ ID NO: 85	HCDR1 (IMGT)	GFTFSSFG
SEQ ID NO: 86	HCDR2 (IMGT)	ISYSGSDT
SEQ ID NO: 87	HCDR3 (IMGT)	ARDVGVMDY
SEQ ID NO: 88	VH	QVQLLESGGGLVQPGGSLRLSCAASGFTFSSFGMSWVR QAPGKGLEWVSAISYSGSDTYYADSVKGRFTISRDNSK NTLYLQMNSLRAEDTAVYYCARDVGVMDYWGQGTLVTV SS
SEQ ID NO: 89	DNA VH	CAGGTTCAGCTGCTGGAATCTGGCGGAGGACTGGTTCA ACCTGGCGGCTCTCTGAGACTGTCTTGTGCCGCCAGCG GCTTCACCTTCAGCAGCTTTGGCATGAGCTGGGTCCGA CAGGCCCCTGGCAAAGGACTTGAATGGGTGTCCGCCAT CAGCTACAGCGGCAGCGATACCTACTACGCCGACAGCG TGAAGGGCAGATTCACCATCTCCAGAGACAACAGCAAG AACACCCTGTACCTGCAGATGAACAGCCTGAGAGCCGA GGACACCGCCGTGTACTACTGTGCCAGAGATGTGGGCG

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		TGATGGACTATTGGGGCCAGGGCACACTGGTCACCGTT AGCTCT
SEQ ID NO: 90	Heavy Chain	QVQLLESGGGLVQPGGSLRLSCAASGFTFSSFGMSWVR QAPGKGLEWVSAISYSGSDTYYADSVKGRFTISRDNSK NTLYLQMNSLRAEDTAVYYCARDVGVMDYWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPCP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPC PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSREEMTKNQVSLTCLVKGFYPCDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 91	DNA Heavy Chain	CAGGTTCAGCTGCTGGAATCTGGCGGAGGACTGGTTCA ACCTGGCGGCTCTCTGAGACTGTCTTGTGCCGCCAGCG GCTTCACCTTCAGCAGCTTTGGCATGAGCTGGGTCCGA CAGGCCCCTGGCAAAGGACTTGAATGGGTGTCCGCCAT CAGCTACAGCGGCAGCGATACCTACTACGCCGACAGCG TGAAGGGCAGATTCACCATCTCCAGAGACAACAGCAAG AACACCCTGTACCTGCAGATGAACAGCCTGAGAGCCGA GGACACCGCCGTGTACTACTGTGCCAGAGATGTGGGCG TGATGGACTATTGGGGCCAGGGCACACTGGTCACCGTT AGCTCTGCTAGCACCAAGGGCCCAAGTGTGTTTCCCCT GGCCCCCAGCAGCAAGTCTACTTCCGGCGGAACTGCTG CCCTGGGTTGCCTGGTGAAGGACTACTTCCCCTGTCCC GTGACAGTGTCCTGGAACTCTGGGGCTCTGACTTCCGG CGTGCACACCTTCCCCGCCGTGCTGCAGAGCAGCGGCC TGTACAGCCTGAGCAGCGTGGTGACAGTGCCCTCCAGC TCTCTGGGAACCCAGACCTATATCTGCAACGTGAACCA CAAGCCCAGCAACACCAAGGTGGACAAGAGAGTGGAGC CCAAGAGCTGCGACAAGACCCACACCTGCCCCCCCTGC CCAGCTCCAGAACTGCTGGGAGGGCCTTCCGTGTTCCT GTTCCCCCCCAAGCCCAAGGACACCCTGATGATCAGCA GGACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGTCC CACGAGGACCCAGAGGTGAAGTTCAACTGGTACGTGGA CGGCGTGGAGGTGCACAACGCCAAGACCAAGCCCAGAG AGGAGCAGTACAACAGCACCTACAGGGTGGTGTCCGTG CTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGA ATACAAGTGCAAAGTCTCCAACAAGGCCCTGCCAGCCC CAATCGAAAAGACAATCAGCAAGGCCAAGGGCCAGCCA CGGGAGCCCCAGGTGTACACCCTGCCCCCCAGCCGGGA GGAGATGACCAAGAACCAGGTGTCCCTGACCTGTCTGG TGAAGGGCTTCTACCCCTGTGATATCGCCGTGGAGTGG GAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCAC CCCCCCAGTGCTGGACAGCGACGGCAGCTTCTTCCTGT ACAGCAAGCTGACCGTGGACAAGTCCAGGTGGCAGCAG GGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCT GCACAACCACTACACCCAGAAGTCCCTGAGCCTGAGCC CCGGCAAG
SEQ ID NO: 92	LCDR1 (Combined)	SGDNLGTYYAH
SEQ ID NO: 93	LCDR2 (Combined)	SQSHRPS
SEQ ID NO: 94	LCDR3 (Combined)	GAWDAPSPELV
SEQ ID NO: 92	LCDR1 (Kabat)	SGDNLGTYYAH

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SEQ ID NO: 93	LCDR2 (Kabat)	SQSHRPS
SEQ ID NO: 94	LCDR3 (Kabat)	GAWDAPSPELV
SEQ ID NO: 95	LCDR1 (Chothia)	DNLGTYY
SEQ ID NO: 96	LCDR2 (Chothia)	SQS
SEQ ID NO: 97	LCDR3 (Chothia)	WDAPSPEL
SEQ ID NO: 98	LCDR1 (IMGT)	NLGTYY
SEQ ID NO: 96	LCDR2 (IMGT)	SQS
SEQ ID NO: 94	LCDR3 (IMGT)	GAWDAPSPELV
SEQ ID NO: 99	VL	SYELTQPLSVSVALGQTARITCSGDNLGTYYAHWYQQK PGQAPVLVIYSQSHRPSGIPERFSGSNSGNTATLTISR AQAGDEADYYCGAWDAPSPELVFGGGTKLTVL
SEQ ID NO: 100	DNA VL	AGCTATGAGCTGACACAGCCTCTGTCCGTGTCTGTGGC TCTGGGACAGACCGCCAGAATCACCTGTAGCGGCGATA ACCTGGGCACCTACTACGCCCACTGGTATCAGCAGAAG CCTGGACAGGCTCCCGTGCTGGTCATCTACAGCCAGTC TCACAGACCCAGCGGCATCCCCGAGAGATTCAGCGGCA GCAATAGCGGCAATACCGCCACACTGACCATCAGCAGA GCACAGGCTGGCGACGAGGCCGATTACTATTGTGGCGC TTGGGACGCCCCATCTCCTGAGCTTGTTTTTGGCGGAG GCACCAAGCTGACAGTGCTG
SEQ ID NO: 101	Light Chain	SYELTQPLSVSVALGQTARITCSGDNLGTYYAHWYQQK PGQAPVLVIYSQSHRPSGIPERFSGSNSGNTATLTISR AQAGDEADYYCGAWDAPSPELVFGGGTKLTVLGQPKAA PSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKA DSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSH RSYSCQVTHEGSTVEKTVAPTECS
SEQ ID NO: 102	DNA Light Chain	AGCTATGAGCTGACACAGCCTCTGTCCGTGTCTGTGGC TCTGGGACAGACCGCCAGAATCACCTGTAGCGGCGATA ACCTGGGCACCTACTACGCCCACTGGTATCAGCAGAAG CCTGGACAGGCTCCCGTGCTGGTCATCTACAGCCAGTC TCACAGACCCAGCGGCATCCCCGAGAGATTCAGCGGCA GCAATAGCGGCAATACCGCCACACTGACCATCAGCAGA GCACAGGCTGGCGACGAGGCCGATTACTATTGTGGCGC TTGGGACGCCCCATCTCCTGAGCTTGTTTTTGGCGGAG GCACCAAGCTGACAGTGCTGGGACAGCCTAAGGCCGCT CCCTCCGTGACCCTGTTCCCCCCCAGCTCCGAGGAACT GCAGGCCAACAAGGCCACCCTGGTGTGCCTGATCAGCG ACTTCTACCCTGGCGCCGTGACCGTGGCCTGGAAGGCC GACAGCAGCCCCGTGAAGGCCGGCGTGGAGACAACCAC CCCCAGCAAGCAGAGCAACAACAAGTACGCCGCCAGCA GCTACCTGAGCCTGACCCCCGAGCAGTGGAAGAGCCAC AGAAGCTACAGCTGCCAGGTCACCCACGAGGGCAGCAC CGTGGAGAAAACCGTGGCCCCCACCGAGTGCAGC
Y1 E152C_S375C		
SEQ ID NO: 103	HCDR1 (Combined)	GFTFSSYAMS
SEQ ID NO: 104	HCDR2 (Combined)	AISGSGGSTYYADSVKG
SEQ ID NO: 105	HCDR3 (Combined)	AFRLYWLDV
SEQ ID NO: 106	HCDR1 (Kabat)	SYAMS
SEQ ID NO: 104	HCDR2 (Kabat)	AISGSGGSTYYADSVKG
SEQ ID NO: 105	HCDR3 (Kabat)	AFRLYWLDV
SEQ ID NO: 107	HCDR1 (Chothia)	GFTFSSY

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SEQ ID NO: 108	HCDR2 (Chothia)	SGSGGS
SEQ ID NO: 105	HCDR3(Chothia)	AFRLYWLDV
SEQ ID NO: 109	HCDR1 (IMGT)	GFTFSSYA
SEQ ID NO: 110	HCDR2 (IMGT)	ISGSGGST
SEQ ID NO: 111	HCDR3 (IMGT)	ARAFRLYWLDV
SEQ ID NO: 112	VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVR QAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSK NTLYLQMNSLRAEDTAVYYCARAFRLYWLDVWGQGTLV TVSS
SEQ ID NO: 113	DNA VH	GAAGTTCAGCTGCTGGAATCTGGCGGAGGACTGGTTCA ACCTGGCGGCTCTCTGAGACTGTCTTGTGCCGCCAGCG GCTTCACCTTTAGCAGCTACGCCATGAGCTGGGTCCGA CAGGCTCCTGGCAAAGGCCTTGAATGGGTGTCCGCCAT CTCTGGCTCTGGCGGCAGCACATATTACGCCGACTCTG TGAAGGGCAGATTCACCATCAGCCGGGACAACAGCAAG AACACCCTGTACCTGCAGATGAACAGCCTGAGAGCCGA GGACACCGCCGTGTACTATTGTGCCAGAGCCTTCCGGC TGTACTGGCTGGATGTTTGGGGACAGGGCACCCTGGTC ACAGTGTCATCT
SEQ ID NO: 114	Heavy Chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVR QAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSK NTLYLQMNSLRAEDTAVYYCARAFRLYWLDVWGQGTLV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP CPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPCDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 115	DNA Heavy Chain	GAAGTTCAGCTGCTGGAATCTGGCGGAGGACTGGTTCA ACCTGGCGGCTCTCTGAGACTGTCTTGTGCCGCCAGCG GCTTCACCTTTAGCAGCTACGCCATGAGCTGGGTCCGA CAGGCTCCTGGCAAAGGCCTTGAATGGGTGTCCGCCAT CTCTGGCTCTGGCGGCAGCACATATTACGCCGACTCTG TGAAGGGCAGATTCACCATCAGCCGGGACAACAGCAAG AACACCCTGTACCTGCAGATGAACAGCCTGAGAGCCGA GGACACCGCCGTGTACTATTGTGCCAGAGCCTTCCGGC TGTACTGGCTGGATGTTTGGGGACAGGGCACCCTGGTC ACAGTGTCATCTGCTAGCACCAAGGGCCCAAGTGTGTT TCCCCTGGCCCCCAGCAGCAAGTCTACTTCCGGCGGAA CTGCTGCCCTGGGTTGCCTGGTGAAGGACTACTTCCCC TGTCCCGTGACAGTGTCCTGGAACTCTGGGGCTCTGAC TTCCGGCGTGCACACCTTCCCCGCCGTGCTGCAGAGCA GCGGCCTGTACAGCCTGAGCAGCGTGGTGACAGTGCCC TCCAGCTCTCTGGGAACCCAGACCTATATCTGCAACGT GAACCACAAGCCCAGCAACACCAAGGTGGACAAGAGAG TGGAGCCCAAGAGCTGCGACAAGACCCACACCTGCCCC CCCTGCCCAGCTCCAGAACTGCTGGGAGGGCCTTCCGT GTTCCTGTTCCCCCCCAAGCCCAAGGACACCCTGATGA TCAGCAGGACCCCCGAGGTGACCTGCGTGGTGGTGGAC GTGTCCCACGAGGACCCAGAGGTGAAGTTCAACTGGTA CGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGC

Date Reçue/Date Received 2024-04-11

		CCAGAGAGGAGCAGTACAACAGCACCTACAGGGTGGTG TCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGG CAAAGAATACAAGTGCAAAGTCTCCAACAAGGCCCTGC CAGCCCCAATCGAAAAGACAATCAGCAAGGCCAAGGGC CAGCCACGGGAGCCCCAGGTGTACACCCTGCCCCCCAG CCGGGAGGAGATGACCAAGAACCAGGTGTCCCTGACCT GTCTGGTGAAGGGCTTCTACCCCTGTGATATCGCCGTG GAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAA GACCACCCCCCCAGTGCTGGACAGCGACGGCAGCTTCT TCCTGTACAGCAAGCTGACCGTGGACAAGTCCAGGTGG CAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGA GGCCCTGCACAACCACTACACCCAGAAGTCCCTGAGCC TGAGCCCCGGCAAG
SEQ ID NO: 116	LCDR1 (Combined)	RASQSISSYLN
SEQ ID NO: 47	LCDR2 (Combined)	AASSLQS
SEQ ID NO: 117	LCDR3 (Combined)	QQVYSAPVT
SEQ ID NO: 116	LCDR1 (Kabat)	RASQSISSYLN
SEQ ID NO: 47	LCDR2 (Kabat)	AASSLQS
SEQ ID NO: 117	LCDR3 (Kabat)	QQVYSAPVT
SEQ ID NO: 49	LCDR1 (Chothia)	SQSISSY
SEQ ID NO: 50	LCDR2 (Chothia)	AAS
SEQ ID NO: 118	LCDR3 (Chothia)	VYSAPV
SEQ ID NO: 52	LCDR1 (IMGT)	QSISSY
SEQ ID NO: 50	LCDR2 (IMGT)	AAS
SEQ ID NO: 117	LCDR3 (IMGT)	QQVYSAPVT
SEQ ID NO: 119	VL	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQ KPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCQQVYSAPVTFGQGTKVEIK
SEQ ID NO: 120	DNA VL	GACATCCAGATGACACAGAGCCCTAGCAGCCTGTCTGC CAGCGTGGGAGACAGAGTGACCATCACCTGTAGAGCCA GCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAG AAGCCCGGCAAGGCCCCTAAACTGCTGATCTATGCCGC CAGCTCTCTGCAGTCTGGCGTGCCAAGCAGATTTTCTG GCAGCGGCTCTGGCACCGACTTCACCCTGACCATATCT AGCCTGCAGCCAGAGGACTTCGCCACCTACTACTGCCA GCAGGTCTACAGCGCCCCTGTGACATTTGGCCAGGGCA CCAAGGTGGAAATCAAG
SEQ ID NO: 121	Light Chain	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQ KPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCQQVYSAPVTFGQGTKVEIKRTVAAPS VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 122	DNA Light Chain	GACATCCAGATGACACAGAGCCCTAGCAGCCTGTCTGC CAGCGTGGGAGACAGAGTGACCATCACCTGTAGAGCCA GCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAG AAGCCCGGCAAGGCCCCTAAACTGCTGATCTATGCCGC CAGCTCTCTGCAGTCTGGCGTGCCAAGCAGATTTTCTG GCAGCGGCTCTGGCACCGACTTCACCCTGACCATATCT AGCCTGCAGCCAGAGGACTTCGCCACCTACTACTGCCA GCAGGTCTACAGCGCCCCTGTGACATTTGGCCAGGGCA CCAAGGTGGAAATCAAGCGTACGGTGGCCGCTCCCAGC

Date Reçue/Date Received 2024-04-11

		GTGTTCATCTTCCCCCCCAGCGACGAGCAGCTGAAGAG TGGCACCGCCAGCGTGGTGTGCCTGCTGAACAACTTCT ACCCCCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAAC GCCCTGCAGAGCGGCAACAGCCAGGAGAGCGTCACCGA GCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCA CCCTGACCCTGAGCAAGGCCGACTACGAGAAGCATAAG GTGTACGCCTGCGAGGTGACCCACCAGGGCCTGTCCAG CCCCGTGACCAAGAGCTTCAACAGGGGCGAGTGC
Y2 E152C_S375C		
SEQ ID NO: 123	HCDR1 (Combined)	GFTFSNYWIS
SEQ ID NO: 124	HCDR2 (Combined)	RIKSKTYGGTTDYAEPVKG
SEQ ID NO: 125	HCDR3 (Combined)	TSRRSYAFDY
SEQ ID NO: 126	HCDR1 (Kabat)	NYWIS
SEQ ID NO: 124	HCDR2 (Kabat)	RIKSKTYGGTTDYAEPVKG
SEQ ID NO: 125	HCDR3 (Kabat)	TSRRSYAFDY
SEQ ID NO: 127	HCDR1 (Chothia)	GFTFSNY
SEQ ID NO: 128	HCDR2 (Chothia)	KSKTYGGT
SEQ ID NO: 125	HCDR3(Chothia)	TSRRSYAFDY
SEQ ID NO: 129	HCDR1 (IMGT)	GFTFSNYW
SEQ ID NO: 130	HCDR2 (IMGT)	IKSKTYGGTT
SEQ ID NO: 131	HCDR3 (IMGT)	ARTSRRSYAFDY
SEQ ID NO: 132	VH	EVQLVESGGGLVKPGGSLRLSCAASGFTFSNYWISWVR QAPGKGLEWVGRIKSKTYGGTTDYAEPVKGRFTISRDD SKNTLYLQMNSLKTEDTAVYYCARTSRRSYAFDYWGQG TLVTVSS
SEQ ID NO: 133	DNA VH	GAAGTGCAGCTGGTGGAATCTGGCGGCGGACTTGTGAA ACCTGGCGGCTCTCTGAGACTGAGCTGTGCCGCTTCCG GCTTCACCTTCAGCAACTACTGGATCAGCTGGGTCCGA CAGGCCCCTGGCAAAGGACTTGAGTGGGTCGGACGGAT CAAGAGCAAGACCTACGGCGGCACCACCGATTATGCCG AGCCTGTGAAGGGCAGATTCACCATCAGCCGGGACGAC AGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGAA AACCGAGGACACCGCCGTGTACTACTGCGCCAGAACCA GCAGAAGAAGCTACGCCTTCGACTACTGGGGCCAGGGC ACACTGGTTACCGTTAGCTCT
SEQ ID NO: 134	Heavy Chain	EVQLVESGGGLVKPGGSLRLSCAASGFTFSNYWISWVR QAPGKGLEWVGRIKSKTYGGTTDYAEPVKGRFTISRDD SKNTLYLQMNSLKTEDTAVYYCARTSRRSYAFDYWGQG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD YFPCPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPCD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 135	DNA Heavy Chain	GAAGTGCAGCTGGTGGAATCTGGCGGCGGACTTGTGAA ACCTGGCGGCTCTCTGAGACTGAGCTGTGCCGCTTCCG GCTTCACCTTCAGCAACTACTGGATCAGCTGGGTCCGA CAGGCCCCTGGCAAAGGACTTGAGTGGGTCGGACGGAT CAAGAGCAAGACCTACGGCGGCACCACCGATTATGCCG

Date Reçue/Date Received 2024-04-11

		AGCCTGTGAAGGGCAGATTCACCATCAGCCGGGACGAC AGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGAA AACCGAGGACACCGCCGTGTACTACTGCGCCAGAACCA GCAGAAGAAGCTACGCCTTCGACTACTGGGGCCAGGGC ACACTGGTTACCGTTAGCTCTGCTAGCACCAAGGGCCC AAGTGTGTTTCCCCTGGCCCCCAGCAGCAAGTCTACTT CCGGCGGAACTGCTGCCCTGGGTTGCCTGGTGAAGGAC TACTTCCCCTGTCCCGTGACAGTGTCCTGGAACTCTGG GGCTCTGACTTCCGGCGTGCACACCTTCCCCGCCGTGC TGCAGAGCAGCGGCCTGTACAGCCTGAGCAGCGTGGTG ACAGTGCCCTCCAGCTCTCTGGGAACCCAGACCTATAT CTGCAACGTGAACCACAAGCCCAGCAACACCAAGGTGG ACAAGAGAGTGGAGCCCAAGAGCTGCGACAAGACCCAC ACCTGCCCCCCCTGCCCAGCTCCAGAACTGCTGGGAGG GCCTTCCGTGTTCCTGTTCCCCCCCAAGCCCAAGGACA CCCTGATGATCAGCAGGACCCCCGAGGTGACCTGCGTG GTGGTGGACGTGTCCCACGAGGACCCAGAGGTGAAGTT CAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCA AGACCAAGCCCAGAGAGGAGCAGTACAACAGCACCTAC AGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTG GCTGAACGGCAAAGAATACAAGTGCAAAGTCTCCAACA AGGCCCTGCCAGCCCCAATCGAAAAGACAATCAGCAAG GCCAAGGGCCAGCCACGGGAGCCCCAGGTGTACACCCT GCCCCCCAGCCGGGAGGAGATGACCAAGAACCAGGTGT CCCTGACCTGTCTGGTGAAGGGCTTCTACCCCTGTGAT ATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAA CAACTACAAGACCACCCCCCCCAGTGCTGGACAGCGACG GCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAG TCCAGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGT GATGCACGAGGCCCTGCACAACCACTACACCCAGAAGT CCCTGAGCCTGAGCCCCGGCAAG
SEQ ID NO: 136	LCDR1 (Combined)	RASQSISSWLA
SEQ ID NO: 137	LCDR2 (Combined)	DASSLES
SEQ ID NO: 138	LCDR3 (Combined)	QQITRYPVT
SEQ ID NO: 136	LCDR1 (Kabat)	RASQSISSWLA
SEQ ID NO: 137	LCDR2 (Kabat)	DASSLES
SEQ ID NO: 138	LCDR3 (Kabat)	QQITRYPVT
SEQ ID NO: 139	LCDR1 (Chothia)	SQSISSW
SEQ ID NO: 140	LCDR2 (Chothia)	DAS
SEQ ID NO: 141	LCDR3 (Chothia)	ITRYPV
SEQ ID NO: 142	LCDR1 (IMGT)	QSISSW
SEQ ID NO: 140	LCDR2 (IMGT)	DAS
SEQ ID NO: 138	LCDR3 (IMGT)	QQITRYPVT
SEQ ID NO: 143	VL	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQ KPGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTIS SLQPEDFATYYCQQITRYPVTFGQGTKVEIK
SEQ ID NO: 144	DNA VL	GACATCCAGATGACACAGAGCCCCAGCACACTGTCTGC CAGCGTGGGAGACAGAGTGACCATCACCTGTAGAGCCA GCCAGAGCATCTCCTCTTGGCTGGCCTGGTATCAGCAG AAGCCTGGCAAGGCCCCTAAGCTGCTGATCTACGATGC CAGCAGCCTGGAAAGCGGCGTGCCAAGCAGATTTTCTG GCAGCGGCTCTGGCACCGAGTTCACCCTGACCATATCT

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		AGCCTGCAGCCAGAGGACTTCGCCACCTACTACTGCCA GCAGATCACAAGATACCCCGTGACCTTTGGCCAGGGCA CCAAGGTGGAAATCAAG
SEQ ID NO: 145	Light Chain	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQ KPGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTIS SLQPEDFATYYCQQITRYPVTFGQGTKVEIKRTVAAPS VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 146	DNA Light Chain	GACATCCAGATGACACAGAGCCCCAGCACACTGTCTGC CAGCGTGGGAGACAGAGTGACCATCACCTGTAGAGCCA GCCAGAGCATCTCCTCTTGGCTGGCCTGGTATCAGCAG AAGCCTGGCAAGGCCCCTAAGCTGCTGATCTACGATGC CAGCAGCCTGGAAAGCGGCGTGCCAAGCAGATTTTCTG GCAGCGGCTCTGGCACCGAGTTCACCCTGACCATATCT AGCCTGCAGCCAGAGGACTTCGCCACCTACTACTGCCA GCAGATCACAAGATACCCCGTGACCTTTGGCCAGGGCA CCAAGGTGGAAATCAAGCGTACGGTGGCCGCTCCCAGC GTGTTCATCTTCCCCCCCAGCGACGAGCAGCTGAAGAG TGGCACCGCCAGCGTGGTGTGCCTGCTGAACAACTTCT ACCCCCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAAC GCCCTGCAGAGCGGCAACAGCCAGGAGAGCGTCACCGA GCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCA CCCTGACCCTGAGCAAGGCCGACTACGAGAAGCATAAG GTGTACGCCTGCGAGGTGACCCACCAGGGCCTGTCCAG CCCCGTGACCAAGAGCTTCAACAGGGGCGAGTGC
Y3 E152C_S375C		
SEQ ID NO: 123	HCDR1 (Combined)	GFTFSNYWIS
SEQ ID NO: 124	HCDR2 (Combined)	RIKSKTYGGTTDYAEPVKG
SEQ ID NO: 147	HCDR3 (Combined)	VSGYYSHSGGFDV
SEQ ID NO: 126	HCDR1 (Kabat)	NYWIS
SEQ ID NO: 124	HCDR2 (Kabat)	RIKSKTYGGTTDYAEPVKG
SEQ ID NO: 147	HCDR3 (Kabat)	VSGYYSHSGGFDV
SEQ ID NO: 127	HCDR1 (Chothia)	GFTFSNY
SEQ ID NO: 128	HCDR2 (Chothia)	KSKTYGGT
SEQ ID NO: 147	HCDR3(Chothia)	VSGYYSHSGGFDV
SEQ ID NO: 129	HCDR1 (IMGT)	GFTFSNYW
SEQ ID NO: 130	HCDR2 (IMGT)	IKSKTYGGTT
SEQ ID NO: 148	HCDR3 (IMGT)	ARVSGYYSHSGGFDV
SEQ ID NO: 149	VH	EVQLVESGGGLVKPGGSLRLSCAASGFTFSNYWISWVR QAPGKGLEWVGRIKSKTYGGTTDYAEPVKGRFTISRDD SKNTLYLQMNSLKTEDTAVYYCARVSGYYSHSGGFDVW GQGTLVTVSS
SEQ ID NO: 150	DNA VH	GAAGTGCAGCTGGTGGAATCTGGCGGCGGACTTGTGAA ACCTGGCGGCTCTCTGAGACTGAGCTGTGCCGCTTCCG GCTTCACCTTCAGCAACTACTGGATCAGCTGGGTCCGA CAGGCCCCTGGCAAAGGACTTGAGTGGGTCGGACGGAT CAAGAGCAAGACCTACGGCGGCACCACCGATTATGCCG AGCCTGTGAAGGGCAGATTCACCATCAGCCGGGACGAC AGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGAA AACCGAGGACACCGCCGTGTACTACTGCGCCAGAGTGT

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		CTGGCTACTACTCTCACAGCGGCGGCTTTGATGTGTGG GGCCAGGGAACACTGGTCACCGTTAGTTCT
SEQ ID NO: 151	Heavy Chain	EVQLVESGGGLVKPGGSLRLSCAASGFTFSNYWISWVR QAPGKGLEWVGRIKSKTYGGTTDYAEPVKGRFTISRDD SKNTLYLQMNSLKTEDTAVYYCARVSGYYSHSGGFDVW GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPCPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCD KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY PCDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 152	DNA Heavy Chain	GAAGTGCAGCTGGTGGAATCTGGCGGCGGACTTGTGAA ACCTGGCGGCTCTCTGAGACTGAGCTGTGCCGCTTCCG GCTTCACCTTCAGCAACTACTGGATCAGCTGGGTCCGA CAGGCCCCTGGCAAAGGACTTGAGTGGGTCGGACGGAT CAAGAGCAAGACCTACGGCGGCACCACCGATTATGCCG AGCCTGTGAAGGGCAGATTCACCATCAGCCGGGACGAC AGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGAA AACCGAGGACACCGCCGTGTACTACTGCGCCAGAGTGT CTGGCTACTACTCTCACAGCGGCGGCTTTGATGTGTGG GGCCAGGGAACACTGGTCACCGTTAGTTCTGCTAGCAC CAAGGGCCCAAGTGTGTTTCCCCTGGCCCCCAGCAGCA AGTCTACTTCCGGCGGAACTGCTGCCCTGGGTTGCCTG GTGAAGGACTACTTCCCCTGTCCCGTGACAGTGTCCTG GAACTCTGGGGCTCTGACTTCCGGCGTGCACACCTTCC CCGCCGTGCTGCAGAGCAGCGGCCTGTACAGCCTGAGC AGCGTGGTGACAGTGCCCTCCAGCTCTCTGGGAACCCA GACCTATATCTGCAACGTGAACCACAAGCCCAGCAACA CCAAGGTGGACAAGAGAGTGGAGCCCAAGAGCTGCGAC AAGACCCACACCTGCCCCCCCTGCCCAGCTCCAGAACT GCTGGGAGGGCCTTCCGTGTTCCTGTTCCCCCCCAAGC CCAAGGACACCCTGATGATCAGCAGGACCCCCGAGGTG ACCTGCGTGGTGGTGGACGTGTCCCACGAGGACCCAGA GGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGC ACAACGCCAAGACCAAGCCCAGAGAGGAGCAGTACAAC AGCACCTACAGGGTGGTGTCCGTGCTGACCGTGCTGCA CCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAAG TCTCCAACAAGGCCCTGCCAGCCCCAATCGAAAAGACA ATCAGCAAGGCCAAGGGCCAGCCACGGGAGCCCCAGGT GTACACCCTGCCCCCCAGCCGGGAGGAGATGACCAAGA ACCAGGTGTCCCTGACCTGTCTGGTGAAGGGCTTCTAC CCCTGTGATATCGCCGTGGAGTGGGAGAGCAACGGCCA GCCCGAGAACAACTACAAGACCACCCCCCCAGTGCTGG ACAGCGACGGCAGCTTCTTCCTGTACAGCAAGCTGACC GTGGACAAGTCCAGGTGGCAGCAGGGCAACGTGTTCAG CTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACA CCCAGAAGTCCCTGAGCCTGAGCCCCGGCAAG
SEQ ID NO: 153	LCDR1 (Combined)	RASQGISNYLA
SEQ ID NO: 154	LCDR2 (Combined)	AASTLQS
SEQ ID NO: 155	LCDR3 (Combined)	QKTWRTPGT
SEQ ID NO: 153	LCDR1 (Kabat)	RASQGISNYLA

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SEQ ID NO: 154	LCDR2 (Kabat)	AASTLQS
SEQ ID NO: 155	LCDR3 (Kabat)	QKTWRTPGT
SEQ ID NO: 156	LCDR1 (Chothia)	SQGISNY
SEQ ID NO: 50	LCDR2 (Chothia)	AAS
SEQ ID NO: 157	LCDR3 (Chothia)	TWRTPG
SEQ ID NO: 158	LCDR1 (IMGT)	QGISNY
SEQ ID NO: 50	LCDR2 (IMGT)	AAS
SEQ ID NO: 155	LCDR3 (IMGT)	QKTWRTPGT
SEQ ID NO: 159	VL	DIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQ KPGKVPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTIS SLQPEDVATYYCQKTWRTPGTFGQGTKVEIK
SEQ ID NO: 160	DNA VL	GACATCCAGATGACACAGAGCCCTAGCAGCCTGTCTGC CAGCGTGGGAGACAGAGTGACCATCACCTGTAGAGCCA GCCAGGGCATCAGCAACTACCTGGCCTGGTATCAGCAG AAACCCGGCAAGGTGCCCAAGCTGCTGATCTACGCTGC CAGCACACTGCAGAGCGGAGTGCCTAGCAGATTTTCTG GCAGCGGCTCCGGCACCGATTTCACCCTGACCATATCT AGCCTGCAGCCAGAGGACGTGGCCACCTACTACTGTCA GAAAACCTGGCGGACCCCTGGCACATTTGGCCAGGGAA CAAAGGTGGAAATCAAG
SEQ ID NO: 161	Light Chain	DIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQ KPGKVPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTIS SLQPEDVATYYCQKTWRTPGTFGQGTKVEIKRTVAAPS VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 162	DNA Light Chain	GACATCCAGATGACACAGAGCCCTAGCAGCCTGTCTGC CAGCGTGGGAGACAGAGTGACCATCACCTGTAGAGCCA GCCAGGGCATCAGCAACTACCTGGCCTGGTATCAGCAG AAACCCGGCAAGGTGCCCAAGCTGCTGATCTACGCTGC CAGCACACTGCAGAGCGGAGTGCCTAGCAGATTTTCTG GCAGCGGCTCCGGCACCGATTTCACCCTGACCATATCT AGCCTGCAGCCAGAGGACGTGGCCACCTACTACTGTCA GAAAACCTGGCGGACCCCTGGCACATTTGGCCAGGGAA CAAAGGTGGAAATCAAGCGTACGGTGGCCGCTCCCAGC GTGTTCATCTTCCCCCCCAGCGACGAGCAGCTGAAGAG TGGCACCGCCAGCGTGGTGTGCCTGCTGAACAACTTCT ACCCCCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAAC GCCCTGCAGAGCGGCAACAGCCAGGAGAGCGTCACCGA GCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCA CCCTGACCCTGAGCAAGGCCGACTACGAGAAGCATAAG GTGTACGCCTGCGAGGTGACCCACCAGGGCCTGTCCAG CCCCGTGACCAAGAGCTTCAACAGGGGCGAGTGC
Y4 E152C_S375C		
SEQ ID NO: 103	HCDR1 (Combined)	GFTFSSYAMS
SEQ ID NO: 104	HCDR2 (Combined)	AISGSGGSTYYADSVKG
SEQ ID NO: 163	HCDR3 (Combined)	SRLIAPWLDY
SEQ ID NO: 106	HCDR1 (Kabat)	SYAMS
SEQ ID NO: 104	HCDR2 (Kabat)	AISGSGGSTYYADSVKG
SEQ ID NO: 163	HCDR3 (Kabat)	SRLIAPWLDY
SEQ ID NO: 107	HCDR1 (Chothia)	GFTFSSY

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SEQ ID NO: 108	HCDR2 (Chothia)	SGSGGS
SEQ ID NO: 163	HCDR3(Chothia)	SRLIAPWLDY
SEQ ID NO: 109	HCDR1 (IMGT)	GFTFSSYA
SEQ ID NO: 110	HCDR2 (IMGT)	ISGSGGST
SEQ ID NO: 164	HCDR3 (IMGT)	ARSRLIAPWLDY
SEQ ID NO: 165	VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVR QAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSK NTLYLQMNSLRAEDTAVYYCARSRLIAPWLDYWGQGTL VTVSS
SEQ ID NO: 166	DNA VH	GAAGTTCAGCTGCTGGAATCTGGCGGAGGACTGGTTCA ACCTGGCGGCTCTCTGAGACTGTCTTGTGCCGCCAGCG GCTTCACCTTTAGCAGCTACGCCATGAGCTGGGTCCGA CAGGCTCCTGGCAAAGGCCTTGAATGGGTGTCCGCCAT CTCTGGCTCTGGCGGCAGCACATATTACGCCGACTCTG TGAAGGGCAGATTCACCATCAGCCGGGACAACAGCAAG AACACCCTGTACCTGCAGATGAACAGCCTGAGAGCCGA GGACACCGCCGTGTACTACTGTGCCAGAAGCAGACTGA TCGCCCCTTGGCTGGATTATTGGGGCCAGGGCACACTG GTCACCGTGTCATCT
SEQ ID NO: 167	Heavy Chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVR QAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSK NTLYLQMNSLRAEDTAVYYCARSRLIAPWLDYWGQGTL VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF PCPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPCDIA VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 168	DNA Heavy Chain	GAAGTTCAGCTGCTGGAATCTGGCGGAGGACTGGTTCA ACCTGGCGGCTCTCTGAGACTGTCTTGTGCCGCCAGCG GCTTCACCTTTAGCAGCTACGCCATGAGCTGGGTCCGA CAGGCTCCTGGCAAAGGCCTTGAATGGGTGTCCGCCAT CTCTGGCTCTGGCGGCAGCACATATTACGCCGACTCTG TGAAGGGCAGATTCACCATCAGCCGGGACAACAGCAAG AACACCCTGTACCTGCAGATGAACAGCCTGAGAGCCGA GGACACCGCCGTGTACTACTGTGCCAGAAGCAGACTGA TCGCCCCTTGGCTGGATTATTGGGGCCAGGGCACACTG GTCACCGTGTCATCTGCTAGCACCAAGGGCCCAAGTGT GTTTCCCCTGGCCCCCAGCAGCAAGTCTACTTCCGGCG GAACTGCTGCCCTGGGTTGCCTGGTGAAGGACTACTTC CCCTGTCCCGTGACAGTGTCCTGGAACTCTGGGGCTCT GACTTCCGGCGTGCACACCTTCCCCGCCGTGCTGCAGA GCAGCGGCCTGTACAGCCTGAGCAGCGTGGTGACAGTG CCCTCCAGCTCTCTGGGAACCCAGACCTATATCTGCAA CGTGAACCACAAGCCCAGCAACACCAAGGTGGACAAGA GAGTGGAGCCCAAGAGCTGCGACAAGACCCACACCTGC CCCCCCTGCCCAGCTCCAGAACTGCTGGGAGGGCCTTC CGTGTTCCTGTTCCCCCCCAAGCCCAAGGACACCCTGA TGATCAGCAGGACCCCCGAGGTGACCTGCGTGGTGGTG GACGTGTCCCACGAGGACCCAGAGGTGAAGTTCAACTG GTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCA

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		AGCCCAGAGAGGAGCAGTACAACAGCACCTACAGGGTG GTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAA CGGCAAAGAATACAAGTGCAAAGTCTCCAACAAGGCCC TGCCAGCCCCAATCGAAAAGACAATCAGCAAGGCCAAG GGCCAGCCACGGGAGCCCCAGGTGTACACCCTGCCCCC CAGCCGGGAGGAGATGACCAAGAACCAGGTGTCCCTGA CCTGTCTGGTGAAGGGCTTCTACCCCTGTGATATCGCC GTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTA CAAGACCACCCCCCCAGTGCTGGACAGCGACGGCAGCT TCTTCCTGTACAGCAAGCTGACCGTGGACAAGTCCAGG TGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCA CGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGA GCCTGAGCCCCGGCAAG
SEQ ID NO: 116	LCDR1 (Combined)	RASQSISSYLN
SEQ ID NO: 47	LCDR2 (Combined)	AASSLQS
SEQ ID NO: 169	LCDR3 (Combined)	QQVYGSPPT
SEQ ID NO: 116	LCDR1 (Kabat)	RASQSISSYLN
SEQ ID NO: 47	LCDR2 (Kabat)	AASSLQS
SEQ ID NO: 169	LCDR3 (Kabat)	QQVYGSPPT
SEQ ID NO: 49	LCDR1 (Chothia)	SQSISSY
SEQ ID NO: 50	LCDR2 (Chothia)	AAS
SEQ ID NO: 170	LCDR3 (Chothia)	VYGSPP
SEQ ID NO: 52	LCDR1 (IMGT)	QSISSY
SEQ ID NO: 50	LCDR2 (IMGT)	AAS
SEQ ID NO: 169	LCDR3 (IMGT)	QQVYGSPPT
SEQ ID NO: 171	VL	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQ KPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCQQVYGSPPTFGQGTKVEIK
SEQ ID NO: 172	DNA VL	GACATCCAGATGACACAGAGCCCTAGCAGCCTGTCTGC CAGCGTGGGAGACAGAGTGACCATCACCTGTAGAGCCA GCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAG AAGCCCGGCAAGGCCCCTAAACTGCTGATCTATGCCGC CAGCTCTCTGCAGTCTGGCGTGCCAAGCAGATTTTCTG GCAGCGGCTCTGGCACCGACTTCACCCTGACCATATCT AGCCTGCAGCCAGAGGACTTCGCCACCTACTACTGCCA GCAGGTCTACGGCAGCCCTCCTACATTTGGCCAGGGCA CCAAGGTGGAAATCAAG
SEQ ID NO: 173	Light Chain	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQ KPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCQQVYGSPPTFGQGTKVEIKRTVAAPS VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 174	DNA Light Chain	GACATCCAGATGACACAGAGCCCTAGCAGCCTGTCTGC CAGCGTGGGAGACAGAGTGACCATCACCTGTAGAGCCA GCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAG AAGCCCGGCAAGGCCCCTAAACTGCTGATCTATGCCGC CAGCTCTCTGCAGTCTGGCGTGCCAAGCAGATTTTCTG GCAGCGGCTCTGGCACCGACTTCACCCTGACCATATCT AGCCTGCAGCCAGAGGACTTCGCCACCTACTACTGCCA GCAGGTCTACGGCAGCCCTCCTACATTTGGCCAGGGCA CCAAGGTGGAAATCAAGCGTACGGTGGCCGCTCCCAGC

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		GTGTTCATCTTCCCCCCCAGCGACGAGCAGCTGAAGAG TGGCACCGCCAGCGTGGTGTGCCTGCTGAACAACTTCT ACCCCCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAAC GCCCTGCAGAGCGGCAACAGCCAGGAGAGCGTCACCGA GCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCA CCCTGACCCTGAGCAAGGCCGACTACGAGAAGCATAAG GTGTACGCCTGCGAGGTGACCCACCAGGGCCTGTCCAG CCCCGTGACCAAGAGCTTCAACAGGGGCGAGTGC
Y5 E152C_S375C		
SEQ ID NO: 175	HCDR1 (Combined)	GYSFTSYWIG
SEQ ID NO: 176	HCDR2 (Combined)	IIYPGDSDTRYSPSFQG
SEQ ID NO: 177	HCDR3 (Combined)	GSSAASGLSGDL
SEQ ID NO: 178	HCDR1 (Kabat)	SYWIG
SEQ ID NO: 176	HCDR2 (Kabat)	IIYPGDSDTRYSPSFQG
SEQ ID NO: 177	HCDR3 (Kabat)	GSSAASGLSGDL
SEQ ID NO: 179	HCDR1 (Chothia)	GYSFTSY
SEQ ID NO: 180	HCDR2 (Chothia)	YPGDSD
SEQ ID NO: 177	HCDR3(Chothia)	GSSAASGLSGDL
SEQ ID NO: 181	HCDR1 (IMGT)	GYSFTSYW
SEQ ID NO: 182	HCDR2 (IMGT)	IYPGDSDT
SEQ ID NO: 183	HCDR3 (IMGT)	ARGSSAASGLSGDL
SEQ ID NO: 184	VH	EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVR QMPGKGLEWMGIIYPGDSDTRYSPSFQGQVTISADKSI STAYLQWSSLKASDTAMYYCARGSSAASGLSGDLWGQG TLVTVSS
SEQ ID NO: 185	DNA VH	GAAGTTCAGCTGGTGCAGTCTGGCGCCGAAGTGAAGAA GCCTGGCGAGAGCCTGAAGATCTCCTGCAAAGGCAGCG GCTACAGCTTCACCAGCTACTGGATCGGCTGGGTCCGA CAGATGCCTGGCAAAGGCCTTGAGTGGATGGGCATCAT CTACCCCGGCGACAGCGACACCAGATACAGCCCTAGCT TTCAGGGCCAAGTGACCATCAGCGCCGACAAGAGCATC AGCACAGCCTACCTGCAGTGGTCCAGCCTGAAGGCCTC TGACACCGCCATGTACTACTGTGCCAGAGGAAGCTCTG CCGCCTCTGGACTGTCTGGCGATCTTTGGGGACAGGGC ACACTGGTCACAGTGTCTAGT
SEQ ID NO: 186	Heavy Chain	EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVR QMPGKGLEWMGIIYPGDSDTRYSPSFQGQVTISADKSI STAYLQWSSLKASDTAMYYCARGSSAASGLSGDLWGQG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD YFPCPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPCD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 187	DNA Heavy Chain	GAAGTTCAGCTGGTGCAGTCTGGCGCCGAAGTGAAGAA GCCTGGCGAGAGCCTGAAGATCTCCTGCAAAGGCAGCG GCTACAGCTTCACCAGCTACTGGATCGGCTGGGTCCGA CAGATGCCTGGCAAAGGCCTTGAGTGGATGGGCATCAT CTACCCCGGCGACAGCGACACCAGATACAGCCCTAGCT

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		TTCAGGGCCAAGTGACCATCAGCGCCGACAAGAGCATC AGCACAGCCTACCTGCAGTGGTCCAGCCTGAAGGCCTC TGACACCGCCATGTACTACTGTGCCAGAGGAAGCTCTG CCGCCTCTGGACTGTCTGGCGATCTTTGGGGACAGGGC ACACTGGTCACAGTGTCTAGTGCTAGCACCAAGGGCCC AAGTGTGTTTCCCCTGGCCCCCAGCAGCAAGTCTACTT CCGGCGGAACTGCTGCCCTGGGTTGCCTGGTGAAGGAC TACTTCCCCTGTCCCGTGACAGTGTCCTGGAACTCTGG GGCTCTGACTTCCGGCGTGCACACCTTCCCCGCCGTGC TGCAGAGCAGCGGCCTGTACAGCCTGAGCAGCGTGGTG ACAGTGCCCTCCAGCTCTCTGGGAACCCAGACCTATAT CTGCAACGTGAACCACAAGCCCAGCAACACCAAGGTGG ACAAGAGAGTGGAGCCCAAGAGCTGCGACAAGACCCAC ACCTGCCCCCCCTGCCCAGCTCCAGAACTGCTGGGAGG GCCTTCCGTGTTCCTGTTCCCCCCCAAGCCCAAGGACA CCCTGATGATCAGCAGGACCCCCGAGGTGACCTGCGTG GTGGTGGACGTGTCCCACGAGGACCCAGAGGTGAAGTT CAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCA AGACCAAGCCCAGAGAGGAGCAGTACAACAGCACCTAC AGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTG GCTGAACGGCAAAGAATACAAGTGCAAAGTCTCCAACA AGGCCCTGCCAGCCCCAATCGAAAAGACAATCAGCAAG GCCAAGGGCCAGCCACGGGAGCCCCAGGTGTACACCCT GCCCCCCAGCCGGGAGGAGATGACCAAGAACCAGGTGT CCCTGACCTGTCTGGTGAAGGGCTTCTACCCCTGTGAT ATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAA CAACTACAAGACCACCCCCCCCAGTGCTGGACAGCGACG GCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAG TCCAGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGT GATGCACGAGGCCCTGCACAACCACTACACCCAGAAGT CCCTGAGCCTGAGCCCCGGCAAG
SEQ ID NO: 116	LCDR1 (Combined)	RASQSISSYLN
SEQ ID NO: 47	LCDR2 (Combined)	AASSLQS
SEQ ID NO: 188	LCDR3 (Combined)	QQDYYSPFT
SEQ ID NO: 116	LCDR1 (Kabat)	RASQSISSYLN
SEQ ID NO: 47	LCDR2 (Kabat)	AASSLQS
SEQ ID NO: 188	LCDR3 (Kabat)	QQDYYSPFT
SEQ ID NO: 49	LCDR1 (Chothia)	SQSISSY
SEQ ID NO: 50	LCDR2 (Chothia)	AAS
SEQ ID NO: 189	LCDR3 (Chothia)	DYYSPF
SEQ ID NO: 52	LCDR1 (IMGT)	QSISSY
SEQ ID NO: 50	LCDR2 (IMGT)	AAS
SEQ ID NO: 188	LCDR3 (IMGT)	QQDYYSPFT
SEQ ID NO: 190	VL	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQ KPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCQQDYYSPFTFGQGTKVEIK
SEQ ID NO: 191	DNA VL	GACATCCAGATGACACAGAGCCCTAGCAGCCTGTCTGC CAGCGTGGGAGACAGAGTGACCATCACCTGTAGAGCCA GCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAG AAGCCCGGCAAGGCCCCTAAACTGCTGATCTATGCCGC CAGCTCTCTGCAGTCTGGCGTGCCAAGCAGATTTTCTG GCAGCGGCTCTGGCACCGACTTCACCCTGACCATATCT

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		AGCCTGCAGCCAGAGGACTTCGCCACCTACTACTGCCA GCAGGACTACTACAGCCCCTTCACCTTTGGCCAGGGCA CCAAGGTGGAAATCAAG
SEQ ID NO: 192	Light Chain	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQ KPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCQQDYYSPFTFGQGTKVEIKRTVAAPS VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 193	DNA Light Chain	GACATCCAGATGACACAGAGCCCTAGCAGCCTGTCTGC CAGCGTGGGAGACAGAGTGACCATCACCTGTAGAGCCA GCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAG AAGCCCGGCAAGGCCCCTAAACTGCTGATCTATGCCGC CAGCTCTCTGCAGTCTGGCGTGCCAAGCAGATTTTCTG GCAGCGGCTCTGGCACCGACTTCACCCTGACCATATCT AGCCTGCAGCCAGAGGACTTCGCCACCTACTACTGCCA GCAGGACTACTACAGCCCCTTCACCTTTGGCCAGGGCA CCAAGGTGGAAATCAAGCGTACGGTGGCCGCTCCCAGC GTGTTCATCTTCCCCCCCAGCGACGAGCAGCTGAAGAG TGGCACCGCCAGCGTGGTGTGCCTGCTGAACAACTTCT ACCCCCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAAC GCCCTGCAGAGCGGCAACAGCCAGGAGAGCGTCACCGA GCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCA CCCTGACCCTGAGCAAGGCCGACTACGAGAAGCATAAG GTGTACGCCTGCGAGGTGACCCACCAGGGCCTGTCCAG CCCCGTGACCAAGAGCTTCAACAGGGGCGAGTGC
Y6 E152C_S375C		
SEQ ID NO: 103	HCDR1 (Combined)	GFTFSSYAMS
SEQ ID NO: 104	HCDR2 (Combined)	AISGSGGSTYYADSVKG
SEQ ID NO: 194	HCDR3 (Combined)	AYKLSWLDL
SEQ ID NO: 106	HCDR1 (Kabat)	SYAMS
SEQ ID NO: 104	HCDR2 (Kabat)	AISGSGGSTYYADSVKG
SEQ ID NO: 194	HCDR3 (Kabat)	AYKLSWLDL
SEQ ID NO: 107	HCDR1 (Chothia)	GFTFSSY
SEQ ID NO: 108	HCDR2 (Chothia)	SGSGGS
SEQ ID NO: 194	HCDR3(Chothia)	AYKLSWLDL
SEQ ID NO: 109	HCDR1 (IMGT)	GFTFSSYA
SEQ ID NO: 110	HCDR2 (IMGT)	ISGSGGST
SEQ ID NO: 195	HCDR3 (IMGT)	ARAYKLSWLDL
SEQ ID NO: 196	VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVR QAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSK NTLYLQMNSLRAEDTAVYYCARAYKLSWLDLWGQGTLV TVSS
SEQ ID NO: 197	DNA VH	GAAGTTCAGCTGCTGGAATCTGGCGGAGGACTGGTTCA ACCTGGCGGCTCTCTGAGACTGTCTTGTGCCGCCAGCG GCTTCACCTTTAGCAGCTACGCCATGAGCTGGGTCCGA CAGGCTCCTGGCAAAGGCCTTGAATGGGTGTCCGCCAT CTCTGGCTCTGGCGGCAGCACATATTACGCCGACTCTG TGAAGGGCAGATTCACCATCAGCCGGGACAACAGCAAG AACACCCTGTACCTGCAGATGAACAGCCTGAGAGCCGA GGACACCGCCGTGTACTATTGTGCCAGAGCCTACAAGC

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		TGAGCTGGCTGGATCTTTGGGGCCAGGGCACACTGGTC ACAGTGTCATCT
SEQ ID NO: 198	Heavy Chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVR QAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSK NTLYLQMNSLRAEDTAVYYCARAYKLSWLDLWGQGTLV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP CPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPCDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 199	DNA Heavy Chain	GAAGTTCAGCTGCTGGAATCTGGCGGAGGACTGGTTCA ACCTGGCGGCTCTCTGAGACTGTCTTGTGCCGCCAGCG GCTTCACCTTTAGCAGCTACGCCATGAGCTGGGTCCGA CAGGCTCCTGGCAAAGGCCTTGAATGGGTGTCCGCCAT CTCTGGCTCTGGCGGCAGCACATATTACGCCGACTCTG TGAAGGGCAGATTCACCATCAGCCGGGACAACAGCAAG AACACCCTGTACCTGCAGATGAACAGCCTGAGAGCCGA GGACACCGCCGTGTACTATTGTGCCAGAGCCTACAAGC TGAGCTGGCTGGATCTTTGGGGCCAGGGCACACTGGTC ACAGTGTCATCTGCTAGCACCAAGGGCCCAAGTGTGTT TCCCCTGGCCCCCAGCAGCAAGTCTACTTCCGGCGGAA CTGCTGCCCTGGGTTGCCTGGTGAAGGACTACTTCCCC TGTCCCGTGACAGTGTCCTGGAACTCTGGGGCTCTGAC TTCCGGCGTGCACACCTTCCCCGCCGTGCTGCAGAGCA GCGGCCTGTACAGCCTGAGCAGCGTGGTGACAGTGCCC TCCAGCTCTCTGGGAACCCAGACCTATATCTGCAACGT GAACCACAAGCCCAGCAACACCAAGGTGGACAAGAGAG TGGAGCCCAAGAGCTGCGACAAGACCCACACCTGCCCC CCCTGCCCAGCTCCAGAACTGCTGGGAGGGCCTTCCGT GTTCCTGTTCCCCCCCAAGCCCAAGGACACCCTGATGA TCAGCAGGACCCCCGAGGTGACCTGCGTGGTGGTGGAC GTGTCCCACGAGGACCCAGAGGTGAAGTTCAACTGGTA CGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGC CCAGAGAGGAGCAGTACAACAGCACCTACAGGGTGGTG TCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGG CAAAGAATACAAGTGCAAAGTCTCCAACAAGGCCCTGC CAGCCCCAATCGAAAAGACAATCAGCAAGGCCAAGGGC CAGCCACGGGAGCCCCAGGTGTACACCCTGCCCCCCAG CCGGGAGGAGATGACCAAGAACCAGGTGTCCCTGACCT GTCTGGTGAAGGGCTTCTACCCCTGTGATATCGCCGTG GAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAA GACCACCCCCCCAGTGCTGGACAGCGACGGCAGCTTCT TCCTGTACAGCAAGCTGACCGTGGACAAGTCCAGGTGG CAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGA GGCCCTGCACAACCACTACACCCAGAAGTCCCTGAGCC TGAGCCCCGGCAAG
SEQ ID NO: 116	LCDR1 (Combined)	RASQSISSYLN
SEQ ID NO: 47	LCDR2 (Combined)	AASSLQS
SEQ ID NO: 200	LCDR3 (Combined)	QQVWYAPVT
SEQ ID NO: 116	LCDR1 (Kabat)	RASQSISSYLN

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SEQ ID NO: 47	LCDR2 (Kabat)	AASSLQS
SEQ ID NO: 200	LCDR3 (Kabat)	QQVWYAPVT
SEQ ID NO: 49	LCDR1 (Chothia)	SQSISSY
SEQ ID NO: 50	LCDR2 (Chothia)	AAS
SEQ ID NO: 201	LCDR3 (Chothia)	VWYAPV
SEQ ID NO: 52	LCDR1 (IMGT)	QSISSY
SEQ ID NO: 50	LCDR2 (IMGT)	AAS
SEQ ID NO: 200	LCDR3 (IMGT)	QQVWYAPVT
SEQ ID NO: 202	VL	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQ KPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCQQVWYAPVTFGQGTKVEIK
SEQ ID NO: 203	DNA VL	GACATCCAGATGACACAGAGCCCTAGCAGCCTGTCTGC CAGCGTGGGAGACAGAGTGACCATCACCTGTAGAGCCA GCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAG AAGCCCGGCAAGGCCCCTAAACTGCTGATCTATGCCGC CAGCTCTCTGCAGTCTGGCGTGCCAAGCAGATTTTCTG GCAGCGGCTCTGGCACCGACTTCACCCTGACCATATCT AGCCTGCAGCCAGAGGACTTCGCCACCTACTACTGCCA GCAAGTTTGGTACGCCCCTGTGACCTTTGGCCAGGGCA CCAAGGTGGAAATCAAG
SEQ ID NO: 204	Light Chain	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQ KPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCQQVWYAPVTFGQGTKVEIKRTVAAPS VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 205	DNA Light Chain	GACATCCAGATGACACAGAGCCCTAGCAGCCTGTCTGC CAGCGTGGGAGACAGAGTGACCATCACCTGTAGAGCCA GCCAGAGCATCAGCAGCTACCTGAACTGGTATCAGCAG AAGCCCGGCAAGGCCCCTAAACTGCTGATCTATGCCGC CAGCTCTCTGCAGTCTGGCGTGCCAAGCAGATTTTCTG GCAGCGGCTCTGGCACCGACTTCACCCTGACCATATCT AGCCTGCAGCCAGAGGACTTCGCCACCTACTACTGCCA GCAAGTTTGGTACGCCCCTGTGACCTTTGGCCAGGGCA CCAAGGTGGAAATCAAGCGTACGGTGGCCGCTCCCAGC GTGTTCATCTTCCCCCCCAGCGACGAGCAGCTGAAGAG TGGCACCGCCAGCGTGGTGTGCCTGCTGAACAACTTCT ACCCCCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAAC GCCCTGCAGAGCGGCAACAGCCAGGAGAGCGTCACCGA GCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCA CCCTGACCCTGAGCAAGGCCGACTACGAGAAGCATAAG GTGTACGCCTGCGAGGTGACCCACCAGGGCCTGTCCAG CCCCGTGACCAAGAGCTTCAACAGGGGCGAGTGC
Y7 E152C_S375C		
SEQ ID NO: 206	HCDR1 (Combined)	GFTFSNAWMS
SEQ ID NO: 207	HCDR2 (Combined)	RIKSKTDAGTTDYAAPVKG
SEQ ID NO: 208	HCDR3 (Combined)	TIYPSAPSSSLDY
SEQ ID NO: 209	HCDR1 (Kabat)	NAWMS
SEQ ID NO: 207	HCDR2 (Kabat)	RIKSKTDAGTTDYAAPVKG
SEQ ID NO: 208	HCDR3 (Kabat)	TIYPSAPSSSLDY
SEQ ID NO: 210	HCDR1 (Chothia)	GFTFSNA

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SEQ ID NO: 211	HCDR2 (Chothia)	KSKTDAGT
SEQ ID NO: 208	HCDR3(Chothia)	TIYPSAPSSSLDY
SEQ ID NO: 212	HCDR1 (IMGT)	GFTFSNAW
SEQ ID NO: 213	HCDR2 (IMGT)	IKSKTDAGTT
SEQ ID NO: 214	HCDR3 (IMGT)	ARTIYPSAPSSSLDY
SEQ ID NO: 215	VH	EVQLVESGGGLVKPGGSLRLSCAASGFTFSNAWMSWVR QAPGKGLEWVGRIKSKTDAGTTDYAAPVKGRFTISRDD SKNTLYLQMNSLKTEDTAVYYCARTIYPSAPSSSLDYW GQGTLVTVSS
SEQ ID NO: 216	DNA VH	GAAGTGCAGCTGGTGGAATCTGGCGGCGGACTTGTGAA ACCTGGCGGCTCTCTGAGACTGAGCTGTGCCGCTTCCG GCTTCACCTTCAGCAATGCCTGGATGAGCTGGGTCCGA CAGGCCCCTGGAAAAGGCCTTGAGTGGGTCGGACGGAT CAAGAGCAAGACCGATGCCGGCACCACCGATTATGCTG CCCCTGTGAAGGGCAGATTCACCATCAGCAGGGACGAC AGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGAA AACCGAGGACACCGCCGTGTACTACTGCGCCAGAACAA TCTACCCCAGCGCTCCTAGCAGCAGCCTGGATTATTGG GGCCAGGGCACACTGGTCACCGTGTCATCT
SEQ ID NO: 217	Heavy Chain	EVQLVESGGGLVKPGGSLRLSCAASGFTFSNAWMSWVR QAPGKGLEWVGRIKSKTDAGTTDYAAPVKGRFTISRDD SKNTLYLQMNSLKTEDTAVYYCARTIYPSAPSSSLDYW GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPCPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCD KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY PCDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 218	DNA Heavy Chain	GAAGTGCAGCTGGTGGAATCTGGCGGCGGACTTGTGAA ACCTGGCGGCTCTCTGAGACTGAGCTGTGCCGCTTCCG GCTTCACCTTCAGCAATGCCTGGATGAGCTGGGTCCGA CAGGCCCCTGGAAAAGGCCTTGAGTGGGTCGGACGGAT CAAGAGCAAGACCGATGCCGGCACCACCGATTATGCTG CCCCTGTGAAGGGCAGATTCACCATCAGCAGGGACGAC AGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGAA AACCGAGGACACCGCCGTGTACTACTGCGCCAGAACAA TCTACCCCAGCGCTCCTAGCAGCAGCCTGGATTATTGG GGCCAGGGCACACTGGTCACCGTGTCATCTGCTAGCAC CAAGGGCCCAAGTGTGTTTCCCCTGGCCCCCAGCAGCA AGTCTACTTCCGGCGGAACTGCTGCCCTGGGTTGCCTG GTGAAGGACTACTTCCCCTGTCCCGTGACAGTGTCCTG GAACTCTGGGGCTCTGACTTCCGGCGTGCACACCTTCC CCGCCGTGCTGCAGAGCAGCGGCCTGTACAGCCTGAGC AGCGTGGTGACAGTGCCCTCCAGCTCTCTGGGAACCCA GACCTATATCTGCAACGTGAACCACAAGCCCAGCAACA CCAAGGTGGACAAGAGAGTGGAGCCCAAGAGCTGCGAC AAGACCCACACCTGCCCCCCCTGCCCAGCTCCAGAACT GCTGGGAGGGCCTTCCGTGTTCCTGTTCCCCCCCAAGC CCAAGGACACCCTGATGATCAGCAGGACCCCCGAGGTG ACCTGCGTGGTGGTGGACGTGTCCCACGAGGACCCAGA GGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGC

Date Reçue/Date Received 2024-04-11

		ACAACGCCAAGACCAAGCCCAGAGAGGAGCAGTACAAC AGCACCTACAGGGTGGTGTCCGTGCTGACCGTGCTGCA CCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAAG TCTCCAACAAGGCCCTGCCAGCCCCAATCGAAAAGACA ATCAGCAAGGCCAAGGGCCAGCCACGGGAGCCCCAGGT GTACACCCTGCCCCCCAGCCGGGAGGAGATGACCAAGA ACCAGGTGTCCCTGACCTGTCTGGTGAAGGGCTTCTAC CCCTGTGATATCGCCGTGGAGTGGGAGAGCAACGGCCA GCCCGAGAACAACTACAAGACCACCCCCCCAGTGCTGG ACAGCGACGGCAGCTTCTTCCTGTACAGCAAGCTGACC GTGGACAAGTCCAGGTGGCAGCAGGGCAACGTGTTCAG CTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACA CCCAGAAGTCCCTGAGCCTGAGCCCCGGCAAG
SEQ ID NO: 153	LCDR1 (Combined)	RASQGISNYLA
SEQ ID NO: 154	LCDR2 (Combined)	AASTLQS
SEQ ID NO: 219	LCDR3 (Combined)	QQLIFFPLT
SEQ ID NO: 153	LCDR1 (Kabat)	RASQGISNYLA
SEQ ID NO: 154	LCDR2 (Kabat)	AASTLQS
SEQ ID NO: 219	LCDR3 (Kabat)	QQLIFFPLT
SEQ ID NO: 156	LCDR1 (Chothia)	SQGISNY
SEQ ID NO: 50	LCDR2 (Chothia)	AAS
SEQ ID NO: 220	LCDR3 (Chothia)	LIFFPL
SEQ ID NO: 158	LCDR1 (IMGT)	QGISNY
SEQ ID NO: 50	LCDR2 (IMGT)	AAS
SEQ ID NO: 219	LCDR3 (IMGT)	QQLIFFPLT
SEQ ID NO: 221	VL	DIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQ KPGKVPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTIS SLQPEDVATYYCQQLIFFPLTFGQGTKVEIK
SEQ ID NO: 222	DNA VL	GACATCCAGATGACACAGAGCCCTAGCAGCCTGTCTGC CAGCGTGGGAGACAGAGTGACCATCACCTGTAGAGCCA GCCAGGGCATCAGCAACTACCTGGCCTGGTATCAGCAG AAACCCGGCAAGGTGCCCAAGCTGCTGATCTACGCTGC CAGCACACTGCAGAGCGGAGTGCCTAGCAGATTTTCTG GCAGCGGCTCCGGCACCGATTTCACCCTGACCATATCT AGCCTGCAGCCAGAGGACGTGGCCACCTACTATTGCCA GCAGCTGATCTTCTTCCCTCTGACCTTTGGCCAGGGCA CCAAGGTGGAAATCAAG
SEQ ID NO: 223	Light Chain	DIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQ KPGKVPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTIS SLQPEDVATYYCQQLIFFPLTFGQGTKVEIKRTVAAPS VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 224	DNA Light Chain	GACATCCAGATGACACAGAGCCCTAGCAGCCTGTCTGC CAGCGTGGGAGACAGAGTGACCATCACCTGTAGAGCCA GCCAGGGCATCAGCAACTACCTGGCCTGGTATCAGCAG AAACCCGGCAAGGTGCCCAAGCTGCTGATCTACGCTGC CAGCACACTGCAGAGCGGAGTGCCTAGCAGATTTTCTG GCAGCGGCTCCGGCACCGATTTCACCCTGACCATATCT AGCCTGCAGCCAGAGGACGTGGCCACCTACTATTGCCA GCAGCTGATCTTCTTCCCTCTGACCTTTGGCCAGGGCA CCAAGGTGGAAATCAAGCGTACGGTGGCCGCTCCCAGC

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		GTGTTCATCTTCCCCCCCAGCGACGAGCAGCTGAAGAG TGGCACCGCCAGCGTGGTGTGCCTGCTGAACAACTTCT ACCCCCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAAC GCCCTGCAGAGCGGCAACAGCCAGGAGAGCGTCACCGA GCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCA CCCTGACCCTGAGCAAGGCCGACTACGAGAAGCATAAG GTGTACGCCTGCGAGGTGACCCACCAGGGCCTGTCCAG CCCCGTGACCAAGAGCTTCAACAGGGGCGAGTGC
Y8 E152C_S375C		
SEQ ID NO: 206	HCDR1 (Combined)	GFTFSNAWMS
SEQ ID NO: 207	HCDR2 (Combined)	RIKSKTDAGTTDYAAPVKG
SEQ ID NO: 225	HCDR3 (Combined)	ASHRLHSLFDV
SEQ ID NO: 209	HCDR1 (Kabat)	NAWMS
SEQ ID NO: 207	HCDR2 (Kabat)	RIKSKTDAGTTDYAAPVKG
SEQ ID NO: 225	HCDR3 (Kabat)	ASHRLHSLFDV
SEQ ID NO: 210	HCDR1 (Chothia)	GFTFSNA
SEQ ID NO: 211	HCDR2 (Chothia)	KSKTDAGT
SEQ ID NO: 225	HCDR3(Chothia)	ASHRLHSLFDV
SEQ ID NO: 212	HCDR1 (IMGT)	GFTFSNAW
SEQ ID NO: 213	HCDR2 (IMGT)	IKSKTDAGTT
SEQ ID NO: 226	HCDR3 (IMGT)	ARASHRLHSLFDV
SEQ ID NO: 227	VH	EVQLVESGGGLVKPGGSLRLSCAASGFTFSNAWMSWVR QAPGKGLEWVGRIKSKTDAGTTDYAAPVKGRFTISRDD SKNTLYLQMNSLKTEDTAVYYCARASHRLHSLFDVWGQ GTLVTVSS
SEQ ID NO: 228	DNA VH	GAAGTGCAGCTGGTGGAATCTGGCGGCGGACTTGTGAA ACCTGGCGGCTCTCTGAGACTGAGCTGTGCCGCTTCCG GCTTCACCTTCAGCAATGCCTGGATGAGCTGGGTCCGA CAGGCCCCTGGAAAAGGCCTTGAGTGGGTCGGACGGAT CAAGAGCAAGACCGATGCCGGCACCACCGATTATGCTG CCCCTGTGAAGGGCAGATTCACCATCAGCAGGGACGAC AGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGAA AACCGAGGACACCGCCGTGTACTACTGTGCCAGAGCCT CTCACAGACTGCACAGCCTGTTTGACGTGTGGGGCCAG GGAACACTGGTCACCGTTAGTTCT
SEQ ID NO: 229	Heavy Chain	EVQLVESGGGLVKPGGSLRLSCAASGFTFSNAWMSWVR QAPGKGLEWVGRIKSKTDAGTTDYAAPVKGRFTISRDD SKNTLYLQMNSLKTEDTAVYYCARASHRLHSLFDVWGQ GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPCPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKT HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPC DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 230	DNA Heavy Chain	GAAGTGCAGCTGGTGGAATCTGGCGGCGGACTTGTGAA ACCTGGCGGCTCTCTGAGACTGAGCTGTGCCGCTTCCG GCTTCACCTTCAGCAATGCCTGGATGAGCTGGGTCCGA CAGGCCCCTGGAAAAGGCCTTGAGTGGGTCGGACGGAT CAAGAGCAAGACCGATGCCGGCACCACCGATTATGCTG

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		CCCCTGTGAAGGGCAGATTCACCATCAGCAGGGACGAC AGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGAA AACCGAGGACACCGCCGTGTACTACTGTGCCAGAGCCT CTCACAGACTGCACAGCCTGTTTGACGTGTGGGGCCAG GGAACACTGGTCACCGTTAGTTCTGCTAGCACCAAGGG CCCAAGTGTGTTTCCCCTGGCCCCCAGCAGCAAGTCTA CTTCCGGCGGAACTGCTGCCCTGGGTTGCCTGGTGAAG GACTACTTCCCCTGTCCCGTGACAGTGTCCTGGAACTC TGGGGCTCTGACTTCCGGCGTGCACACCTTCCCCGCCG TGCTGCAGAGCAGCGGCCTGTACAGCCTGAGCAGCGTG GTGACAGTGCCCTCCAGCTCTCTGGGAACCCAGACCTA TATCTGCAACGTGAACCACAAGCCCAGCAACACCAAGG TGGACAAGAGAGTGGAGCCCAAGAGCTGCGACAAGACC CACACCTGCCCCCCCTGCCCAGCTCCAGAACTGCTGGG AGGGCCTTCCGTGTTCCTGTTCCCCCCCAAGCCCAAGG ACACCCTGATGATCAGCAGGACCCCCGAGGTGACCTGC GTGGTGGTGGACGTGTCCCACGAGGACCCAGAGGTGAA GTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACG CCAAGACCAAGCCCAGAGAGGAGCAGTACAACAGCACC TACAGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGA CTGGCTGAACGGCAAAGAATACAAGTGCAAAGTCTCCA ACAAGGCCCTGCCAGCCCCAATCGAAAAGACAATCAGC AAGGCCAAGGGCCAGCCACGGGAGCCCCAGGTGTACAC CCTGCCCCCCCAGCCGGGAGGAGATGACCAAGAACCAGG TGTCCCTGACCTGTCTGGTGAAGGGCTTCTACCCCTGT GATATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGA GAACAACTACAAGACCACCCCCCCAGTGCTGGACAGCG ACGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGAC AAGTCCAGGTGGCAGCAGGGCAACGTGTTCAGCTGCAG CGTGATGCACGAGGCCCTGCACAACCACTACACCCAGA AGTCCCTGAGCCTGAGCCCCGGCAAG
SEQ ID NO: 136	LCDR1 (Combined)	RASQSISSWLA
SEQ ID NO: 137	LCDR2 (Combined)	DASSLES
SEQ ID NO: 231	LCDR3 (Combined)	QQGLFYPHT
SEQ ID NO: 136	LCDR1 (Kabat)	RASQSISSWLA
SEQ ID NO: 137	LCDR2 (Kabat)	DASSLES
SEQ ID NO: 231	LCDR3 (Kabat)	QQGLFYPHT
SEQ ID NO: 139	LCDR1 (Chothia)	SQSISSW
SEQ ID NO: 140	LCDR2 (Chothia)	DAS
SEQ ID NO: 232	LCDR3 (Chothia)	GLFYPH
SEQ ID NO: 142	LCDR1 (IMGT)	QSISSW
SEQ ID NO: 140	LCDR2 (IMGT)	DAS
SEQ ID NO: 231	LCDR3 (IMGT)	QQGLFYPHT
SEQ ID NO: 233	VL	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQ KPGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTIS SLQPEDFATYYCQQGLFYPHTFGQGTKVEIK
SEQ ID NO: 234	DNA VL	GACATCCAGATGACACAGAGCCCCAGCACACTGTCTGC CAGCGTGGGAGACAGAGTGACCATCACCTGTAGAGCCA GCCAGAGCATCTCCTCTTGGCTGGCCTGGTATCAGCAG AAGCCTGGCAAGGCCCCTAAGCTGCTGATCTACGATGC CAGCAGCCTGGAAAGCGGCGTGCCAAGCAGATTTTCTG GCAGCGGCTCTGGCACCGAGTTCACCCTGACCATATCT

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		AGCCTGCAGCCAGAGGACTTCGCCACCTACTATTGTCA GCAGGGCCTGTTCTACCCTCACACCTTTGGCCAGGGCA CCAAGGTGGAAATCAAG
SEQ ID NO: 235	Light Chain	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQ KPGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTIS SLQPEDFATYYCQQGLFYPHTFGQGTKVEIKRTVAAPS VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 236	DNA Light Chain	GACATCCAGATGACACAGAGCCCCAGCACACTGTCTGC CAGCGTGGGAGACAGAGTGACCATCACCTGTAGAGCCA GCCAGAGCATCTCCTCTTGGCTGGCCTGGTATCAGCAG AAGCCTGGCAAGGCCCCTAAGCTGCTGATCTACGATGC CAGCAGCCTGGAAAGCGGCGTGCCAAGCAGATTTTCTG GCAGCGGCTCTGGCACCGAGTTCACCCTGACCATATCT AGCCTGCAGCCAGAGGACTTCGCCACCTACTATTGTCA GCAGGGCCTGTTCTACCCTCACACCTTTGGCCAGGGCA CCAAGGTGGAAATCAAGCGTACGGTGGCCGCTCCCAGC GTGTTCATCTTCCCCCCCAGCGACGAGCAGCTGAAGAG TGGCACCGCCAGCGTGGTGTGCCTGCTGAACAACTTCT ACCCCCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAAC GCCCTGCAGAGCGGCAACAGCCAGGAGAGCGTCACCGA GCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCA CCCTGACCCTGAGCAAGGCCGACTACGAGAAGCATAAG GTGTACGCCTGCGAGGTGACCCACCAGGGCCTGTCCAG CCCCGTGACCAAGAGCTTCAACAGGGGCGAGTGC
Y9 E152C_S375C		
SEQ ID NO: 206	HCDR1 (Combined)	GFTFSNAWMS
SEQ ID NO: 207	HCDR2 (Combined)	RIKSKTDAGTTDYAAPVKG
SEQ ID NO: 237	HCDR3 (Combined)	DEYPWGWFDV
SEQ ID NO: 209	HCDR1 (Kabat)	NAWMS
SEQ ID NO: 207	HCDR2 (Kabat)	RIKSKTDAGTTDYAAPVKG
SEQ ID NO: 237	HCDR3 (Kabat)	DEYPWGWFDV
SEQ ID NO: 210	HCDR1 (Chothia)	GFTFSNA
SEQ ID NO: 211	HCDR2 (Chothia)	KSKTDAGT
SEQ ID NO: 237	HCDR3(Chothia)	DEYPWGWFDV
SEQ ID NO: 212	HCDR1 (IMGT)	GFTFSNAW
SEQ ID NO: 213	HCDR2 (IMGT)	IKSKTDAGTT
SEQ ID NO: 238	HCDR3 (IMGT)	ARDEYPWGWFDV
SEQ ID NO: 239	VH	EVQLVESGGGLVKPGGSLRLSCAASGFTFSNAWMSWVR QAPGKGLEWVGRIKSKTDAGTTDYAAPVKGRFTISRDD SKNTLYLQMNSLKTEDTAVYYCARDEYPWGWFDVWGQG TLVTVSS
SEQ ID NO: 240	DNA VH	GAAGTGCAGCTGGTGGAATCTGGCGGCGGACTTGTGAA ACCTGGCGGCTCTCTGAGACTGAGCTGTGCCGCTTCCG GCTTCACCTTCAGCAATGCCTGGATGAGCTGGGTCCGA CAGGCCCCTGGAAAAGGCCTTGAGTGGGTCGGACGGAT CAAGAGCAAGACCGATGCCGGCACCACCGATTATGCTG CCCCTGTGAAGGGCAGATTCACCATCAGCAGGGACGAC AGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGAA AACCGAGGACACCGCCGTGTACTACTGCGCCAGAGATG

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		AGTACCCCTGGGGATGGTTCGATGTGTGGGGACAGGGA ACCCTGGTCACCGTTAGTTCT
SEQ ID NO: 241	Heavy Chain	EVQLVESGGGLVKPGGSLRLSCAASGFTFSNAWMSWVR QAPGKGLEWVGRIKSKTDAGTTDYAAPVKGRFTISRDD SKNTLYLQMNSLKTEDTAVYYCARDEYPWGWFDVWGQG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD YFPCPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPCD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 242	DNA Heavy Chain	GAAGTGCAGCTGGTGGAATCTGGCGGCGGACTTGTGAA ACCTGGCGGCTCTCTGAGACTGAGCTGTGCCGCTTCCG GCTTCACCTTCAGCAATGCCTGGATGAGCTGGGTCCGA CAGGCCCCTGGAAAAGGCCTTGAGTGGGTCGGACGGAT CAAGAGCAAGACCGATGCCGGCACCACCGATTATGCTG CCCCTGTGAAGGGCAGATTCACCATCAGCAGGGACGAC AGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGAA AACCGAGGACACCGCCGTGTACTACTGCGCCAGAGATG AGTACCCCTGGGGATGGTTCGATGTGTGGGGACAGGGA ACCCTGGTCACCGTTAGTTCTGCTAGCACCAAGGGCCC AAGTGTGTTTCCCCTGGCCCCCAGCAGCAAGTCTACTT CCGGCGGAACTGCTGCCCTGGGTTGCCTGGTGAAGGAC TACTTCCCCTGTCCCGTGACAGTGTCCTGGAACTCTGG GGCTCTGACTTCCGGCGTGCACACCTTCCCCGCCGTGC TGCAGAGCAGCGGCCTGTACAGCCTGAGCAGCGTGGTG ACAGTGCCCTCCAGCTCTCTGGGAACCCAGACCTATAT CTGCAACGTGAACCACAAGCCCAGCAACACCAAGGTGG ACAAGAGAGTGGAGCCCAAGAGCTGCGACAAGACCCAC ACCTGCCCCCCCTGCCCAGCTCCAGAACTGCTGGGAGG GCCTTCCGTGTTCCTGTTCCCCCCCAAGCCCAAGGACA CCCTGATGATCAGCAGGACCCCCGAGGTGACCTGCGTG GTGGTGGACGTGTCCCACGAGGACCCAGAGGTGAAGTT CAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCA AGACCAAGCCCAGAGAGGAGCAGTACAACAGCACCTAC AGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTG GCTGAACGGCAAAGAATACAAGTGCAAAGTCTCCAACA AGGCCCTGCCAGCCCCAATCGAAAAGACAATCAGCAAG GCCAAGGGCCAGCCACGGGAGCCCCAGGTGTACACCCT GCCCCCCAGCCGGGAGGAGATGACCAAGAACCAGGTGT CCCTGACCTGTCTGGTGAAGGGCTTCTACCCCTGTGAT ATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAA CAACTACAAGACCACCCCCCCAGTGCTGGACAGCGACG GCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAG TCCAGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGT GATGCACGAGGCCCTGCACAACCACTACACCCAGAAGT CCCTGAGCCTGAGCCCCGGCAAG
SEQ ID NO: 243	LCDR1 (Combined)	RASQGISSWLA
SEQ ID NO: 47	LCDR2 (Combined)	AASSLQS
SEQ ID NO: 244	LCDR3 (Combined)	QQYIFYPLT
SEQ ID NO: 243	LCDR1 (Kabat)	RASQGISSWLA

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SEQ ID NO: 47	LCDR2 (Kabat)	AASSLQS
SEQ ID NO: 244	LCDR3 (Kabat)	QQYIFYPLT
SEQ ID NO: 245	LCDR1 (Chothia)	SQGISSW
SEQ ID NO: 50	LCDR2 (Chothia)	AAS
SEQ ID NO: 246	LCDR3 (Chothia)	YIFYPL
SEQ ID NO: 247	LCDR1 (IMGT)	QGISSW
SEQ ID NO: 50	LCDR2 (IMGT)	AAS
SEQ ID NO: 244	LCDR3 (IMGT)	QQYIFYPLT
SEQ ID NO: 248	VL	DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQ KPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCQQYIFYPLTFGQGTKVEIK
SEQ ID NO: 249	DNA VL	GACATCCAGATGACACAGAGCCCTAGCTCCGTGTCTGC CAGCGTGGGAGACAGAGTGACCATCACCTGTAGAGCCA GCCAGGGCATCTCTTCTTGGCTGGCCTGGTATCAGCAG AAGCCTGGCAAGGCCCCTAAGCTGCTGATCTATGCCGC TTCCAGTCTGCAGAGCGGCGTGCCAAGCAGATTTTCTG GCAGCGGCTCTGGCACCGACTTCACCCTGACCATATCT AGCCTGCAGCCAGAGGACTTCGCCACCTACTACTGCCA GCAGTACATCTTCTACCCTCTGACCTTCGGCCAGGGCA CCAAGGTGGAAATCAAG
SEQ ID NO: 250	Light Chain	DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQ KPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCQQYIFYPLTFGQGTKVEIKRTVAAPS VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 251	DNA Light Chain	GACATCCAGATGACACAGAGCCCTAGCTCCGTGTCTGC CAGCGTGGGAGACAGAGTGACCATCACCTGTAGAGCCA GCCAGGGCATCTCTTCTTGGCTGGCCTGGTATCAGCAG AAGCCTGGCAAGGCCCCTAAGCTGCTGATCTATGCCGC TTCCAGTCTGCAGAGCGGCGTGCCAAGCAGATTTTCTG GCAGCGGCTCTGGCACCGACTTCACCCTGACCATATCT AGCCTGCAGCCAGAGGACTTCGCCACCTACTACTGCCA GCAGTACATCTTCTACCCTCTGACCTTCGGCCAGGGCA CCAAGGTGGAAATCAAGCGTACGGTGGCCGCTCCCAGC GTGTTCATCTTCCCCCCCAGCGACGAGCAGCTGAAGAG TGGCACCGCCAGCGTGGTGTGCCTGCTGAACAACTTCT ACCCCCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAAC GCCCTGCAGAGCGGCAACAGCCAGGAGAGCGTCACCGA GCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCA CCCTGACCCTGAGCAAGGCCGACTACGAGAAGCATAAG GTGTACGCCTGCGAGGTGACCCACCAGGGCCTGTCCAG CCCCGTGACCAAGAGCTTCAACAGGGGCGAGTGC
Y10 E152C_S375C		
SEQ ID NO: 206	HCDR1 (Combined)	GFTFSNAWMS
SEQ ID NO: 207	HCDR2 (Combined)	RIKSKTDAGTTDYAAPVKG
SEQ ID NO: 252	HCDR3 (Combined)	VASPSAPGGFDY
SEQ ID NO: 209	HCDR1 (Kabat)	NAWMS
SEQ ID NO: 207	HCDR2 (Kabat)	RIKSKTDAGTTDYAAPVKG
SEQ ID NO: 252	HCDR3 (Kabat)	VASPSAPGGFDY
SEQ ID NO: 210	HCDR1 (Chothia)	GFTFSNA

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SEQ ID NO: 211	HCDR2 (Chothia)	KSKTDAGT
SEQ ID NO: 252	HCDR3(Chothia)	VASPSAPGGFDY
SEQ ID NO: 212	HCDR1 (IMGT)	GFTFSNAW
SEQ ID NO: 213	HCDR2 (IMGT)	IKSKTDAGTT
SEQ ID NO: 253	HCDR3 (IMGT)	ARVASPSAPGGFDY
SEQ ID NO: 254	VH	EVQLVESGGGLVKPGGSLRLSCAASGFTFSNAWMSWVR QAPGKGLEWVGRIKSKTDAGTTDYAAPVKGRFTISRDD SKNTLYLQMNSLKTEDTAVYYCARVASPSAPGGFDYWG QGTLVTVSS
SEQ ID NO: 255	DNA VH	GAAGTGCAGCTGGTGGAATCTGGCGGCGGACTTGTGAA ACCTGGCGGCTCTCTGAGACTGAGCTGTGCCGCTTCCG GCTTCACCTTCAGCAATGCCTGGATGAGCTGGGTCCGA CAGGCCCCTGGAAAAGGCCTTGAGTGGGTCGGACGGAT CAAGAGCAAGACCGATGCCGGCACCACCGATTATGCTG CCCCTGTGAAGGGCAGATTCACCATCAGCAGGGACGAC AGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGAA AACCGAGGACACCGCCGTGTACTACTGCGCCAGAGTGG CTTCTCCTTCTGCTCCCGGCGGATTCGATTATTGGGGC CAGGGAACACTGGTCACCGTGTCTAGT
SEQ ID NO: 256	Heavy Chain	EVQLVESGGGLVKPGGSLRLSCAASGFTFSNAWMSWVR QAPGKGLEWVGRIKSKTDAGTTDYAAPVKGRFTISRDD SKNTLYLQMNSLKTEDTAVYYCARVASPSAPGGFDYWG QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV KDYFPCPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP CDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 257	DNA Heavy Chain	GAAGTGCAGCTGGTGGAATCTGGCGGCGGACTTGTGAA ACCTGGCGGCTCTCTGAGACTGAGCTGTGCCGCTTCCG GCTTCACCTTCAGCAATGCCTGGATGAGCTGGGTCCGA CAGGCCCCTGGAAAAGGCCTTGAGTGGGTCGGACGGAT CAAGAGCAAGACCGATGCCGGCACCACCGATTATGCTG CCCCTGTGAAGGGCAGATTCACCATCAGCAGGGACGAC AGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGAA AACCGAGGACACCGCCGTGTACTACTGCGCCAGAGTGG CTTCTCCTTCTGCTCCCGGCGGATTCGATTATTGGGGC CAGGGAACACTGGTCACCGTGTCTAGTGCTAGCACCAA GGGCCCAAGTGTGTTTCCCCTGGCCCCCAGCAGCAAGT CTACTTCCGGCGGAACTGCTGCCCTGGGTTGCCTGGTG AAGGACTACTTCCCCTGTCCCGTGACAGTGTCCTGGAA CTCTGGGGCTCTGACTTCCGGCGTGCACACCTTCCCCG CCGTGCTGCAGAGCAGCGGCCTGTACAGCCTGAGCAGC GTGGTGACAGTGCCCTCCAGCTCTCTGGGAACCCAGAC CTATATCTGCAACGTGAACCACAAGCCCAGCAACACCA AGGTGGACAAGAGAGTGGAGCCCAAGAGCTGCGACAAG ACCCACACCTGCCCCCCCTGCCCAGCTCCAGAACTGCT GGGAGGGCCTTCCGTGTTCCTGTTCCCCCCCAAGCCCA AGGACACCCTGATGATCAGCAGGACCCCCGAGGTGACC TGCGTGGTGGTGGACGTGTCCCACGAGGACCCAGAGGT GAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACA

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		ACGCCAAGACCAAGCCCAGAGAGGAGCAGTACAACAGC ACCTACAGGGTGGTGTCCGTGCTGACCGTGCTGCACCA GGACTGGCTGAACGGCAAAGAATACAAGTGCAAAGTCT CCAACAAGGCCCTGCCAGCCCCAATCGAAAAGACAATC AGCAAGGCCAAGGGCCAGCCACGGGAGCCCCAGGTGTA CACCCTGCCCCCCAGCCGGGAGGAGATGACCAAGAACC AGGTGTCCCTGACCTGTCTGGTGAAGGGCTTCTACCCC TGTGATATCGCCGTGGAGTGGGAGAGCAACGGCCAGCC CGAGAACAACTACAAGACCACCCCCCCAGTGCTGGACA GCGACGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTG GACAAGTCCAGGTGGCAGCAGGGCAACGTGTTCAGCTG CAGCGTGATGCACGAGGCCCTGCACAACCACTACACCC AGAAGTCCCTGAGCCTGAGCCCCGGCAAG
SEQ ID NO: 153	LCDR1 (Combined)	RASQGISNYLA
SEQ ID NO: 154	LCDR2 (Combined)	AASTLQS
SEQ ID NO: 258	LCDR3 (Combined)	QQSLFAPFT
SEQ ID NO: 153	LCDR1 (Kabat)	RASQGISNYLA
SEQ ID NO: 154	LCDR2 (Kabat)	AASTLQS
SEQ ID NO: 258	LCDR3 (Kabat)	QQSLFAPFT
SEQ ID NO: 156	LCDR1 (Chothia)	SQGISNY
SEQ ID NO: 50	LCDR2 (Chothia)	AAS
SEQ ID NO: 259	LCDR3 (Chothia)	SLFAPF
SEQ ID NO: 158	LCDR1 (IMGT)	QGISNY
SEQ ID NO: 50	LCDR2 (IMGT)	AAS
SEQ ID NO: 258	LCDR3 (IMGT)	QQSLFAPFT
SEQ ID NO: 260	VL	DIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQ KPGKVPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTIS SLQPEDVATYYCQQSLFAPFTFGQGTKVEIK
SEQ ID NO: 261	DNA VL	GACATCCAGATGACACAGAGCCCTAGCAGCCTGTCTGC CAGCGTGGGAGACAGAGTGACCATCACCTGTAGAGCCA GCCAGGGCATCAGCAACTACCTGGCCTGGTATCAGCAG AAACCCGGCAAGGTGCCCAAGCTGCTGATCTACGCTGC CAGCACACTGCAGAGCGGAGTGCCTAGCAGATTTTCTG GCAGCGGCTCCGGCACCGATTTCACCCTGACCATATCT AGCCTGCAGCCAGAGGACGTGGCCACCTACTACTGTCA GCAGAGCCTGTTCGCCCCTTTCACCTTTGGCCAGGGCA CCAAGGTGGAAATCAAG
SEQ ID NO: 262	Light Chain	DIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQ KPGKVPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTIS SLQPEDVATYYCQQSLFAPFTFGQGTKVEIKRTVAAPS VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 263	DNA Light Chain	GACATCCAGATGACACAGAGCCCTAGCAGCCTGTCTGC CAGCGTGGGAGACAGAGTGACCATCACCTGTAGAGCCA GCCAGGGCATCAGCAACTACCTGGCCTGGTATCAGCAG AAACCCGGCAAGGTGCCCAAGCTGCTGATCTACGCTGC CAGCACACTGCAGAGCGGAGTGCCTAGCAGATTTTCTG GCAGCGGCTCCGGCACCGATTTCACCCTGACCATATCT AGCCTGCAGCCAGAGGACGTGGCCACCTACTACTGTCA GCAGAGCCTGTTCGCCCCTTTCACCTTTGGCCAGGGCA CCAAGGTGGAAATCAAGCGTACGGTGGCCGCTCCCAGC

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		GTGTTCATCTTCCCCCCCAGCGACGAGCAGCTGAAGAG TGGCACCGCCAGCGTGGTGTGCCTGCTGAACAACTTCT ACCCCCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAAC GCCCTGCAGAGCGGCAACAGCCAGGAGAGCGTCACCGA GCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCA CCCTGACCCTGAGCAAGGCCGACTACGAGAAGCATAAG GTGTACGCCTGCGAGGTGACCCACCAGGGCCTGTCCAG CCCCGTGACCAAGAGCTTCAACAGGGGCGAGTGC
G1 E152C_3J LC		
SEQ ID NO: 1	HCDR1 (Combined)	GGTFSDYAIT
SEQ ID NO: 2	HCDR2 (Combined)	GIIPIFGTANYAQKFQG
SEQ ID NO: 3	HCDR3 (Combined)	EGGLLTDISYSRYWFAY
SEQ ID NO: 4	HCDR1 (Kabat)	DYAIT
SEQ ID NO: 2	HCDR2 (Kabat)	GIIPIFGTANYAQKFQG
SEQ ID NO: 3	HCDR3 (Kabat)	EGGLLTDISYSRYWFAY
SEQ ID NO: 5	HCDR1 (Chothia)	GGTFSDY
SEQ ID NO: 6	HCDR2 (Chothia)	IPIFGT
SEQ ID NO: 3	HCDR3(Chothia)	EGGLLTDISYSRYWFAY
SEQ ID NO: 7	HCDR1 (IMGT)	GGTFSDYA
SEQ ID NO: 8	HCDR2 (IMGT)	IIPIFGTA
SEQ ID NO: 9	HCDR3 (IMGT)	AREGGLLTDISYSRYWFAY
SEQ ID NO: 10	VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSDYAITWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYCAREGGLLTDISYSRYWFA YWGQGTLVTVSS
SEQ ID NO: 11	DNA VH	CAGGTGCAATTGGTGCAGAGCGGTGCCGAAGTGAAAAA ACCGGGCAGCAGCGTGAAAGTTAGCTGCAAAGCATCCG GAGGGACGTTTTCTGACTACGCTATCACTTGGGTGCGC CAGGCCCCGGGCCAGGGCCTCGAGTGGATGGGCGGTAT CATCCCGATCTTCGGCACTGCGAACTACGCCCAGAAAT TTCAGGGCCGGGTGACCATTACCGCCGATGAAAGCACC AGCACCGCCTATATGGAACTGAGCAGCCTGCGCAGCGA AGATACGGCCGTGTATTATTGCGCGCGTGAAGGTGGTC TGCTGACTGACATCTCTTACTCTCGTTACTGGTTCGCT TACTGGGGCCAAGGCACCCTGGTGACTGTTAGCTCA
SEQ ID NO: 283	Heavy Chain. Glycosylation site is bolded and italicized.	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSDYAITWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYCAREGGLLTDISYSRYWFA YWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPCPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKS CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ Y***NST***YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 319	DNA Heavy Chain	CAGGTGCAGCTGGTGCAGTCCGGCGCCGAGGTGAAAAA ACCCGGCTCCTCCGTGAAAGTGTCCTGCAAAGCCTCCG GCGGCACATTCTCCGACTACGCCATCACATGGGTGAGG CAGGCCCCCGGCCAGGGCCTGGAGTGGATGGGCGGCAT CATCCCCATCTTCGGCACAGCCAACTACGCCCAGAAAT

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		TCCAGGGCAGGGTGACAATCACAGCCGACGAGTCCACA TCCACAGCCTACATGGAGCTGTCCTCCCTGAGGTCCGA GGACACAGCCGTGTACTACTGCGCCAGGGAGGGCGGCC TGCTGACAGACATCTCCTACTCCAGGTACTGGTTCGCC TACTGGGGCCAGGGCACACTGGTGACAGTGTCCTCCGC CTCCACAAAGGGCCCCTCCGTGTTCCCCCTGGCCCCCT CCTCCAAATCCACATCCGGCGGCACAGCCGCCCTGGGC TGCCTGGTGAAAGACTACTTCCCCTGCCCCGTGACAGT GTCCTGGAACTCCGGCGCCCTGACATCCGGCGTGCACA CATTCCCCGCCGTGCTGCAGTCCTCCGGCCTGTACTCC CTGTCCTCCGTGGTGACAGTGCCCTCCTCCTCCCTGGG CACACAGACATACATCTGCAACGTGAACCACAAACCCT CCAACACAAAAGTGGACAAAAGGGTGGAGCCCAAATCC TGCGACAAAACACACACATGCCCCCCCTGCCCCGCCCC CGAGCTGCTGGGCGGCCCCTCCGTGTTCCTGTTCCCCC CCAAACCCAAAGACACACTGATGATCTCCAGGACACCC GAGGTGACATGCGTGGTGGTGGACGTGTCCCACGAGGA CCCCGAGGTGAAATTCAACTGGTACGTGGACGGCGTGG AGGTGCACAACGCCAAAACAAAACCCAGGGAGGAGCAG TACAACTCCACATACAGGGTGGTGTCCGTGCTGACAGT GCTGCACCAGGACTGGCTGAACGGCAAAGAGTACAAAT GCAAAGTGTCCAACAAAGCCCTGCCCGCCCCCATCGAG AAAACAATCTCCAAAGCCAAAGGCCAGCCCAGGGAGCC CCAGGTGTACACACTGCCCCCCTCCAGGGAGGAGATGA CAAAAAACCAGGTGTCCCTGACATGCCTGGTGAAAGGC TTCTACCCCTCCGACATCGCCGTGGAGTGGGAGTCCAA CGGCCAGCCCGAGAACAACTACAAAACAACACCCCCCG TGCTGGACTCCGACGGCTCCTTCTTCCTGTACTCCAAA CTGACAGTGGACAAATCCAGGTGGCAGCAGGGCAACGT GTTCTCCTGCTCCGTGATGCACGAGGCCCTGCACAACC ACTACACACAGAAATCCCTGTCCCTGTCCCCCGGCAAA
SEQ ID NO: 14	LCDR1 (Combined)	SGDALRDKFVY
SEQ ID NO: 15	LCDR2 (Combined)	DDNNRPS
SEQ ID NO: 16	LCDR3 (Combined)	QSWDHSYSLVV
SEQ ID NO: 14	LCDR1 (Kabat)	SGDALRDKFVY
SEQ ID NO: 15	LCDR2 (Kabat)	DDNNRPS
SEQ ID NO: 16	LCDR3 (Kabat)	QSWDHSYSLVV
SEQ ID NO: 17	LCDR1 (Chothia)	DALRDKF
SEQ ID NO: 18	LCDR2 (Chothia)	DDN
SEQ ID NO: 19	LCDR3 (Chothia)	WDHSYSLV
SEQ ID NO: 20	LCDR1 (IMGT)	ALRDKF
SEQ ID NO: 18	LCDR2 (IMGT)	DDN
SEQ ID NO: 16	LCDR3 (IMGT)	QSWDHSYSLVV
SEQ ID NO: 25	VL	SYELTQPLSVSVALGQTARITCSGDALRDKFVYWYQQK PGQAPVLVIYDDNNRPSGIPERFSGSNSGNTATLTISR AQAGDEADYYCQSWDHSYSLVVFGGGTKLTVL
SEQ ID NO: 26	DNA VL	AGCTACGAGCTGACCCAGCCGCTGTCGGTGTCAGTCGC CCTGGGACAAACTGCCAGGATCACTTGTTCCGGGGACG CATTGCGGGACAAGTTCGTGTACTGGTACCAGCAGAAG CCGGGTCAAGCCCCAGTGCTCGTGATCTACGACGACAA CAACCGGCCTTCCGGTATCCCCGAACGCTTCTCCGGAT CCAATAGCGGAAACACCGCCACCCTGACCATTTCGAGA

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		GCTCAGGCCGGGGATGAAGCGGACTACTACTGCCAGTC ATGGGATCACTCGTACTCCCTCGTCGTGTTTGGAGGCG GCACGAAGCTTACTGTGCTG
SEQ ID NO: 27	Light Chain	SYELTQPLSVSVALGQTARITCSGDALRDKFVYWYQQK PGQAPVLVIYDDNNRPSGIPERFSGSNSGNTATLTISR AQAGDEADYYCQSWDHSYSLVVFGGGTKLTVLGQPKAA PSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKA DSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSH RSYSCQVTHEGSTVEKTVAPTECS
SEQ ID NO: 28	DNA Light Chain	AGCTACGAGCTGACCCAGCCGCTGTCGGTGTCAGTCGC CCTGGGACAAACTGCCAGGATCACTTGTTCCGGGGACG CATTGCGGGACAAGTTCGTGTACTGGTACCAGCAGAAG CCGGGTCAAGCCCCAGTGCTCGTGATCTACGACGACAA CAACCGGCCTTCCGGTATCCCCGAACGCTTCTCCGGAT CCAATAGCGGAAACACCGCCACCCTGACCATTTCGAGA GCTCAGGCCGGGGATGAAGCGGACTACTACTGCCAGTC ATGGGATCACTCGTACTCCCTCGTCGTGTTTGGAGGCG GCACGAAGCTTACTGTGCTGGGCCAGCCTAAGGCCGCT CCCTCCGTGACCCTGTTCCCCCCCAGCTCCGAGGAACT GCAGGCCAACAAGGCCACCCTGGTGTGCCTGATCAGCG ACTTCTACCCTGGCGCCGTGACCGTGGCCTGGAAGGCC GACAGCAGCCCCGTGAAGGCCGGCGTGGAGACAACCAC CCCCAGCAAGCAGAGCAACAACAAGTACGCCGCCAGCA GCTACCTGAGCCTGACCCCCGAGCAGTGGAAGAGCCAC AGAAGCTACAGCTGCCAGGTCACCCACGAGGGCAGCAC CGTGGAGAAAACCGTGGCCCCCACCGAGTGCAGC
G1 E152C_3R LC		
SEQ ID NO: 1	HCDR1 (Combined)	GGTFSDYAIT
SEQ ID NO: 2	HCDR2 (Combined)	GIIPIFGTANYAQKFQG
SEQ ID NO: 3	HCDR3 (Combined)	EGGLLTDISYSRYWFAY
SEQ ID NO: 4	HCDR1 (Kabat)	DYAIT
SEQ ID NO: 2	HCDR2 (Kabat)	GIIPIFGTANYAQKFQG
SEQ ID NO: 3	HCDR3 (Kabat)	EGGLLTDISYSRYWFAY
SEQ ID NO: 5	HCDR1 (Chothia)	GGTFSDY
SEQ ID NO: 6	HCDR2 (Chothia)	IPIFGT
SEQ ID NO: 3	HCDR3(Chothia)	EGGLLTDISYSRYWFAY
SEQ ID NO: 7	HCDR1 (IMGT)	GGTFSDYA
SEQ ID NO: 8	HCDR2 (IMGT)	IIPIFGTA
SEQ ID NO: 9	HCDR3 (IMGT)	AREGGLLTDISYSRYWFAY
SEQ ID NO: 10	VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSDYAITWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYCAREGGLLTDISYSRYWFA YWGQGTLVTVSS
SEQ ID NO: 11	DNA VH	CAGGTGCAATTGGTGCAGAGCGGTGCCGAAGTGAAAAA ACCGGGCAGCAGCGTGAAAGTTAGCTGCAAAGCATCCG GAGGGACGTTTTCTGACTACGCTATCACTTGGGTGCGC CAGGCCCCGGGCCAGGGCCTCGAGTGGATGGGCGGTAT CATCCCGATCTTCGGCACTGCGAACTACGCCCAGAAAT TTCAGGGCCGGGTGACCATTACCGCCGATGAAAGCACC AGCACCGCCTATATGGAACTGAGCAGCCTGCGCAGCGA AGATACGGCCGTGTATTATTGCGCGCGTGAAGGTGGTC

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		TGCTGACTGACATCTCTTACTCTCGTTACTGGTTCGCT TACTGGGGCCAAGGCACCCTGGTGACTGTTAGCTCA
SEQ ID NO: 283	Heavy Chain. Glycosylation site is bolded and italicized.	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSDYAITWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYCAREGGLLTDISYSRYWFA YWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPCPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKS CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ Y***NST***YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 319	DNA Heavy Chain	CAGGTGCAGCTGGTGCAGTCCGGCGCCGAGGTGAAAAA ACCCGGCTCCTCCGTGAAAGTGTCCTGCAAAGCCTCCG GCGGCACATTCTCCGACTACGCCATCACATGGGTGAGG CAGGCCCCCGGCCAGGGCCTGGAGTGGATGGGCGGCAT CATCCCCATCTTCGGCACAGCCAACTACGCCCAGAAAT TCCAGGGCAGGGTGACAATCACAGCCGACGAGTCCACA TCCACAGCCTACATGGAGCTGTCCTCCCTGAGGTCCGA GGACACAGCCGTGTACTACTGCGCCAGGGAGGGCGGCC TGCTGACAGACATCTCCTACTCCAGGTACTGGTTCGCC TACTGGGGCCAGGGCACACTGGTGACAGTGTCCTCCGC CTCCACAAAGGGCCCCTCCGTGTTCCCCCTGGCCCCCT CCTCCAAATCCACATCCGGCGGCACAGCCGCCCTGGGC TGCCTGGTGAAAGACTACTTCCCCTGCCCCGTGACAGT GTCCTGGAACTCCGGCGCCCTGACATCCGGCGTGCACA CATTCCCCGCCGTGCTGCAGTCCTCCGGCCTGTACTCC CTGTCCTCCGTGGTGACAGTGCCCTCCTCCTCCCTGGG CACACAGACATACATCTGCAACGTGAACCACAAACCCT CCAACACAAAAGTGGACAAAAGGGTGGAGCCCAAATCC TGCGACAAAACACACACATGCCCCCCCTGCCCCGCCCC CGAGCTGCTGGGCGGCCCCTCCGTGTTCCTGTTCCCCC CCAAACCCAAAGACACACTGATGATCTCCAGGACACCC GAGGTGACATGCGTGGTGGTGGACGTGTCCCACGAGGA CCCCGAGGTGAAATTCAACTGGTACGTGGACGGCGTGG AGGTGCACAACGCCAAAACAAAACCCAGGGAGGAGCAG TACAACTCCACATACAGGGTGGTGTCCGTGCTGACAGT GCTGCACCAGGACTGGCTGAACGGCAAAGAGTACAAAT GCAAAGTGTCCAACAAAGCCCTGCCCGCCCCCATCGAG AAAACAATCTCCAAAGCCAAAGGCCAGCCCAGGGAGCC CCAGGTGTACACACTGCCCCCCTCCAGGGAGGAGATGA CAAAAAACCAGGTGTCCCTGACATGCCTGGTGAAAGGC TTCTACCCCTCCGACATCGCCGTGGAGTGGGAGTCCAA CGGCCAGCCCGAGAACAACTACAAAACAACACCCCCCG TGCTGGACTCCGACGGCTCCTTCTTCCTGTACTCCAAA CTGACAGTGGACAAATCCAGGTGGCAGCAGGGCAACGT GTTCTCCTGCTCCGTGATGCACGAGGCCCTGCACAACC ACTACACACAGAAATCCCTGTCCCTGTCCCCCGGCAAA
SEQ ID NO: 14	LCDR1 (Combined)	SGDALRDKFVY
SEQ ID NO: 15	LCDR2 (Combined)	DDNNRPS
SEQ ID NO: 16	LCDR3 (Combined)	QSWDHSYSLVV
SEQ ID NO: 14	LCDR1 (Kabat)	SGDALRDKFVY

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SEQ ID NO: 15	LCDR2 (Kabat)	DDNNRPS
SEQ ID NO: 16	LCDR3 (Kabat)	QSWDHSYSLVV
SEQ ID NO: 17	LCDR1 (Chothia)	DALRDKF
SEQ ID NO: 18	LCDR2 (Chothia)	DDN
SEQ ID NO: 19	LCDR3 (Chothia)	WDHSYSLV
SEQ ID NO: 20	LCDR1 (IMGT)	ALRDKF
SEQ ID NO: 18	LCDR2 (IMGT)	DDN
SEQ ID NO: 16	LCDR3 (IMGT)	QSWDHSYSLVV
SEQ ID NO: 29	VL	SYELTQPPSVSVSPGQTASITCSGDALRDKFVYWYQQK PGQSPVLVIYDDNNRPSGIPERFSGSNSGNTATLTISG TQAMDEADYYCQSWDHSYSLVVFGGGTKLTVL
SEQ ID NO: 30	DNA VL	TCGTACGAGCTCACTCAACCGCCTTCTGTGTCCGTGTC ACCCGGGCAGACTGCCTCCATTACCTGTTCGGGAGATG CCCTGCGCGACAAGTTTGTGTACTGGTACCAGCAGAAG CCCGGACAGTCGCCAGTGCTCGTCATCTATGACGACAA CAACAGACCTTCCGGTATCCCGGAACGGTTCAGCGGAA GCAATTCCGGCAACACCGCTACCCTGACCATTAGCGGC ACTCAGGCCATGGACGAAGCGGATTACTACTGCCAATC CTGGGACCACTCATACTCCCTTGTGGTGTTCGGTGGCG GAACGAAGCTGACCGTCCTG
SEQ ID NO: 31	Light Chain	SYELTQPPSVSVSPGQTASITCSGDALRDKFVYWYQQK PGQSPVLVIYDDNNRPSGIPERFSGSNSGNTATLTISG TQAMDEADYYCQSWDHSYSLVVFGGGTKLTVLGQPKAA PSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKA DSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSH RSYSCQVTHEGSTVEKTVAPTECS
SEQ ID NO: 32	DNA Light Chain	TCGTACGAGCTCACTCAACCGCCTTCTGTGTCCGTGTC ACCCGGGCAGACTGCCTCCATTACCTGTTCGGGAGATG CCCTGCGCGACAAGTTTGTGTACTGGTACCAGCAGAAG CCCGGACAGTCGCCAGTGCTCGTCATCTATGACGACAA CAACAGACCTTCCGGTATCCCGGAACGGTTCAGCGGAA GCAATTCCGGCAACACCGCTACCCTGACCATTAGCGGC ACTCAGGCCATGGACGAAGCGGATTACTACTGCCAATC CTGGGACCACTCATACTCCCTTGTGGTGTTCGGTGGCG GAACGAAGCTGACCGTCCTGGGCCAGCCTAAGGCCGCT CCCTCCGTGACCCTGTTCCCCCCCAGCTCCGAGGAACT GCAGGCCAACAAGGCCACCCTGGTGTGCCTGATCAGCG ACTTCTACCCTGGCGCCGTGACCGTGGCCTGGAAGGCC GACAGCAGCCCCGTGAAGGCCGGCGTGGAGACAACCAC CCCCAGCAAGCAGAGCAACAACAAGTACGCCGCCAGCA GCTACCTGAGCCTGACCCCCGAGCAGTGGAAGAGCCAC AGAAGCTACAGCTGCCAGGTCACCCACGAGGGCAGCAC CGTGGAGAAAACCGTGGCCCCCACCGAGTGCAGC
M2 E152C		
SEQ ID NO: 79	HCDR1 (Combined)	GFTFSSFGMS
SEQ ID NO: 80	HCDR2 (Combined)	AISYSGSDTYYADSVKG
SEQ ID NO: 81	HCDR3 (Combined)	DVGVMDY
SEQ ID NO: 82	HCDR1 (Kabat)	SFGMS
SEQ ID NO: 80	HCDR2 (Kabat)	AISYSGSDTYYADSVKG
SEQ ID NO: 81	HCDR3 (Kabat)	DVGVMDY
SEQ ID NO: 83	HCDR1 (Chothia)	GFTFSSF

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SEQ ID NO: 84	HCDR2 (Chothia)	SYSGSD
SEQ ID NO: 81	HCDR3(Chothia)	DVGVMDY
SEQ ID NO: 85	HCDR1 (IMGT)	GFTFSSFG
SEQ ID NO: 86	HCDR2 (IMGT)	ISYSGSDT
SEQ ID NO: 87	HCDR3 (IMGT)	ARDVGVMDY
SEQ ID NO: 88	VH	QVQLLESGGGLVQPGGSLRLSCAASGFTFSSFGMSWVR QAPGKGLEWVSAISYSGSDTYYADSVKGRFTISRDNSK NTLYLQMNSLRAEDTAVYYCARDVGVMDYWGQGTLVTV SS
SEQ ID NO: 89	DNA VH	CAGGTTCAGCTGCTGGAATCTGGCGGAGGACTGGTTCA ACCTGGCGGCTCTCTGAGACTGTCTTGTGCCGCCAGCG GCTTCACCTTCAGCAGCTTTGGCATGAGCTGGGTCCGA CAGGCCCCTGGCAAAGGACTTGAATGGGTGTCCGCCAT CAGCTACAGCGGCAGCGATACCTACTACGCCGACAGCG TGAAGGGCAGATTCACCATCTCCAGAGACAACAGCAAG AACACCCTGTACCTGCAGATGAACAGCCTGAGAGCCGA GGACACCGCCGTGTACTACTGTGCCAGAGATGTGGGCG TGATGGACTATTGGGGCCAGGGCACACTGGTCACCGTT AGCTCT
SEQ ID NO: 315	Heavy Chain	QVQLLESGGGLVQPGGSLRLSCAASGFTFSSFGMSWVR QAPGKGLEWVSAISYSGSDTYYADSVKGRFTISRDNSK NTLYLQMNSLRAEDTAVYYCARDVGVMDYWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPCP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPC PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 320	DNA Heavy Chain	CAGGTTCAGCTGCTGGAATCTGGCGGAGGACTGGTTCA ACCTGGCGGCTCTCTGAGACTGTCTTGTGCCGCCAGCG GCTTCACCTTCAGCAGCTTTGGCATGAGCTGGGTCCGA CAGGCCCCTGGCAAAGGACTTGAATGGGTGTCCGCCAT CAGCTACAGCGGCAGCGATACCTACTACGCCGACAGCG TGAAGGGCAGATTCACCATCTCCAGAGACAACAGCAAG AACACCCTGTACCTGCAGATGAACAGCCTGAGAGCCGA GGACACCGCCGTGTACTACTGTGCCAGAGATGTGGGCG TGATGGACTATTGGGGCCAGGGCACACTGGTCACCGTT AGCTCTGCTAGCACCAAGGGCCCAAGTGTGTTTCCCCT GGCCCCCAGCAGCAAGTCTACTTCCGGCGGAACTGCTG CCCTGGGTTGCCTGGTGAAGGACTACTTCCCCTGTCCC GTGACAGTGTCCTGGAACTCTGGGGCTCTGACTTCCGG CGTGCACACCTTCCCCGCCGTGCTGCAGAGCAGCGGCC TGTACAGCCTGAGCAGCGTGGTGACAGTGCCCTCCAGC TCTCTGGGAACCCAGACCTATATCTGCAACGTGAACCA CAAGCCCAGCAACACCAAGGTGGACAAGAGAGTGGAGC CCAAGAGCTGCGACAAGACCCACACCTGCCCCCCCTGC CCAGCTCCAGAACTGCTGGGAGGGCCTTCCGTGTTCCT GTTCCCCCCCAAGCCCAAGGACACCCTGATGATCAGCA GGACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGTCC CACGAGGACCCAGAGGTGAAGTTCAACTGGTACGTGGA CGGCGTGGAGGTGCACAACGCCAAGACCAAGCCCAGAG

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		AGGAGCAGTACAACAGCACCTACAGGGTGGTGTCCGTG CTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGA ATACAAGTGCAAAGTCTCCAACAAGGCCCTGCCAGCCC CAATCGAAAAGACAATCAGCAAGGCCAAGGGCCAGCCA CGGGAGCCCCAGGTGTACACCCTGCCCCCCAGCCGGGA GGAGATGACCAAGAACCAGGTGTCCCTGACCTGTCTGG TGAAGGGCTTCTACCCCAGTGATATCGCCGTGGAGTGG GAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCAC CCCCCCAGTGCTGGACAGCGACGGCAGCTTCTTCCTGT ACAGCAAGCTGACCGTGGACAAGTCCAGGTGGCAGCAG GGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCT GCACAACCACTACACCCAGAAGTCCCTGAGCCTGAGCC CCGGCAAG
SEQ ID NO: 92	LCDR1 (Combined)	SGDNLGTYYAH
SEQ ID NO: 93	LCDR2 (Combined)	SQSHRPS
SEQ ID NO: 94	LCDR3 (Combined)	GAWDAPSPELV
SEQ ID NO: 92	LCDR1 (Kabat)	SGDNLGTYYAH
SEQ ID NO: 93	LCDR2 (Kabat)	SQSHRPS
SEQ ID NO: 94	LCDR3 (Kabat)	GAWDAPSPELV
SEQ ID NO: 95	LCDR1 (Chothia)	DNLGTYY
SEQ ID NO: 96	LCDR2 (Chothia)	SQS
SEQ ID NO: 97	LCDR3 (Chothia)	WDAPSPEL
SEQ ID NO: 98	LCDR1 (IMGT)	NLGTYY
SEQ ID NO: 96	LCDR2 (IMGT)	SQS
SEQ ID NO: 94	LCDR3 (IMGT)	GAWDAPSPELV
SEQ ID NO: 99	VL	SYELTQPLSVSVALGQTARITCSGDNLGTYYAHWYQQK PGQAPVLVIYSQSHRPSGIPERFSGSNSGNTATLTISR AQAGDEADYYCGAWDAPSPELVFGGGTKLTVL
SEQ ID NO: 100	DNA VL	AGCTATGAGCTGACACAGCCTCTGTCCGTGTCTGTGGC TCTGGGACAGACCGCCAGAATCACCTGTAGCGGCGATA ACCTGGGCACCTACTACGCCCACTGGTATCAGCAGAAG CCTGGACAGGCTCCCGTGCTGGTCATCTACAGCCAGTC TCACAGACCCAGCGGCATCCCCGAGAGATTCAGCGGCA GCAATAGCGGCAATACCGCCACACTGACCATCAGCAGA GCACAGGCTGGCGACGAGGCCGATTACTATTGTGGCGC TTGGGACGCCCCATCTCCTGAGCTTGTTTTTGGCGGAG GCACCAAGCTGACAGTGCTG
SEQ ID NO: 101	Light Chain	SYELTQPLSVSVALGQTARITCSGDNLGTYYAHWYQQK PGQAPVLVIYSQSHRPSGIPERFSGSNSGNTATLTISR AQAGDEADYYCGAWDAPSPELVFGGGTKLTVLGQPKAA PSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKA DSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSH RSYSCQVTHEGSTVEKTVAPTECS
SEQ ID NO: 102	DNA Light Chain	AGCTATGAGCTGACACAGCCTCTGTCCGTGTCTGTGGC TCTGGGACAGACCGCCAGAATCACCTGTAGCGGCGATA ACCTGGGCACCTACTACGCCCACTGGTATCAGCAGAAG CCTGGACAGGCTCCCGTGCTGGTCATCTACAGCCAGTC TCACAGACCCAGCGGCATCCCCGAGAGATTCAGCGGCA GCAATAGCGGCAATACCGCCACACTGACCATCAGCAGA GCACAGGCTGGCGACGAGGCCGATTACTATTGTGGCGC TTGGGACGCCCCATCTCCTGAGCTTGTTTTTGGCGGAG GCACCAAGCTGACAGTGCTGGGACAGCCTAAGGCCGCT

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		CCCTCCGTGACCCTGTTCCCCCCCCAGCTCCGAGGAACT GCAGGCCAACAAGGCCACCCTGGTGTGCCTGATCAGCG ACTTCTACCCTGGCGCCGTGACCGTGGCCTGGAAGGCC GACAGCAGCCCCGTGAAGGCCGGCGTGGAGACAACCAC CCCCAGCAAGCAGAGCAACAACAAGTACGCCGCCAGCA GCTACCTGAGCCTGACCCCCGAGCAGTGGAAGAGCCAC AGAAGCTACAGCTGCCAGGTCACCCACGAGGGCAGCAC CGTGGAGAAAACCGTGGCCCCCACCGAGTGCAGC

Generation of anti-PMEL17 antibodies are also described in Example 2 of International Application Publication No. WO 2020/128612, which is incorporated by reference in its entirety.

Other antibodies of the disclosure include those where the amino acids or nucleic acids encoding the amino acids have been mutated, yet have at least about 60, about 70, about 80, about 90 or about 95 percent identity to the sequences described in **Table 2**. In some embodiments, about 1, about 2, about 3, about 4 or about 5 amino acids have been mutated in the variable regions when compared with the variable regions depicted in the sequence described in **Table 2**, while retaining substantially the same therapeutic activity as the antibodies listed in **Table 2**.

Since each of these antibodies can bind to PMEL17, the VH, VL, full length light chain, and full-length heavy chain sequences (amino acid sequences and the nucleotide sequences encoding the amino acid sequences) can be "mixed and matched" to create other PMEL17-binding antibodies of the disclosure. Such "mixed and matched" PMEL17-binding antibodies can be tested using the binding assays known in the art (*e.g.*, ELISAs, and other assays described in the Example section). When these chains are mixed and matched, a VH sequence from a particular VH/VL pairing should be replaced with a structurally similar VH sequence. Likewise, a full length heavy chain sequence from a particular full length heavy chain / full length light chain pairing should be replaced with a structurally similar full length heavy chain sequence. Likewise, a VL sequence from a particular VH/VL pairing should be replaced with a structurally similar VL sequence. Likewise a full length light chain sequence from a particular full length heavy chain / full length light chain pairing should be replaced with a structurally similar full length light chain sequence. Accordingly, in some embodiments, the disclosure provides an isolated monoclonal antibody or antigen binding region thereof having: a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 42, 64, 88, 112, 132, 149, 165, 184, 196, 215, 227, 239 or 254; and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 21, 25, 29, 53, 75, 99, 119, 143, 159, 171, 190, 202, 221, 233, 248 or 260; wherein the antibody specifically binds to PMEL17.

In some embodiments, the disclosure provides (i) an isolated monoclonal antibody having: a full length heavy chain comprising an amino acid sequence that has been optimized for expression in the cell of a mammalian expression system selected from the group consisting of SEQ ID NOs: 12,

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44, 66, 90, 114, 134, 151, 167, 186, 198, 217, 229, 241,256, or 315; and a full length light chain comprising an amino acid sequence that has been optimized for expression in the cell of a mammalian selected from the group consisting of SEQ ID NOs: 23, 27, 31, 55, 77, 101, 121, 145, 161, 173, 192, 204, 223, 235, 250 or 262; or (ii) a functional protein comprising an antigen binding portion thereof.

In some embodiments, the disclosure provides PMEL17-binding antibodies that comprise the heavy chain and light chain CDR1s, CDR2s and CDR3s as described in **Table 2**, or combinations thereof. The amino acid sequences of the VH CDR1s of the antibodies are shown, for example, in SEQ ID NOs: 1, 4, 5, 7, 33, 36, 37, 39, 57, 60, 79, 82, 83, 85, 103, 106, 107, 109, 123, 126, 127, 129, 175, 178, 179, 181, 206, 209, 210, and 212. The amino acid sequences of the VH CDR2s of the antibodies and are shown, for example, in SEQ ID NOs: 2, 6, 8, 34, 38, 40, 58, 61, 62, 80, 84, 86, 104, 108, 110, 124, 128, 130, 176, 180, 182, 207, 211, and 213. The amino acid sequences of the VH CDR3s of the antibodies are shown, for example, in SEQ ID NOs: 3, 9, 35, 41, 59, 63, 81, 87, 105, 111, 125, 131, 147, 148, 163, 164, 177, 183, 194, 195, 208, 214, 225, 226, 237, 238, 252, and 253. The amino acid sequences of the VL CDR1s of the antibodies are shown, for example, in SEQ ID NOs: 14, 17, 20, 46, 49, 52, 68, 71, 74, 92, 95, 98, 116, 136, 139, 142, 153, 156, 158, 243, 245, and 247. The amino acid sequences of the VL CDR2s of the antibodies are shown, for example, in SEQ ID Nos: 15, 18, 47, 50, 69, 72, 93, 96, 137, 140, and 154. The amino acid sequences of the VL CDR3s of the antibodies are shown, for example, in SEQ ID NOs: 16, 19, 48, 51, 70, 73, 94, 97, 117, 118, 138, 141, 155, 157, 169, 170188, 189, 200, 201, 219, 220, 231, 232, 244, 246, 258, and 259.

Given that each of these antibodies can bind to PMEL17 and that antigen-binding specificity is provided primarily by the CDR1, 2 and 3 regions, the VH CDR1, CDR2 and CDR3 sequences and VL CDR1, CDR2 and CDR3 sequences can be "mixed and matched" (*i.e.*, CDRs from different antibodies can be mixed and matched. Such "mixed and matched" PMEL17-binding antibodies can be tested using the binding assays known in the art and those described in the Examples (*e.g.*, ELISAs). When VH CDR sequences are mixed and matched, the CDR1, CDR2 and/or CDR3 sequence from a particular VH sequence should be replaced with a structurally similar CDR sequence(s). Likewise, when VL CDR sequences are mixed and matched, the CDR1, CDR2 and/or CDR3 sequence from a particular VL sequence should be replaced with a structurally similar CDR sequence(s). It will be readily apparent to the ordinarily skilled artisan that novel VH and VL sequences can be created by substituting one or more VH and/or VL CDR region sequences with structurally similar sequences from the CDR sequences shown herein for monoclonal antibodies of the present disclosure.

Accordingly, in some embodiments, the present disclosure provides an isolated monoclonal antibody or antigen binding region thereof comprising a heavy chain CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 4, 5, 7, 33, 36, 37, 39, 57, 60, 79, 82, 83, 85, 103, 106, 107, 109, 123, 126, 127, 129, 175, 178, 179, 181, 206, 209, 210, and 212; a heavy chain CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:

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2, 6, 8, 34, 38, 40, 58, 61, 62, 80, 84, 86, 104, 108, 110, 124, 128, 130, 176, 180, 182, 207, 211, and 213; a heavy chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 9, 35, 41, 59, 63, 81, 87, 105, 111, 125, 131, 147, 148, 163, 164, 177, 183, 194, 195, 208, 214, 225, 226, 237, 238, 252, and 253; a light chain CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 17, 20, 46, 49, 52, 68, 71, 74, 92, 95, 98, 116, 136, 139, 142, 153, 156, 158, 243, 245, and 247; a light chain CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 18, 47, 50, 69, 72, 93, 96, 137, 140, and 154; and a light chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 19, 48, 51, 70, 73, 94, 97, 117, 118, 138, 141, 155, 157, 169, 170, 188, 189, 200, 201, 219, 220, 231, 232, 244, 246, 258, and 259; wherein the antibody specifically binds PMEL17.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain CDR1 of SEQ ID NO:1, 4, 5 or 7, a heavy chain CDR2 of SEQ ID NO:2, 6 or 8; a heavy chain CDR3 of SEQ ID NO:3 or 9; a light chain CDR1 of SEQ ID NO:14, 17 or 20; a light chain CDR2 of SEQ ID NO:15 or 18; and a light chain CDR3 of SEQ ID NO:16 or 19.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain CDR1 of SEQ ID NO:33, 36, 37 or 39, a heavy chain CDR2 of SEQ ID NO:34, 38 or 40; a heavy chain CDR3 of SEQ ID NO:35 or 41; a light chain CDR1 of SEQ ID NO:46, 49 or 52; a light chain CDR2 of SEQ ID NO:47 or 50; and a light chain CDR3 of SEQ ID NO:48 or 51.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain CDR1 of SEQ ID NO:5, 7, 57 or 60, a heavy chain CDR2 of SEQ ID NO:58, 61 or 62; a heavy chain CDR3 of SEQ ID NO:59 or 63; a light chain CDR1 of SEQ ID NO:68, 71 or 74; a light chain CDR2 of SEQ ID NO:69 or 72; and a light chain CDR3 of SEQ ID NO:70 or 73.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain CDR1 of SEQ ID NO:79, 82, 83 or 85, a heavy chain CDR2 of SEQ ID NO:80, 84 or 86; a heavy chain CDR3 of SEQ ID NO:81 or 87; a light chain CDR1 of SEQ ID NO:92, 95 or 98; a light chain CDR2 of SEQ ID NO:93 or 96; and a light chain CDR3 of SEQ ID NO:94 or 97.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain CDR1 of SEQ ID NO:103, 106, 107 or 109, a heavy chain CDR2 of SEQ ID NO:104, 108 or 110; a heavy chain CDR3 of SEQ ID NO:105 or 111; a light chain CDR1 of SEQ ID NO:49, 52 or 116; a light chain CDR2 of SEQ ID NO:47 or 50; and a light chain CDR3 of SEQ ID NO:117 or 118.

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In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain CDR1 of SEQ ID NO:123, 126, 127 or 129, a heavy chain CDR2 of SEQ ID NO:124, 128 or 130; a heavy chain CDR3 of SEQ ID NO:125 or 131; a light chain CDR1 of SEQ ID NO:136, 139 or 142; a light chain CDR2 of SEQ ID NO:137 or 140; and a light chain CDR3 of SEQ ID NO:138 or 141.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain CDR1 of SEQ ID NO:123, 126, 127 or 129, a heavy chain CDR2 of SEQ ID NO:124, 128 or 130; a heavy chain CDR3 of SEQ ID NO:147 or 148; a light chain CDR1 of SEQ ID NO:153, 156 or 158; a light chain CDR2 of SEQ ID NO:50 or 154; and a light chain CDR3 of SEQ ID NO:155 or 157.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain CDR1 of SEQ ID NO:103, 106, 107 or 109, a heavy chain CDR2 of SEQ ID NO:104, 108 or 110; a heavy chain CDR3 of SEQ ID NO:163 or 164; a light chain CDR1 of SEQ ID NO:49, 52 or 116; a light chain CDR2 of SEQ ID NO:47 or 50; and a light chain CDR3 of SEQ ID NO:169 or 170.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain CDR1 of SEQ ID NO:175, 178, 179 or 181, a heavy chain CDR2 of SEQ ID NO:176, 180 or 182; a heavy chain CDR3 of SEQ ID NO:177 or 183; a light chain CDR1 of SEQ ID NO:49, 52 or 116; a light chain CDR2 of SEQ ID NO:47 or 50; and a light chain CDR3 of SEQ ID NO:188 or 189.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain CDR1 of SEQ ID NO: 103, 106, 107 or 109, a heavy chain CDR2 of SEQ ID NO: 104, 108 or 110; a heavy chain CDR3 of SEQ ID NO:194 or 195; a light chain CDR1 of SEQ ID NO: 49, 52 or 116; a light chain CDR2 of SEQ ID NO: 47 or 50; and a light chain CDR3 of SEQ ID NO:200 or 201.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain CDR1 of SEQ ID NO:206, 209, 210 or 212, a heavy chain CDR2 of SEQ ID NO:207, 211 or 213; a heavy chain CDR3 of SEQ ID NO:208 or 214; a light chain CDR1 of SEQ ID NO:153, 156 or 158; a light chain CDR2 of SEQ ID NO:50 or 154; and a light chain CDR3 of SEQ ID NO:219 or 220.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain CDR1 of SEQ ID NO: 206, 209, 210 or 212, a heavy chain CDR2 of SEQ ID NO: 207, 211 or 213; a heavy chain CDR3 of SEQ ID NO:225 or 226; a light chain CDR1 of SEQ ID NO:136, 139 or 142; a light chain CDR2 of SEQ ID NO:137 or 140; and a light chain CDR3 of SEQ ID NO:231 or 232.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain variable region that comprises an HCDR1

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of SEQ ID NO: 206, 209, 210 or 212, an HCDR2 of SEQ ID NO: 207, 211 or 213, and an HCDR3 of SEQ ID NO:237 or 238; and a light chain variable region that comprises an LCDR1 of SEQ ID NO:243, 245 or 247, an LCDR2 of SEQ ID NO:47 or 50, and an LCDR3 of SEQ ID NO:244 or 246.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain variable region that comprises an HCDR1 of SEQ ID NO: 206, 209, 210 or 212, an HCDR2 of SEQ ID NO: 207, 211 or 213, and an HCDR3 of SEQ ID NO:252 or 253; and a light chain variable region that comprises an LCDR1 of SEQ ID NO:153, 156 or 158, an LCDR2 of SEQ ID NO:50 or 154, and an LCDR3 of SEQ ID NO:258 or 259.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises CDR sequences selected from:

a heavy chain CDR1 of SEQ ID NO:1, , a heavy chain CDR2 of SEQ ID NO:2, a heavy chain CDR3 of SEQ ID NO:3, a light chain CDR1 of SEQ ID NO:14, a light chain CDR2 of SEQ ID NO:15, and a light chain CDR3 of SEQ ID NO:16;

a heavy chain CDR1 of SEQ ID NO: 4, a heavy chain CDR2 of SEQ ID NO:2, a heavy chain CDR3 of SEQ ID NO:3, a light chain CDR1 of SEQ ID NO:14, a light chain CDR2 of SEQ ID NO:15, and a light chain CDR3 of SEQ ID NO:16;

a heavy chain CDR1 of SEQ ID NO:5, a heavy chain CDR2 of SEQ ID NO:6, a heavy chain CDR3 of SEQ ID NO:3, a light chain CDR1 of SEQ ID NO:17, a light chain CDR2 of SEQ ID NO: 18, and a light chain CDR3 of SEQ ID NO: 19; or

a heavy chain CDR1 of SEQ ID NO:7, a heavy chain CDR2 of SEQ ID NO:8, a heavy chain CDR3 of SEQ ID NO:9, a light chain CDR1 of SEQ ID NO:20, a light chain CDR2 of SEQ ID NO:18, and a light chain CDR3 of SEQ ID NO:16.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises CDR sequences selected from:

a heavy chain CDR1 of SEQ ID NO:33, a heavy chain CDR2 of SEQ ID NO:34, a heavy chain CDR3 of SEQ ID NO:35, a light chain CDR1 of SEQ ID NO:46, a light chain CDR2 of SEQ ID NO:47, and a light chain CDR3 of SEQ ID NO:48;

a heavy chain CDR1 of SEQ ID NO:36, a heavy chain CDR2 of SEQ ID NO:34, a heavy chain CDR3 of SEQ ID NO:35, a light chain CDR1 of SEQ ID NO:46, a light chain CDR2 of SEQ ID NO:47, and a light chain CDR3 of SEQ ID NO:48;

a heavy chain CDR1 of SEQ ID NO:37, a heavy chain CDR2 of SEQ ID NO:38, a heavy chain CDR3 of SEQ ID NO:35, a light chain CDR1 of SEQ ID NO:49, a light chain CDR2 of SEQ ID NO:50, and a light chain CDR3 of SEQ ID NO:51; or

a heavy chain CDR1 of SEQ ID NO: 39, a heavy chain CDR2 of SEQ ID NO:40, a heavy chain CDR3 of SEQ ID NO:41, a light chain CDR1 of SEQ ID NO:52, a light chain CDR2 of SEQ ID NO:50, and a light chain CDR3 of SEQ ID NO:48.

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In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises CDR sequences selected from:

a) a heavy chain CDR1 of SEQ ID NO:57, a heavy chain CDR2 of SEQ ID NO:58, a heavy chain CDR3 of SEQ ID NO:59, a light chain CDR1 of SEQ ID NO:68, a light chain CDR2 of SEQ ID NO:69, and a light chain CDR3 of SEQ ID NO:70;

b) a heavy chain CDR1 of SEQ ID NO:60, a heavy chain CDR2 of SEQ ID NO:58, a heavy chain CDR3 of SEQ ID NO:59, a light chain CDR1 of SEQ ID NO:68, a light chain CDR2 of SEQ ID NO:69, and a light chain CDR3 of SEQ ID NO:70;

c) a heavy chain CDR1 of SEQ ID NO:5, a heavy chain CDR2 of SEQ ID NO:61, a heavy chain CDR3 of SEQ ID NO:59, a light chain CDR1 of SEQ ID NO:71, a light chain CDR2 of SEQ ID NO:72, and a light chain CDR3 of SEQ ID NO:73; or

d) a heavy chain CDR1 of SEQ ID NO:7, a heavy chain CDR2 of SEQ ID NO:62, a heavy chain CDR3 of SEQ ID NO:63, a light chain CDR1 of SEQ ID NO:74, a light chain CDR2 of SEQ ID NO:72, and a light chain CDR3 of SEQ ID NO:70.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises CDR sequences selected from:

a heavy chain CDR1 of SEQ ID NO:79, , a heavy chain CDR2 of SEQ ID NO:80, a heavy chain CDR3 of SEQ ID NO:81, a light chain CDR1 of SEQ ID NO:92, a light chain CDR2 of SEQ ID NO:93, and a light chain CDR3 of SEQ ID NO:94;

a heavy chain CDR1 of SEQ ID NO:82, a heavy chain CDR2 of SEQ ID NO:80, a heavy chain CDR3 of SEQ ID NO:81, a light chain CDR1 of SEQ ID NO:92, a light chain CDR2 of SEQ ID NO:93, and a light chain CDR3 of SEQ ID NO:94;

a heavy chain CDR1 of SEQ ID NO:83, a heavy chain CDR2 of SEQ ID NO:84, a heavy chain CDR3 of SEQ ID NO:81, a light chain CDR1 of SEQ ID NO:95, a light chain CDR2 of SEQ ID NO:96, and a light chain CDR3 of SEQ ID NO: 97; or

a heavy chain CDR1 of SEQ ID NO: 85, a heavy chain CDR2 of SEQ ID NO:86, a heavy chain CDR3 of SEQ ID NO:87, a light chain CDR1 of SEQ ID NO:98, a light chain CDR2 of SEQ ID NO:96, and a light chain CDR3 of SEQ ID NO:94.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises CDR sequences selected from:

a heavy chain CDR1 of SEQ ID NO:103, a heavy chain CDR2 of SEQ ID NO:104, a heavy chain CDR3 of SEQ ID NO:105, a light chain CDR1 of SEQ ID NO: 116; a light chain CDR2 of SEQ ID NO:47; and a light chain CDR3 of SEQ ID NO:117;

a heavy chain CDR1 of SEQ ID NO:106, a heavy chain CDR2 of SEQ ID NO:104, a heavy chain CDR3 of SEQ ID NO:105, a light chain CDR1 of SEQ ID NO: 116, a light chain CDR2 of SEQ ID NO:47, and a light chain CDR3 of SEQ ID NO:117;

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a heavy chain CDR1 of SEQ ID NO:107, a heavy chain CDR2 of SEQ ID NO:108, a heavy chain CDR3 of SEQ ID NO:105, a light chain CDR1 of SEQ ID NO:49, a light chain CDR2 of SEQ ID NO:50, and a light chain CDR3 of SEQ ID NO:118; or

a heavy chain CDR1 of SEQ ID NO:109, a heavy chain CDR2 of SEQ ID NO:110, a heavy chain CDR3 of SEQ ID NO:111, a light chain CDR1 of SEQ ID NO:52 a light chain CDR2 of SEQ ID NO:50, and a light chain CDR3 of SEQ ID NO:117.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises CDR sequences selected from:

a heavy chain CDR1 of SEQ ID NO:123, a heavy chain CDR2 of SEQ ID NO:124, a heavy chain CDR3 of SEQ ID NO:125, a light chain CDR1 of SEQ ID NO:136, a light chain CDR2 of SEQ ID NO:137,and a light chain CDR3 of SEQ ID NO:138;

a heavy chain CDR1 of SEQ ID NO:126, a heavy chain CDR2 of SEQ ID NO:124, a heavy chain CDR3 of SEQ ID NO:125, a light chain CDR1 of SEQ ID NO:136, a light chain CDR2 of SEQ ID NO:137, and a light chain CDR3 of SEQ ID NO:138;

a heavy chain CDR1 of SEQ ID NO:127, a heavy chain CDR2 of SEQ ID NO:128, a heavy chain CDR3 of SEQ ID NO:125, a light chain CDR1 of SEQ ID NO:139, a light chain CDR2 of SEQ ID NO:140, and a light chain CDR3 of SEQ ID NO: 141; or

a heavy chain CDR1 of SEQ ID NO: 129, a heavy chain CDR2 of SEQ ID NO:130, a heavy chain CDR3 of SEQ ID NO:131, a light chain CDR1 of SEQ ID NO:142, a light chain CDR2 of SEQ ID NO:140, and a light chain CDR3 of SEQ ID NO:138.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises CDR sequences selected from:

a heavy chain CDR1 of SEQ ID NO:123, a heavy chain CDR2 of SEQ ID NO:124, a heavy chain CDR3 of SEQ ID NO:147, a light chain CDR1 of SEQ ID NO:153, a light chain CDR2 of SEQ ID NO:154, and a light chain CDR3 of SEQ ID NO:155;

a heavy chain CDR1 of SEQ ID NO:126, a heavy chain CDR2 of SEQ ID NO:124, a heavy chain CDR3 of SEQ ID NO:147, a light chain CDR1 of SEQ ID NO:153, a light chain CDR2 of SEQ ID NO: 154, and a light chain CDR3 of SEQ ID NO:155;

a heavy chain CDR1 of SEQ ID NO:127, a heavy chain CDR2 of SEQ ID NO:128, a heavy chain CDR3 of SEQ ID NO:147, a light chain CDR1 of SEQ ID NO:156, a light chain CDR2 of SEQ ID NO:50, and a light chain CDR3 of SEQ ID NO:157; or

a heavy chain CDR1 of SEQ ID NO: 129, a heavy chain CDR2 of SEQ ID NO:130, a heavy chain CDR3 of SEQ ID NO:148, a light chain CDR1 of SEQ ID NO:158, a light chain CDR2 of SEQ ID NO:50, and a light chain CDR3 of SEQ ID NO:155.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises CDR sequences selected from:

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a heavy chain CDR1 of SEQ ID NO:103, a heavy chain CDR2 of SEQ ID NO:104, a heavy chain CDR3 of SEQ ID NO:163, a light chain CDR1 of SEQ ID NO: 116, a light chain CDR2 of SEQ ID NO:47, and a light chain CDR3 of SEQ ID NO:169;

a heavy chain CDR1 of SEQ ID NO:106, a heavy chain CDR2 of SEQ ID NO:104, a heavy chain CDR3 of SEQ ID NO:163, a light chain CDR1 of SEQ ID NO:116, a light chain CDR2 of SEQ ID NO:47, and a light chain CDR3 of SEQ ID NO:169;

a heavy chain CDR1 of SEQ ID NO:107, a heavy chain CDR2 of SEQ ID NO:108, a heavy chain CDR3 of SEQ ID NO:163, a light chain CDR1 of SEQ ID NO:49, a light chain CDR2 of SEQ ID NO:50, and a light chain CDR3 of SEQ ID NO:170; or

a heavy chain CDR1 of SEQ ID NO: 109, a heavy chain CDR2 of SEQ ID NO:110, a heavy chain CDR3 of SEQ ID NO:164, a light chain CDR1 of SEQ ID NO:52, a light chain CDR2 of SEQ ID NO:50, and a light chain CDR3 of SEQ ID NO:169.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises CDR sequences selection from:

a heavy chain CDR1 of SEQ ID NO:175, a heavy chain CDR2 of SEQ ID NO:176, a heavy chain CDR3 of SEQ ID NO:177, a light chain CDR1 of SEQ ID NO:116, a light chain CDR2 of SEQ ID NO:47, and a light chain CDR3 of SEQ ID NO:188;

a heavy chain CDR1 of SEQ ID NO:178, a heavy chain CDR2 of SEQ ID NO:176, a heavy chain CDR3 of SEQ ID NO:177, a light chain CDR1 of SEQ ID NO:116, a light chain CDR2 of SEQ ID NO:47, and a light chain CDR3 of SEQ ID NO:188;

a heavy chain CDR1 of SEQ ID NO:179, a heavy chain CDR2 of SEQ ID NO:180, a heavy chain CDR3 of SEQ ID NO:177, a light chain CDR1 of SEQ ID NO:49, a light chain CDR2 of SEQ ID NO:50, and a light chain CDR3 of SEQ ID NO:189; or

a heavy chain CDR1 of SEQ ID NO: 181, a heavy chain CDR2 of SEQ ID NO:182; a heavy chain CDR3 of SEQ ID NO:183, a light chain CDR1 of SEQ ID NO:52, a light chain CDR2 of SEQ ID NO:50, and a light chain CDR3 of SEQ ID NO:188.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises CDR sequences selected from:

a heavy chain CDR1 of SEQ ID NO: 103, a heavy chain CDR2 of SEQ ID NO: 104, a heavy chain CDR3 of SEQ ID NO:194, a light chain CDR1 of SEQ ID NO: 116, a light chain CDR2 of SEQ ID NO: 47, and a light chain CDR3 of SEQ ID NO:200;

a heavy chain CDR1 of SEQ ID NO: 106, a heavy chain CDR2 of SEQ ID NO: 104, a heavy chain CDR3 of SEQ ID NO:194, a light chain CDR1 of SEQ ID NO: 116, a light chain CDR2 of SEQ ID NO: 47, and a light chain CDR3 of SEQ ID NO:200;

a heavy chain CDR1 of SEQ ID NO: 107, a heavy chain CDR2 of SEQ ID NO: 108, a heavy chain CDR3 of SEQ ID NO:194, a light chain CDR1 of SEQ ID NO: 49, a light chain CDR2 of SEQ ID NO: 50, and a light chain CDR3 of SEQ ID NO: 201; or

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a heavy chain CDR1 of SEQ ID NO: 109, a heavy chain CDR2 of SEQ ID NO: 110, a heavy chain CDR3 of SEQ ID NO:195, a light chain CDR1 of SEQ ID NO: 52, a light chain CDR2 of SEQ ID NO: 50, and a light chain CDR3 of SEQ ID NO:200.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises CDR sequences selected from:

a heavy chain CDR1 of SEQ ID NO:206, a heavy chain CDR2 of SEQ ID NO:207, a heavy chain CDR3 of SEQ ID NO:208, a light chain CDR1 of SEQ ID NO:153, a light chain CDR2 of SEQ ID NO:154, and a light chain CDR3 of SEQ ID NO:219;

a heavy chain CDR1 of SEQ ID NO:209, a heavy chain CDR2 of SEQ ID NO:207, a heavy chain CDR3 of SEQ ID NO:208, a light chain CDR1 of SEQ ID NO:153, a light chain CDR2 of SEQ ID NO: 154, and a light chain CDR3 of SEQ ID NO:219;

a heavy chain CDR1 of SEQ ID NO:210, a heavy chain CDR2 of SEQ ID NO:211, a heavy chain CDR3 of SEQ ID NO:208, a light chain CDR1 of SEQ ID NO:156, a light chain CDR2 of SEQ ID NO:50, and a light chain CDR3 of SEQ ID NO:220; or

a heavy chain CDR1 of SEQ ID NO: 212, a heavy chain CDR2 of SEQ ID NO:213, a heavy chain CDR3 of SEQ ID NO:214, a light chain CDR1 of SEQ ID NO:158, a light chain CDR2 of SEQ ID NO:50, and a light chain CDR3 of SEQ ID NO:219.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises CDR sequences selected from:

a heavy chain CDR1 of SEQ ID NO: 206, a heavy chain CDR2 of SEQ ID NO: 207, a heavy chain CDR3 of SEQ ID NO:225, a light chain CDR1 of SEQ ID NO:136, a light chain CDR2 of SEQ ID NO:137,and a light chain CDR3 of SEQ ID NO:231;

a heavy chain CDR1 of SEQ ID NO: 209, a heavy chain CDR2 of SEQ ID NO: 207, a heavy chain CDR3 of SEQ ID NO:225, a light chain CDR1 of SEQ ID NO:136, a light chain CDR2 of SEQ ID NO:137, and a light chain CDR3 of SEQ ID NO:231;

a heavy chain CDR1 of SEQ ID NO: 210, a heavy chain CDR2 of SEQ ID NO: 211, a heavy chain CDR3 of SEQ ID NO:225, a light chain CDR1 of SEQ ID NO:139, a light chain CDR2 of SEQ ID NO:140, and a light chain CDR3 of SEQ ID NO: 232; or

a heavy chain CDR1 of SEQ ID NO: 212, a heavy chain CDR2 of SEQ ID NO: 213, a heavy chain CDR3 of SEQ ID NO: 226, a light chain CDR1 of SEQ ID NO:142; a light chain CDR2 of SEQ ID NO: 140; and a light chain CDR3 of SEQ ID NO:231.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises CDR sequences selected from:

a heavy chain variable region that comprises an HCDR1 of SEQ ID NO: 206, an HCDR2 of SEQ ID NO: 207, and an HCDR3 of SEQ ID NO:237, and a light chain variable region that comprises an LCDR1 of SEQ ID NO:243, an LCDR2 of SEQ ID NO:47, and an LCDR3 of SEQ ID NO:244;

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a heavy chain variable region that comprises an HCDR1 of SEQ ID NO: 209, an HCDR2 of SEQ ID NO: 207, and an HCDR3 of SEQ ID NO:237, and a light chain variable region that comprises an LCDR1 of SEQ ID NO:243, an LCDR2 of SEQ ID NO:47, and an LCDR3 of SEQ ID NO:244;

a heavy chain variable region that comprises an HCDR1 of SEQ ID NO: 210, an HCDR2 of SEQ ID NO: 211, and an HCDR3 of SEQ ID NO:237, and a light chain variable region that comprises an LCDR1 of SEQ ID NO:245, an LCDR2 of SEQ ID NO:50, and an LCDR3 of SEQ ID NO:246; or

a heavy chain variable region that comprises an HCDR1 of SEQ ID NO: 212, an HCDR2 of SEQ ID NO: 213, and an HCDR3 of SEQ ID NO:238; and a light chain variable region that comprises an LCDR1 of SEQ ID NO:247, an LCDR2 of SEQ ID NO: 50, and an LCDR3 of SEQ ID NO:244.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises CDR sequences selected from:

a heavy chain variable region that comprises an HCDR1 of SEQ ID NO: 206, an HCDR2 of SEQ ID NO: 207, and an HCDR3 of SEQ ID NO:252, and a light chain variable region that comprises an LCDR1 of SEQ ID NO:153, an LCDR2 of SEQ ID NO: 154, and an LCDR3 of SEQ ID NO:258;

a heavy chain variable region that comprises an HCDR1 of SEQ ID NO: 209, an HCDR2 of SEQ ID NO: 207, and an HCDR3 of SEQ ID NO:252, and a light chain variable region that comprises an LCDR1 of SEQ ID NO:153, an LCDR2 of SEQ ID NO:154, and an LCDR3 of SEQ ID NO:258;

a heavy chain variable region that comprises an HCDR1 of SEQ ID NO: 210, an HCDR2 of SEQ ID NO: 211, and an HCDR3 of SEQ ID NO:252, and a light chain variable region that comprises an LCDR1 of SEQ ID NO:156, an LCDR2 of SEQ ID NO:50, and an LCDR3 of SEQ ID NO:259; or

a heavy chain variable region that comprises an HCDR1 of SEQ ID NO: 212, an HCDR2 of SEQ ID NO: 213, and an HCDR3 of SEQ ID NO: 253; and a light chain variable region that comprises an LCDR1 of SEQ ID NO:158, an LCDR2 of SEQ ID NO:50, and an LCDR3 of SEQ ID NO:258.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:10, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:21.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain variable region (VH) comprising the

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amino acid sequence of SEQ ID NO:10, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:25.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:10, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:29.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:42, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:53.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:64, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:75.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:88, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:99.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:112, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:119.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:132, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:143.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:149, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:159.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:165, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:171.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain variable region (VH) comprising the

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amino acid sequence of SEQ ID NO:184, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:190.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:196, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:202.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:215, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:221.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:227, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:233.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:239, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:248.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:254, and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:260.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:12, and a light chain comprising the amino acid sequence of SEQ ID NO:23.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:12, and a light chain comprising the amino acid sequence of SEQ ID NO:27.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:12, and a light chain comprising the amino acid sequence of SEQ ID NO:31.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:44, and a light chain comprising the amino acid sequence of SEQ ID NO:55.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:66, and a light chain comprising the amino acid sequence of SEQ ID NO:77.

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In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:90, and a light chain comprising the amino acid sequence of SEQ ID NO:101.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:114, and a light chain comprising the amino acid sequence of SEQ ID NO:121.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:134, and a light chain comprising the amino acid sequence of SEQ ID NO:145.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:151, and a light chain comprising the amino acid sequence of SEQ ID NO:161.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:167, and a light chain comprising the amino acid sequence of SEQ ID NO:173.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:186, and a light chain comprising the amino acid sequence of SEQ ID NO:192.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:198, and a light chain comprising the amino acid sequence of SEQ ID NO:204.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:217, and a light chain comprising the amino acid sequence of SEQ ID NO:223.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:229, and a light chain comprising the amino acid sequence of SEQ ID NO:235.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:241, and a light chain comprising the amino acid sequence of SEQ ID NO:250.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:256, and a light chain comprising the amino acid sequence of SEQ ID NO:262.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:283, and a light chain comprising the amino acid sequence of SEQ ID NO:27.

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In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:283, and a light chain comprising the amino acid sequence of SEQ ID NO:31.

In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:315, and a light chain comprising the amino acid sequence of SEQ ID NO:101.

In certain embodiments, an antibody that specifically binds to PMEL17 is an antibody or antibody fragment (*e.g.*, antigen binding fragment) that is described in **Table 2**.

In some embodiments, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 belongs to the IgG1/ λ isotype subclass. The N-glycosylation site is located at Asn306 on the heavy chain of the antibody or fragment. In some embodiments, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to PMEL17 comprises an N-glycosylation site located at Asn306 of the heavy chain. Both heavy chains of the antibody or antibody fragment (*e.g.*, antigen binding fragments) comprise oligosaccharide chains linked to the protein backbone at Asn306.

1. Identification of Epitopes and Antibodies That Bind to the Same Epitope

The disclosure also provides antibodies and antibody fragments (*e.g.*, antigen binding fragments) that specifically bind to the same epitope as the anti-PMEL17 antibodies described in **Table 2**, or cross compete with the antibodies described in **Table 2**. Additional antibodies and antibody fragments (*e.g.*, antigen binding fragments) can therefore be identified based on their ability to cross-compete (*e.g.*, to competitively inhibit the binding of, in a statistically significant manner) with other antibodies of the disclosure in PMEL17 binding assays, for example, via BIACORE or assays known to persons skilled in the art for measuring binding. The ability of a test antibody to inhibit the binding of antibodies and antibody fragments (*e.g.*, antigen binding fragments) of the present disclosure to a PMEL17 (*e.g.*, human PMEL17) demonstrates that the test antibody can compete with that antibody or antibody fragment (*e.g.*, antigen binding fragments) for binding to PMEL17; such an antibody may, according to non-limiting theory, bind to the same or a related (*e.g.*, a structurally similar or spatially proximal or overlapping) epitope on the PMEL17 protein as the antibody or antibody fragment (*e.g.*, antigen binding fragments) with which it competes. In certain embodiments, the antibodies that bind to the same epitope on PMEL17 as the antibodies or antibody fragments (*e.g.*, antigen binding fragments) described in **Table 2** are human or humanized monoclonal antibodies. Such human or humanized monoclonal antibodies can be prepared and isolated as described herein.

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2. Further Alteration of the Framework of Fc Region

The immunoconjugates of the disclosure may comprise modified antibodies or antigen binding fragments thereof that further comprise modifications to framework residues within VH and/or VL, *e.g.*, to improve the properties of the antibody. In some embodiments, the framework modifications are made to decrease the immunogenicity of the antibody. For example, one approach is to "back-mutate" one or more framework residues to the corresponding germline sequence. More specifically, an antibody that has undergone somatic mutation may contain framework residues that differ from the germline sequence from which the antibody is derived. Such residues can be identified by comparing the antibody framework sequences to the germline sequences from which the antibody is derived. To return the framework region sequences to their germline configuration, the somatic mutations can be "back-mutated" to the germline sequence by, for example, site-directed mutagenesis. Such "back-mutated" antibodies are also intended to be encompassed by the disclosure.

Another type of framework modification involves mutating one or more residues within the framework region, or even within one or more CDR regions, to remove T-cell epitopes to thereby reduce the potential immunogenicity of the antibody. This approach is also referred to as "deimmunization" and is described in further detail in U.S. Application Publication No. 2003/0153043.

In addition, or in the alternative to modifications made within the framework or CDR regions, antibodies of the disclosure may be engineered to include modifications within the Fc region, typically to alter one or more functional properties of the antibody, such as serum half-life, complement fixation, Fc receptor binding, and/or antigen-dependent cellular cytotoxicity (ADCC). Furthermore, an antibody of the disclosure may be chemically modified (*e.g.*, one or more chemical moieties can be attached to the antibody) or be modified to alter its glycosylation, again to alter one or more functional properties of the antibody. Each of these embodiments is described in further detail below.

In some embodiments, the hinge region of CH1 is modified such that the number of cysteine residues in the hinge region is altered, *e.g.*, increased or decreased. This approach is described further in U.S. Patent No. 5,677,425. The number of cysteine residues in the hinge region of CH1 is altered to, for example, facilitate assembly of the light and heavy chains or to increase or decrease the stability of the antibody.

In some embodiments, the antibody or antibody fragment disclosed herein include modified or engineered amino acid residues, *e.g.*, one or more cysteine residues, as sites for conjugation to a drug moiety (Junutula JR, *et al.*, Nat Biotechnol 2008, 26:925-932). In one embodiment, the disclosure provides a modified antibody or antibody fragment comprising a substitution of one or more amino acids with cysteine at the positions described herein. Sites for cysteine substitution are in the constant regions of the antibody or antibody fragment and are thus applicable to a variety of antibody or antibody fragment, and the sites are selected to provide stable and homogeneous

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conjugates. A modified antibody or fragment can have one, two or more cysteine substitutions, and these substitutions can be used in combination with other modification and conjugation methods as described herein. Methods for inserting cysteine at specific locations of an antibody are known in the art, *see, e.g.*, Lyons *et al.*, (1990) Protein Eng., 3:703-708, WO 2011/005481, WO2014/124316, WO 2015/138615. In certain embodiments, a modified antibody comprises a substitution of one or more amino acids with cysteine on its constant region selected from positions 117, 119, 121, 124, 139, 152, 153, 155, 157, 164, 169, 171, 174, 189, 191, 195, 197, 205, 207, 246, 258, 269, 274, 286, 288, 290, 292, 293, 320, 322, 326, 333, 334, 335, 337, 344, 355, 360, 375, 382, 390, 392, 398, 400 and 422 of a heavy chain of the antibody, and wherein the positions are numbered according to the EU system. In some embodiments a modified antibody or antibody fragment comprises a substitution of one or more amino acids with cysteine on its constant region selected from positions 107, 108, 109, 114, 129, 142, 143, 145, 152, 154, 156, 159, 161, 165, 168, 169, 170, 182, 183, 197, 199, and 203 of a light chain of the antibody or antibody fragment, wherein the positions are numbered according to the EU system, and wherein the light chain is a human kappa light chain. In certain embodiments a modified antibody or antibody fragment thereof comprises a combination of substitution of two or more amino acids with cysteine on its constant regions wherein the combinations comprise substitutions at positions 375 of an antibody heavy chain, position 152 of an antibody heavy chain, position 360 of an antibody heavy chain, or position 107 of an antibody light chain and wherein the positions are numbered according to the EU system. In certain embodiments a modified antibody or antibody fragment thereof comprises a substitution of one amino acid with cysteine on its constant regions wherein the substitution is position 375 of an antibody heavy chain, position 152 of an antibody heavy chain, position 360 of an antibody heavy chain, position 107 of an antibody light chain, position 165 of an antibody light chain or position 159 of an antibody light chain and wherein the positions are numbered according to the EU system, and wherein the light chain is a kappa chain. In particular embodiments, a modified antibody or antibody fragment thereof comprises a combination of substitution of two amino acids with cysteine on its constant regions wherein the combinations comprise substitutions at positions 375 of an antibody heavy chain and position 152 of an antibody heavy chain, wherein the positions are numbered according to the EU system. In particular embodiments a modified antibody or antibody fragment thereof comprises a substitution of one amino acid with cysteine at position 360 of an antibody heavy chain, wherein the positions are numbered according to the EU system. In other particular embodiments a modified antibody or antibody fragment thereof comprises a substitution of one amino acid with cysteine at position 107 of an antibody light chain and wherein the positions are numbered according to the EU system, and wherein the light chain is a kappa chain.

In additional embodiments antibodies or antibody fragments (*e.g.*, antigen binding fragment) useful in immunoconjugates of the disclosure include modified or engineered antibodies, such as an antibody modified to introduce one or more other reactive amino acid (other than cysteine), including

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Pcl, pyrrolysine, peptide tags (such as S6, A1 and ybbR tags), and non-natural amino acids, in place of at least one amino acid of the native sequence, thus providing a reactive site on the antibody or antigen binding fragment for conjugation to a drug moiety or a linker-drug moiety with complementary reactivity. For example, the antibodies or antibody fragments can be modified to incorporate Pcl or pyrrolysine (W. Ou, *et al.*, (2011) PNAS 108 (26), 10437-10442; WO 2014/124258) or unnatural amino acids (J.Y. Axup, *et al.*, Proc Natl Acad Sci U S A, 109 (2012), pp. 16101–16106; for review, see C.C. Liu and P.G. Schultz (2010) Annu Rev Biochem 79, 413-444; C.H. Kim, *et al.*, (2013) Curr Opin Chem Biol. 17, 412-419) as sites for conjugation to a drug. Similarly, peptide tags for enzymatic conjugation methods can be introduced into an antibody (Strop P., *et al.*, Chem Biol. 2013, 20(2):161-7; Rabuka D., Curr Opin Chem Biol. 2010 Dec;14(6):790-6; Rabuka D, *et al.*, Nat Protoc. 2012, 7(6):1052-67). One other example is the use of 4'-phosphopantetheinyl transferases (PPTase) for the conjugation of Co-enzyme A analogs (WO 2013/184514), and (Grünewald *et al., (2015)* Bioconjugate Chem. 26 (12), 2554-62). Methods for conjugating such modified or engineered antibodies with payloads or linker-payload combinations are known in the art.

In another embodiment, the Fc hinge region of an antibody is mutated to decrease the biological half-life of the antibody. More specifically, one or more amino acid mutations are introduced into the CH2-CH3 domain interface region of the Fc-hinge fragment such that the antibody has impaired Staphylococcyl protein A (SpA) binding relative to native Fc-hinge domain SpA binding. This approach is described in further detail in U.S. Patent No. 6,165,745.

In yet other embodiments, the Fc region is altered by replacing at least one amino acid residue with a different amino acid residue to alter the effector functions of the antibody. For example, one or more amino acids can be replaced with a different amino acid residue such that the antibody has an altered affinity for an effector ligand but retains the antigen-binding ability of the parent antibody. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of complement. This approach is described in, *e.g.*, U.S. Patent Nos. 5,624,821 and 5,648,260.

In another embodiment, one or more amino acids selected from amino acid residues can be replaced with a different amino acid residue such that the antibody has altered C1q binding and/or reduced or abolished complement dependent cytotoxicity (CDC). This approach is described in, *e.g.*, U.S. Patent Nos. 6,194,551.

In another embodiment, one or more amino acid residues are altered to thereby alter the ability of the antibody to fix complement. This approach is described in, *e.g.*, the PCT Publication WO 94/29351. Allotypic amino acid residues include, but are not limited to, constant region of a heavy chain of the IgG1, IgG2, and IgG3 subclasses as well as constant region of a light chain of the kappa isotype as described by Jefferis *et al.*, MAbs. 1:332-338 (2009).

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Antibody fusion protein complexes containing such mutations mediate reduced or no antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC). In some embodiments, amino acid residues L234 and L235 of the IgG1 constant region are substituted to A234 and A235. In some embodiments, amino acid residue N267 of the IgG1 constant region is substituted to A267. In some embodiments, amino acid residues D265 and P329 of the IgG1 constant region are substituted to A265 and A329. Other antibody Fc silencing mutations may also be used.

In another embodiment, one or more amino acid residues are altered to thereby alter the ability of the antibody to fix complement. This approach is described in, *e.g.*, the PCT Publication WO 94/29351 by Bodmer *et al.* In a specific embodiment, one or more amino acids of an antibody or antigen binding fragment thereof of the disclosure are replaced by one or more allotypic amino acid residues. Allotypic amino acid residues also include, but are not limited to, the constant region of the heavy chain of the IgG1, IgG2, and IgG3 subclasses as well as the constant region of the light chain of the kappa isotype as described by Jefferis *et al.*, MAbs. 1:332-338 (2009).

In still another embodiment, the glycosylation of an antibody is modified. For example, an aglycosylated antibody can be made (*i.e.*, the antibody lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for "antigen." Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region framework glycosylation sites to thereby eliminate glycosylation at that site. Such aglycosylation may increase the affinity of the antibody for antigen. Such an approach is described in, *e.g.*, U.S. Patent Nos. 5,714,350 and 6,350,861.

In some embodiments, the antibody is modified to increase its biological half-life. Various approaches are possible. For example, one or more of the following mutations can be introduced: T252L, T254S, T256F, as described in U.S. Patent No. 6,277,375. Alternatively, to increase the biological half-life, the antibody can be altered within the CH1 or CL region to contain a salvage receptor binding epitope taken from two loops of a CH2 domain of an Fc region of an IgG, as described in U.S. Patent Nos. 5,869,046 and 6,121,022.

3. Production of the anti-PMEL17 Antibodies

Anti-PMEL17 antibodies and antibody fragments (*e.g.*, antigen binding fragments) thereof can be produced by any means known in the art, including but not limited to, recombinant expression, chemical synthesis, and enzymatic digestion of antibody tetramers, whereas full-length monoclonal antibodies can be obtained by, *e.g.*, hybridoma or recombinant production. Recombinant expression can be from any appropriate host cells known in the art, for example, mammalian host cells, bacterial host cells, yeast host cells, insect host cells, etc.

The disclosure further provides polynucleotides encoding the antibodies described herein, *e.g.*, polynucleotides encoding heavy or light chain variable regions or segments comprising the

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complementarity determining regions as described herein. In some embodiments, the polynucleotide encoding the heavy chain variable regions has at least 85%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleic acid sequence identity with a polynucleotide selected from the group consisting of SEQ ID NOs: 11, 43, 65, 89, 113, 133, 150, 166, 185, 197, 216, 228, 240, and 255. In some embodiments, the polynucleotide encoding the light chain variable regions has at least 85%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleic acid sequence identity with a polynucleotide selected from the group consisting of SEQ ID NOs: 22, 26, 30, 54, 76, 100, 120, 144, 160, 172, 191, 203, 222, 234, 249, and 261.

In some embodiments, the polynucleotide encoding the heavy chain has at least 85%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleic acid sequence identity with a polynucleotide of SEQ ID NO: 13, 45, 67, 91, 115, 135, 152, 168, 187, 199, 218, 230, 242, 257, 319, and 320. In some embodiments, the polynucleotide encoding the light chain has at least 85%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleic acid sequence identity with a polynucleotide of SEQ ID NO: 24, 28, 32, 56, 78, 102, 122, 146, 162, 174, 193, 205, 224, 236, 251, and 263.

The polynucleotides of the disclosure can encode only the variable region sequence of an anti-PMEL17 antibody. They can also encode both a variable region and a constant region of the antibody. Some of the polynucleotide sequences encode a polypeptide that comprises variable regions of both the heavy chain and the light chain of one of the exemplified mouse anti-PMEL17 antibody. Some other polynucleotides encode two polypeptide segments that respectively are substantially identical to the variable regions of the heavy chain and the light chain of one of the mouse antibodies.

The polynucleotide sequences can be produced by *de novo* solid-phase DNA synthesis or by PCR mutagenesis of an existing sequence (*e.g.*, sequences as described in the Examples below) encoding an anti-PMEL17 antibody or its binding fragment. Direct chemical synthesis of nucleic acids can be accomplished by methods known in the art, such as the phosphotriester method of Narang *et al.*, Meth. Enzymol. 68:90, 1979; the phosphodiester method of Brown *et al.*, Meth. Enzymol. 68:109, 1979; the diethylphosphoramidite method of Beaucage *et al.*, Tetra. Lett., 22:1859, 1981; and the solid support method of U.S. Patent No. 4,458,066. Introducing mutations to a polynucleotide sequence by PCR can be performed as described in, *e.g.*, PCR Technology: Principles and Applications for DNA Amplification, H.A. Erlich (Ed.), Freeman Press, NY, NY, 1992; PCR Protocols: A Guide to Methods and Applications, Innis *et al.* (Ed.), Academic Press, San Diego, CA, 1990; Mattila *et al.*, Nucleic Acids Res. 19:967, 1991; and Eckert *et al.*, PCR Methods and Applications 1:17, 1991.

Also provided in the disclosure are expression vectors and host cells for producing the anti-PMEL17 antibodies described above. Various expression vectors can be employed to express the polynucleotides encoding the anti-PMEL17 antibody chains or binding fragments. Both viral-based

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and nonviral expression vectors can be used to produce the antibodies in a mammalian host cell. Nonviral vectors and systems include plasmids, episomal vectors, typically with an expression cassette for expressing a protein or RNA, and human artificial chromosomes (*see, e.g.*, Harrington *et al.*, Nat Genet 15:345, 1997). For example, nonviral vectors useful for expression of the anti-PMEL17 polynucleotides and polypeptides in mammalian (*e.g.*, human) cells include pThioHis A, B & C, pcDNA™3.1/His, pEBVHis A, B, & C (Invitrogen, San Diego, CA), MPSV vectors, and numerous other vectors known in the art for expressing other proteins. Useful viral vectors include vectors based on retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, vectors based on SV40, papilloma virus, HBP Epstein Barr virus, vaccinia virus vectors and Semliki Forest virus (SFV). *See* Brent *et al.*, *supra*; Smith, Annu. Rev. Microbiol. 49:807, 1995; and Rosenfeld *et al.*, Cell 68:143, 1992.

The choice of expression vector depends on the intended host cells in which the vector is to be expressed. Typically, the expression vectors contain a promoter and other regulatory sequences (*e.g.*, enhancers) that are operably linked to the polynucleotides encoding an anti-PMEL17 antibody chain or fragment. In some embodiments, an inducible promoter is employed to prevent expression of inserted sequences except under inducing conditions. Inducible promoters include, *e.g.*, arabinose, lacZ, metallothionein promoter or a heat shock promoter. Cultures of transformed organisms can be expanded under noninducing conditions without biasing the population for coding sequences whose expression products are better tolerated by the host cells. In addition to promoters, other regulatory elements may also be required or desired for efficient expression of an anti-PMEL17 antibody chain or fragment. These elements typically include an ATG initiation codon and adjacent ribosome binding site or other sequences. In addition, the efficiency of expression may be enhanced by the inclusion of enhancers appropriate to the cell system in use (*see, e.g.*, Scharf *et al.*, Results Probl. Cell Differ. 20:125, 1994; and Bittner *et al.*, Meth. Enzymol., 153:516, 1987). For example, the SV40 enhancer or CMV enhancer may be used to increase expression in mammalian host cells.

The expression vectors may also provide a secretion signal sequence position to form a fusion protein with polypeptides encoded by inserted anti-PMEL17 antibody sequences. More often, the inserted anti-PMEL17 antibody sequences are linked to a signal sequences before inclusion in the vector. Vectors to be used to receive sequences encoding anti-PMEL17 antibody light and heavy chain variable domains sometimes also encode constant regions or parts thereof. Such vectors allow expression of the variable regions as fusion proteins with the constant regions thereby leading to production of intact antibodies or fragments thereof. Typically, such constant regions are human.

The host cells for harboring and expressing the anti-PMEL17 antibody chains can be either prokaryotic or eukaryotic. *E. coli* is one prokaryotic host useful for cloning and expressing the polynucleotides of the disclosure. Other microbial hosts suitable for use include bacilli, such as Bacillus subtilis, and other enterobacteriaceae, such as Salmonella, Serratia, and various Pseudomonas species. In these prokaryotic hosts, one can also make expression vectors, which typically contain

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expression control sequences compatible with the host cell (*e.g.*, an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters typically control expression, optionally with an operator sequence, and have ribosome binding site sequences and the like, for initiating and completing transcription and translation. Other microbes, such as yeast, can also be employed to express anti-PMEL17 polypeptides of the disclosure. Insect cells in combination with baculovirus vectors can also be used.

In some embodiments, mammalian host cells are used to express and produce the anti-PMEL17 polypeptides of the disclosed. For example, they can be either a hybridoma cell line expressing endogenous immunoglobulin genes (*e.g.*, the myeloma hybridoma clones as described in the Examples) or a mammalian cell line harboring an exogenous expression vector (*e.g.*, the SP2/0 myeloma cells exemplified below). These include any normal mortal or normal or abnormal immortal animal or human cell. For example, a number of suitable host cell lines capable of secreting intact immunoglobulins have been developed, including the CHO cell lines, various Cos cell lines, HeLa cells, myeloma cell lines, transformed B-cells and hybridomas. The use of mammalian tissue cell culture to express polypeptides is discussed generally in, *e.g.*, Winnacker, From Genes to Clones, VCH Publishers, N.Y., N.Y., 1987. Expression vectors for mammalian host cells can include expression control sequences, such as an origin of replication, a promoter, and an enhancer (*see, e.g.*, Queen *et al.*, Immunol. Rev. 89:49-68, 1986), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. These expression vectors usually contain promoters derived from mammalian genes or from mammalian viruses. Suitable promoters may be constitutive, cell type-specific, stage-specific, and/or modulatable or regulatable. Useful promoters include, but are not limited to, the metallothionein promoter, the constitutive adenovirus major late promoter, the dexamethasone-inducible MMTV promoter, the SV40 promoter, the MRP polIII promoter, the constitutive MPSV promoter, the tetracycline-inducible CMV promoter (such as the human immediate-early CMV promoter), the constitutive CMV promoter, and promoter-enhancer combinations known in the art.

Methods for introducing expression vectors containing the polynucleotide sequences of interest vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts (*see generally* Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 4th ed.). Other methods include, *e.g.*, electroporation, calcium phosphate treatment, liposome-mediated transformation, injection and microinjection, ballistic methods, virosomes, immunoliposomes, polycation:nucleic acid conjugates, naked DNA, artificial virions, fusion to the herpes virus structural protein VP22 (Elliot and O'Hare, Cell 88:223, 1997), agent-enhanced uptake of DNA, and *ex vivo* transduction. For long-term, high-yield production of recombinant proteins, stable

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expression will often be desired. For example, cell lines which stably express anti-PMEL17 antibody chains or binding fragments can be prepared using expression vectors of the disclosure which contain viral origins of replication or endogenous expression elements and a selectable marker gene. Following introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth of cells which successfully express the introduced sequences in selective media. Resistant, stably transfected cells can be proliferated using tissue culture techniques appropriate to the cell type.

Process for the production of anti-PMEL antibody drug conjugates are also described in Example 4 of International Application Publication No. WO 2020/128612, which is incorporated by reference in its entirety. The PK properties of exemplary ADCs are also described in Example 18 of International Application Publication No. WO 2020/128612, which is incorporated by reference in its entirety. The *in vitro* stability of anti-PMEL17-GNAQ/11i ADCs in buffer, mouse, rat, and human plasma and *in vivo* stability of anti-PMEL17-GNAQ/11i ADCs in mouse are also described in Example 19 of International Application Publication No. WO 2020/128612, which is incorporated by reference in its entirety.

Therapeutic Uses

The antibody drug conjugates or formulations of the disclosure are useful in a variety of applications including, but not limited to, treatment or prevention of cancer, such as solid cancers or heme malignancies. In certain embodiments, the antibody drug conjugates or formulations of the disclosure are useful for inhibiting tumor growth, inducing differentiation, reducing tumor volume, and/or reducing the tumorigenicity of a tumor. The methods of use can be *in vitro*, *ex vivo*, or *in vivo* methods.

In some embodiments, the disclosure provides a method of treating or preventing a disease comprising administering the antibody drug conjugates or formulations of the disclosure to a patient. The disclosure also provides use of the antibody drug conjugates or formulations of the disclosure to treat or prevent disease in a patient. In some embodiments, the disclosure provides antibody drug conjugates or formulations of the disclosure for use in the treatment or prevention of disease in a patient. In further embodiments, the disclosure provides use of the antibody drug conjugates or formulations of the disclosure in the manufacture of a medicament for treatment or prevention of disease in a patient.

In certain embodiments, the disease treated with the antibody drug conjugates or formulations of the disclosure is a cancer. In certain embodiments, the cancer is characterized by PMEL17 expressing cells to which the antibody drug conjugates of the disclosure binds. In certain embodiments, the cancer is characterized by an increase in expression of PMEL17 relative to a

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healthy patient. In some embodiments, the expression of PMEL17 may be measured by an increase in PMEL17 RNA. In other embodiments, the cancer is characterized by an increase in DNA copy number of PMEL17. Other methods of measuring or determining levels of PMEL17 expression are known to persons skilled in the art. In certain embodiments, the cancer is characterized by a mutation, *e.g.*, an activating mutation affecting Q209 or R183, in the GNAQ and/or the GNA11 gene. Examples of diseases which can be treated and/or prevented include, but are not limited to, carcinomas (*e.g.*, hepatocellular carcinoma), sarcomas, leukemias, lymphomas, eye cancers, eye neoplasms, melanomas (*e.g.*, uveal melanoma, non-uveal melanoma, malignant melanoma, ocular melanoma, subcutaneous melanoma, cutaneous melanoma, or mucosal melanoma), or metastatic cancer thereof. In some embodiments, the disease is a melanoma. In some embodiments, the melanoma is a non-uveal melanoma that contains a mutation of GNAQ, GNA11, or both. Without wishing to be bound by theory, it is believed that in some embodiments, non-uveal melanomas harboring GNAQ/11 mutations may be biologically more related to uveal melanoma than melanomas harboring other mutations (*e.g.*, BRAF, NRAS). In some embodiments, the melanoma does not harbor a BRAF mutation, an NRAS mutation, or both. In some embodiments, the melanoma is mucosal melanoma that contains a mutation of GNAQ, GNA11, or both.

The disclosure provides for methods of treating or preventing a cancer comprising administering a therapeutically effective amount of the antibody drug conjugates or formulations of the disclosure. In certain embodiments, the cancer is a solid cancer such as carcinoma, hepatocellular carcinoma, sarcoma, lymphoma, eye cancer, eye neoplasm, melanoma, uveal melanoma, non-uveal melanoma, malignant melanoma, ocular melanoma, subcutaneous melanoma, cutaneous melanoma, mucosal melanoma, or a metastatic cancer thereof. In certain embodiments, the subject is a human. In certain embodiments, the cancer is a resistant cancer and/or relapsed cancer.

In certain embodiments, the disclosure provides for methods of inhibiting tumor growth comprising administering to a subject a therapeutically effective amount of the antibody drug conjugates or formulations of the disclosure. In certain embodiments, the tumor is of a solid cancer such as carcinoma, hepatocellular carcinoma, sarcoma, lymphoma, eye cancer, eye neoplasm, melanoma, uveal melanoma, non-uveal melanoma, malignant melanoma, ocular melanoma, subcutaneous melanoma, cutaneous melanoma, mucosal melanoma, or a metastatic cancer thereof. In certain embodiments, the subject is a human. In certain embodiments, the subject has a tumor or has had a tumor removed.

In certain embodiments, the tumor expresses the PMEL17 to which the antibody drug conjugate binds. In certain embodiments, the tumor overexpresses the human PMEL17. In certain embodiments, the tumor has an increase copy number of the PMEL17 gene. In certain embodiments,

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the tumor is characterized by a mutation, *e.g.*, an activating mutation affecting Q209 or R183, in the GNAQ and/or the GNA11 gene.

The current disclosure also provides for methods of selecting patients for treatment with antibody drug conjugates or formulation of the disclosure comprising administering a therapeutically effective amount of said antibody drug conjugates or formulations. In some embodiments of the disclosure the methods comprise selecting a patient by measuring for expression of PMEL17. In some embodiments of the disclosure the methods comprise selecting a patient by identifying a mutation, *e.g.*, an activating mutation affecting Q209 or R183, in the GNAQ or the GNA11 gene. In certain embodiments, the methods comprise measuring the level of PMEL17 expression in the patient as well as detecting for the GNAQ and/or GNA11 gene.

In certain embodiments, the subject or patient of any of the methods described herein has been treated with a bispecific gp100 peptide-HLA-directed CD3 T cell engager, *e.g.*, tebentafusp, prior to administration of the antibody drug conjugate or formulation of the disclosure. In other embodiments, the subject or patient of any of the methods described herein has not been treated with a bispecific gp100 peptide-HLA-directed CD3 T cell engager, *e.g.*, tebentafusp, prior to administration of the antibody drug conjugate or formulation of the disclosure.

In some embodiments, the subject or patient of any of the methods described herein has a uveal melanoma (*e.g.*, a metastatic uveal melanoma) and has been treated with a bispecific gp100 peptide-HLA-directed CD3 T cell engager, *e.g.*, tebentafusp, prior to administration of the antibody drug conjugate or formulation of the disclosure. In some embodiments, the subject or patient of any of the methods described herein has a uveal melanoma (*e.g.*, a metastatic uveal melanoma) and has not been treated with a bispecific gp100 peptide-HLA-directed CD3 T cell engager, *e.g.*, tebentafusp, prior to administration of the antibody drug conjugate or formulation of the disclosure. Without wishing to be bound by theory, it is believed that in some embodiments, certain tebentafusp-treated patients may have up to a 50% decrease in PMEL17 expression.

For the treatment or prevention of the disease, the appropriate dosage of the antibody drug conjugates or formulations of the disclosure depends on various factors, such as the type of disease to be treated, the severity and course of the disease, the responsiveness of the disease, previous therapy, patient's clinical history, and so on. The antibody-drug conjugate or formulation can be administered one time or over a series of treatments lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved (*e.g.*, reduction in tumor size). Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient and will vary depending on the relative potency of antibody drug conjugates or formulations. The treating physician can estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues.

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The methods described herein can further comprise determining the expression of one or more biomarkers in a sample from the subject or patient. Exemplary biomarkers that can be used in such methods include, but are not limited to, premelanosome protein 17 (PMEL17), phosphorylated ERK (pERK), cluster of differentiation 8 (CD8), programmed death-ligand 1 (PD-L1), Dual specificity phosphatase 6 (DUSP6), Ras guanyl-releasing protein 3 (RASGRP3), or any combination thereof.

In some embodiments, the sample is a tumor sample. In some embodiments, the sample is a sample from tissue adjacent to the tumor. In some embodiments, the sample is a body fluid sample, *e.g.*, blood, spinal fluid, etc. In some embodiments, the sample comprises cell-free DNA (cfDNA). In some embodiments, the sample is a non-diseased tissue to be used as a reference.

In some embodiments, the biomarkers are measured by methods known in the art. For example, expression of biomarkers can be measured and/or monitored by quantifying nucleic acid samples from the tissues or fluid samples from the subject or patient. Typical methods of quantifying include polymerase chain reaction (PCR), real-time PCR, Southern blotting, Northern blotting, in situ hybridization, DNA sequencing, and/or whole transcriptome analysis. In another example, expression of biomarkers can be measured and/or monitored by measuring protein levels of one or more biomarkers by techniques such as Western blotting, enzyme-linked immunosorbent assay (ELISA), and/or mass spectrometry.

The *in vitro* and *in vivo* anti-cancer activities of the GNAQ/11 inhibitors described herein and the antibody drug conjugates described herein are also described in Examples of International Application Publication No. WO 2020/128612, which is incorporated by reference in its entirety.

Methods of Treating or Diagnosing Cancer

In certain instances, an antibody drug conjugate or formulation of the present disclosure is used in a method of treating a cancer.

In one aspect, the disclosure relates to treatment of a subject *in vivo* using an ADC described herein, or a formulation comprising an ADC described herein, such that growth of cancerous tumors is inhibited or reduced.

In certain embodiments, the ADC or formulation is administered or used in accordance with a dosage regimen disclosed herein. In certain embodiments, the the ADC or formulation is administered in an amount effective to treat a cancer or a symptom thereof.

The ADC or formulation described herein can be used alone to inhibit the growth of cancerous tumors. Alternatively, the ADC or formulation described herein can be used in combination with a second therapeutic agent or modality as described herein. The ADC or formulation, and the second therapeutic agent or modality, can be administered in either order or simultaneously.

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Accordingly, in one aspect, the disclosure provides a method of inhibiting growth of tumor cells in a subject, comprising administering to the subject a therapeutically effective amount of an ADC or formulation described herein, *e.g.,* in accordance with a dosage regimen described herein. In an embodiment, the ADC is administered in the form of a formulation described herein.

In another aspect, a method of treating a subject, *e.g.*, reducing or ameliorating, a hyperproliferative condition or disorder (*e.g.*, a cancer or a metastatic lesion thereof) in a subject is provided. The method includes administering to the subject an ADC described herein, or a formulation comprising an ADC described herein, in accordance with a dosage regimen disclosed herein.

In certain embodiments, the cancer expresses PMEL17, contains a mutation of the GNAQ or GNA11 gene, or the melanoma expresses PMEL17 and contains a mutation of GNAQ, GNA11, or both. Exemplary cancers include, but are not limited to, solid tumors, hematological cancers, soft tissue tumors, and metastatic lesions. In an embodiment, the cancer is a melanoma. In certain embodiments, the melanoma is a uveal melanoma, non-uveal melanoma, malignant melanoma, ocular melanoma, subcutaneous melanoma, cutaneous melanoma, or mucosal melanoma. In certain embodiments, the cancer is a carcinoma, a sarcoma, a leukemia, or a lymphoma. In an embodiment, the carcinoma is a hepatocellular carcinoma. In certain embodiments, the cancer is an eye cancer or neoplasm. In some embodiments, the cancer is an MSI-high cancer. In some embodiments, the cancer is a metastatic cancer. In other embodiments, the cancer is an advanced cancer. In other embodiments, the cancer is a relapsed or refractory cancer.

Methods, ADCs, compositions, and formulations disclosed herein are useful for treating metastatic lesions associated with the aforementioned cancers.

In some embodiments, the ADC is administered at a dose of 1 mg/kg to 20 mg/kg (*e.g.*, 1 mg/kg to 16 mg/kg or 2 mg/kg to 15 mg/kg, *e.g.*, once a week, once every two weeks, or once every four weeks, intravenously. In some embodiments, the ADC is administered at a dose of 1 mg/kg, 2 mg/kg, 4 mg/kg, 8 mg/kg, 12 mg/kg, or 16 mg/kg), *e.g.*, once a week, once every two weeks, or once every four weeks, intravenously.

In an embodiment, the subject has been treated with tebentafusp prior to the administration of the ADC. In an embodiment, the subject has not been treated with tebentafusp prior to the administration of the ADC.

In some embodiments, the method further comprises determining the expression of one or more biomarkers in a sample from the subject. Such methods can be used to evaluate (*e.g.*, monitor) a cancer treatment comprising an ADC or formulation described herein, to evaluate (*e.g.*, monitor) a disease described herein (*e.g.*, the progression of a disease described herein), to select a treatment comprising an ADC or formulation described herein for a subject having a cancer, and/or to select a subject for a cancer treatment comprising an ADC or formulation described herein. In some

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embodiments, based on the determination of the expression of the one or more biomarkers, the treatment can be initiated, continued, or discontinued.

Exemplary biomarkers include, but are not limited to, PMEL17, pERK, CD8, PD-L1, DUSP6, RASGRP3, or any combination thereof. Exemplary samples include, but are not limited to, tumor samples, tumor-adjacent tissues, bodily fluids (*e.g.*, blood, serum, spinal fluid, or urine). In some embodiments, the sample comprises cell-free DNA (cfDNA). In some embodiments, the sample is a non-diseased tissue to be used as a reference. The biomarkers can be measured by methods known in the art, *e.g.*, by measuring the nucleic acid and/or protein levels in a sample.

Combination Therapy

In certain instances, an antibody drug conjugate or formulation of the present disclosure is combined with other therapeutic treatments, such as surgery and radiation therapy, therapeutic agents, such as other anti-cancer agents, anti-allergic agents, anti-nausea agents (or anti-emetics), pain relievers, cytoprotective agents, and combinations thereof.

In some embodiments, an antibody drug conjugate or formulation of the present disclosure is combined in a pharmaceutical combination formulation, or dosing regimen as combination therapy, with a second compound having anti-cancer properties. The second compound of the pharmaceutical combination formulation or dosing regimen can have complementary activities to the antibody or immunoconjugate of the combination such that they do not adversely affect each other. For example, an antibody drug conjugate or formulation of the present disclosure can be administered in combination with, but not limited to, a chemotherapeutic agent, immunomodulatory agents, a tyrosine kinase inhibitor, a GNAQ/GNA11 downstream signaling pathway inhibitor, IAP inhibitors, Bcl2 inhibitors, Mcl1 inhibitors, and other GNAQ/GNA11 inhibitors.

The term "pharmaceutical combination" as used herein refers to either a fixed combination in one dosage unit form, or non-fixed combination or a kit of parts for the combined administration where two or more therapeutic agents may be administered independently at the same time or separately within time intervals, especially where these time intervals allow that the combination partners show a cooperative, *e.g.*, synergistic effect.

The term "combination therapy" refers to the administration of two or more therapeutic agents to treat or prevent a therapeutic condition or disorder described in the present disclosure. Such administration encompasses co-administration of these therapeutic agents in a substantially simultaneous manner, such as in a single capsule having a fixed ratio of active ingredients. Alternatively, such administration encompasses co-administration in multiple, or in separate containers (*e.g.*, capsules, powders, and liquids) for each active ingredient. Powders and/or liquids may be reconstituted or diluted to a desired dose prior to administration. In addition, such administration also encompasses use of each type of therapeutic agent in a sequential manner, either at approximately the same time or at different times. In either case, the treatment regimen will

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provide beneficial effects of the drug combination in treating or preventing the conditions or disorders described herein.

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

General Chemotherapeutic agents considered for use in combination therapies include anastrozole (Arimidex®), bicalutamide (Casodex®), bleomycin sulfate (Blenoxane®), busulfan (Myleran®), busulfan injection (Busulfex®), capecitabine (Xeloda®), N4-pentoxycarbonyl-5-deoxy-5-fluorocytidine, carboplatin (Paraplatin®), carmustine (BiCNU®), chlorambucil (Leukeran®), cisplatin (Platinol®), cladribine (Leustatin®), cyclophosphamide (Cytoxan® or Neosar®), cytarabine, cytosine arabinoside (Cytosar-U®), cytarabine liposome injection (DepoCyt®), dacarbazine (DTIC-Dome®), dactinomycin (Actinomycin D, Cosmegan), daunorubicin hydrochloride (Cerubidine®), daunorubicin citrate liposome injection (DaunoXome®), dexamethasone, docetaxel (Taxotere®), doxorubicin hydrochloride (Adriamycin®, Rubex®), etoposide (Vepesid®), fludarabine phosphate (Fludara®), 5-fluorouracil (Adrucil®, Efudex®), flutamide (Eulexin®), tezacitibine, Gemcitabine (difluorodeoxycitidine), hydroxyurea (Hydrea®), Idarubicin (Idamycin®), ifosfamide (IFEX®), irinotecan (Camptosar®), L-asparaginase (ELSPAR®), leucovorin calcium, melphalan (Alkeran®), 6-mercaptopurine (Purinethol®), methotrexate (Folex®), mitoxantrone (Novantrone®), mylotarg, paclitaxel (Taxol®), phoenix (Yttrium90/MX-DTPA), pentostatin, polifeprosan 20 with carmustine implant (Gliadel®), tamoxifen citrate (Nolvadex®), teniposide (Vumon®), 6-thioguanine, thiotepa, tirapazamine (Tirazone®), topotecan hydrochloride for injection (Hycamptin®), vinblastine (Velban®), vincristine (Oncovin®), and vinorelbine (Navelbine®), and pemetrexed.

In some embodiments, the present disclosure provides a method of treating or preventing cancer by administering to a subject in need thereof an antibody drug conjugate of the present disclosure in combination with one or more MDM2 inhibitors, PKC inhibitors, PRC2 inhibitors, MAPK inhibitors, GPCR inhibitors, tyrosine kinase inhibitors, including but not limited to, BTK inhibitors, EGFR inhibitors, Her2 inhibitors, Her3 inhibitors, IGFR inhibitors, and Met inhibitors.

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For example, MDM2 inhibitors include but are not limited to, RG7112 (RO5045337); RG7388 (RO5503781, Idasanutlin); MI-77301 (SAR405838); MK-8242 (SCH-900242); AMG232; CGM097; DS3032b; HDM201; and ALRN-6924.

For example, PKC inhibitors include but are not limited to, Balanol; Riluzole; Staurosporin; Enzastaurin; δV1-1 (KAI-9803 or Delcasertib); εV1-2 (KAI-1678); Aprinocarsen; Midostaurin (PKC412); UCN-01 (7-hydroxy-staurosporin); Rottlerin (5, 7, dihydroxy-2,2-dimethyl-6-(2,4,6-trihydroxy-3-methyl-5-acetylbenzyl)-8-cinnamoyl-1,2-chromene); and Bryostatin 1.

For example, PRC2 inhibitors include but are not limited to, EI1; EPZ011989; EPZ005687; Tetramethylpiperidinyl Benzamides; UNC1999; and GSK126.

For example, MAPK inhibitors include but are not limited to, Vemurafenib (Zelboraf);, dabrafenib (Tafinlar); encorafenib (Braftovi); trametinib (Mekinist); cobimetinib (Cotellic); binimetinib (Mektovi); and ulixertinib.

For example, tyrosine kinase inhibitors include but are not limited to, Ibrutinib (PCI-32765); Erlotinib hydrochloride (Tarceva®); Linifanib (N-[4-(3-amino-1H-indazol-4-yl)phenyl]-N'-(2-fluoro-5-methylphenyl)urea, also known as ABT 869, available from Genentech); Sunitinib malate (Sutent®); Bosutinib (4-[(2,4-dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(4-methylpiperazin-1-yl)propoxy]quinoline-3-carbonitrile, also known as SKI-606, and described in US Patent No. 6,780,996); Dasatinib (Sprycel®); Pazopanib (Votrient®); Sorafenib (Nexavar®); Zactima (ZD6474); and Imatinib or Imatinib mesylate (Gilvec® and Gleevec®).

Epidermal growth factor receptor (EGFR) inhibitors include but are not limited to, Erlotinib hydrochloride (Tarceva®), Gefitinib (Iressa®); N-[4-[(3-Chloro-4-fluorophenyl)amino]-7-[[(3"S")-tetrahydro-3-furanyl]oxy]-6-quinazolinyl]-4(dimethylamino)-2-butenamide, Tovok®); Vandetanib (Caprelsa®); Lapatinib (Tykerb®); (3R,4R)-4-Amino-1-((4-((3-methoxyphenyl)amino)pyrrolo[2,1-f][1,2,4]triazin-5-yl)methyl)piperidin-3-ol (BMS690514); Canertinib dihydrochloride (CI-1033); 6-[4-[(4-Ethyl-1-piperazinyl)methyl]phenyl]-N-[(1R)-1-phenylethyl]- 7H-Pyrrolo[2,3-d]pyrimidin-4-amine (AEE788, CAS 497839-62-0); Mubritinib (TAK165); Pelitinib (EKB569); Afatinib (BIBW2992); Neratinib (HKI-272); N-[4-[[1-[(3-Fluorophenyl)methyl]-1H-indazol-5-yl]amino]-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yl]-carbamic acid, (3S)-3-morpholinylmethyl ester (BMS599626); N-(3,4-Dichloro-2-fluorophenyl)-6-methoxy-7-[[(3aα,5β,6aα)-octahydro-2-methylcyclopenta[c]pyrrol-5-yl]methoxy]- 4-quinazolinamine (XL647, CAS 781613-23-8); and 4-[4-[[(1R)-1-Phenylethyl]amino]-7H-pyrrolo[2,3-d]pyrimidin-6-yl]-phenol (PKI166, CAS 187724-61-4).

EGFR antibodies include but are not limited to, Cetuximab (Erbitux®); Panitumumab (Vectibix®); Matuzumab (EMD-72000); ; Nimotuzumab (hR3); Zalutumumab; TheraCIM h-R3; MDX0447 (CAS 339151-96-1); and ch806 (mAb-806, CAS 946414-09-1).

Human Epidermal Growth Factor Receptor 2 (Her2 receptor) (also known as Neu, ErbB-2, CD340, or p185) inhibitors include but are not limited to, Trastuzumab (Herceptin®); Pertuzumab

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(Omnitarg®); trastuzumab emtansine (Kadcyla®); Neratinib (HKI-272, (2E)-N-[4-[[3-chloro-4-[(pyridin-2-yl)methoxy]phenyl]amino]-3-cyano-7-ethoxyquinolin-6-yl]-4-(dimethylamino)but-2-enamide, and described PCT Publication No. WO 05/028443); Lapatinib or Lapatinib ditosylate (Tykerb®); (3R,4R)-4-amino-1-((4-((3-methoxyphenyl)amino)pyrrolo[2,1-f][1,2,4]triazin-5-yl)methyl)piperidin-3-ol (BMS690514); (2E)-N-[4-[(3-Chloro-4-fluorophenyl)amino]-7-[[(3S)-tetrahydro-3-furanyl]oxy]-6-quinazolinyl]-4-(dimethylamino)-2-butenamide (BIBW-2992, CAS 850140-72-6); N-[4-[[1-[(3-Fluorophenyl)methyl]-1H-indazol-5-yl]amino]-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yl]-carbamic acid, (3S)-3-morpholinylmethyl ester (BMS 599626, CAS 714971-09-2); Canertinib dihydrochloride (PD183805 or CI-1033); and N-(3,4-Dichloro-2-fluorophenyl)-6-methoxy-7-[[(3aα,5β,6aα)-octahydro-2-methylcyclopenta[c]pyrrol-5-yl]methoxy]- 4-quinazolinamine (XL647, CAS 781613-23-8).

Her3 inhibitors include but are not limited to, LJM716, MM-121, AMG-888, RG7116, REGN-1400, AV-203, MP-RM-1, MM-111, and MEHD-7945A.

MET inhibitors include but are not limited to, Cabozantinib (XL184, CAS 849217-68-1); Foretinib (GSK1363089, formerly XL880, CAS 849217-64-7); Tivantinib (ARQ197, CAS 1000873-98-2); 1-(2-Hydroxy-2-methylpropyl)-N-(5-(7-methoxyquinolin-4-yloxy)pyridin-2-yl)-5-methyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazole-4-carboxamide (AMG 458); Cryzotinib (Xalkori®, PF-02341066); (3Z)-5-(2,3-Dihydro-1H-indol-1-ylsulfonyl)-3-({3,5-dimethyl-4-[(4-methylpiperazin-1-yl)carbonyl]-1H-pyrrol-2-yl}methylene)-1,3-dihydro-2H-indol-2-one (SU11271); (3Z)-N-(3-Chlorophenyl)-3-({3,5-dimethyl-4-[(4-methylpiperazin-1-yl)carbonyl]-1H-pyrrol-2-yl}methylene)-N-methyl-2-oxoindoline-5-sulfonamide (SU11274); (3Z)-N-(3-Chlorophenyl)-3-{[3,5-dimethyl-4-(3-morpholin-4-ylpropyl)-1H-pyrrol-2-yl]methylene}-N-methyl-2-oxoindoline-5-sulfonamide (SU11606); 6-[Difluoro[6-(1-methyl-1H-pyrazol-4-yl)-1,2,4-triazolo[4,3-b]pyridazin-3-yl]methyl]-quinoline (JNJ38877605, CAS 943540-75-8); 2-[4-[1-(Quinolin-6-ylmethyl)-1H-[1,2,3]triazolo[4,5-b]pyrazin-6-yl]-1H-pyrazol-1-yl]ethanol (PF04217903, CAS 956905-27-4); N-((2R)-1,4-Dioxan-2-ylmethyl)-N-methyl-N'-[3-(1-methyl-1H-pyrazol-4-yl)-5-oxo-5H-benzo[4,5]cyclohepta[1,2-b]pyridin-7-yl]sulfamide (MK2461, CAS 917879-39-1); 6-[[6-(1-Methyl-1H-pyrazol-4-yl)-1,2,4-triazolo[4,3-b]pyridazin-3-yl]thio]-quinoline (SGX523, CAS 1022150-57-7); and (3Z)-5-[[(2,6-Dichlorophenyl)methyl]sulfonyl]-3-[[3,5-dimethyl-4-[[(2R)-2-(1-pyrrolidinylmethyl)-1-pyrrolidinyl]carbonyl]-1H-pyrrol-2-yl]methylene]-1,3-dihydro-2H-indol-2-one (PHA665752, CAS 477575-56-7).

IGF1R inhibitors include but are not limited to, BMS-754807, XL-228, OSI-906, GSK0904529A, A-928605, AXL1717, KW-2450, MK0646, AMG479, IMCA12, MEDI-573, and BI836845. See *e.g.*, Yee, JNCI, 104; 975 (2012) for review.

In some embodiments, the present disclosure provides a method of treating or preventing cancer by administering to a subject in need thereof an antibody drug conjugate or formulation of the present disclosure in combination with one or more GNAQ/GNA11 downstream signaling pathway

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inhibitors, including but not limited to, β-arrestin inhibitors, GRK inhibitors, MAPK inhibitors, PI3K inhibitors, JAK inhibitors, etc.

For example, phosphoinositide 3-kinase (PI3K) inhibitors include but are not limited to, Idelalisib (Zydelig, GS-1101, Cal-101), 4-[2-(1H-Indazol-4-yl)-6-[[4-(methylsulfonyl)piperazin-1-yl]methyl]thieno[3,2-d]pyrimidin-4-yl]morpholine (also known as GDC 0941 and described in PCT Publication Nos. WO 09/036082 and WO 09/055730); 2-Methyl-2-[4-[3-methyl-2-oxo-8-(quinolin-3-yl)-2,3-dihydroimidazo[4,5-c]quinolin-1-yl]phenyl]propionitrile (also known as BEZ 235 or NVP-BEZ 235, and described in PCT Publication No. WO 06/122806); 4-(trifluoromethyl)-5-(2,6-dimorpholinopyrimidin-4-yl)pyridin-2-amine (also known as BKM120 or NVP-BKM120, and described in PCT Publication No. WO2007/084786); Tozasertib (VX680 or MK-0457, CAS 639089-54-6); (5Z)-5-[[4-(4-Pyridinyl)-6-quinolinyl]methylene]-2,4-thiazolidinedione (GSK1059615, CAS 958852-01-2); (1E,4S,4aR,5R,6aS,9aR)-5-(Acetyloxy)-1-[(di-2-propenylamino)methylene]-4,4a,5,6,6a,8,9,9a-octahydro-11-hydroxy-4-(methoxymethyl)-4a,6a-dimethyl-cyclopenta[5,6]naphtho[1,2-c]pyran-2,7,10(1H)-trione (PX866, CAS 502632-66-8); and 8-Phenyl-2-(morpholin-4-yl)-chromen-4-one (LY294002, CAS 154447-36-6).

In some embodiments, the present disclosure provides a method of treating or preventing cancer by administering to a subject in need thereof an antibody drug conjugate or formulation of the present disclosure in combination with one or more pro-apoptosis, including but not limited to, IAP inhibitors, Bcl2 inhibitors, MCl1 inhibitors, Trail agents, Chk inhibitors.

For examples, IAP inhibitors include but are not limited to, LCL161, GDC-0917, AEG-35156, AT406, and TL32711. Other examples of IAP inhibitors include but are not limited to those disclosed in WO04/005284, WO 04/007529, WO05/097791, WO 05/069894, WO 05/069888, WO 05/094818, US2006/0014700, US2006/0025347, WO 06/069063, WO 06/010118, WO 06/017295, and WO08/134679, all of which are incorporated herein by reference.

BCL-2 inhibitors include but are not limited to, Venetoclax (also known as GDC-0199, ABT-199, RG7601); 4-[4-[[2-(4-Chlorophenyl)-5,5-dimethyl-1-cyclohexen-1-yl]methyl]-1-piperazinyl]-N-[[4-[[(1R)-3-(4-morpholinyl)-1-[(phenylthio)methyl]propyl]amino]-3-[(trifluoromethyl)sulfonyl]phenyl]sulfonyl]benzamide (also known as ABT-263 and described in PCT Publication No. WO 09/155386); Tetrocarcin A; Antimycin; Gossypol ((-)BL-193); Obatoclax; Ethyl-2-amino-6-cyclopentyl-4-(1-cyano-2-ethoxy-2-oxoethyl)-4Hchromone-3-carboxylate (HA14 – 1); Oblimersen (G3139, Genasense®); Bak BH3 peptide; (-)-Gossypol acetic acid (AT-101); 4-[4-[(4'-Chloro[1,1'-biphenyl]-2-yl)methyl]-1-piperazinyl]-*N*-[[4-[[(1*R*)-3-(dimethylamino)-1-[(phenylthio)methyl]propyl]amino]-3-nitrophenyl]sulfonyl]-benzamide (ABT-737, CAS 852808-04-9); and Navitoclax (ABT-263, CAS 923564-51-6).

Proapoptotic receptor agonists (PARAs) including DR4 (TRAILR1) and DR5 (TRAILR2), including but are not limited to, Dulanermin (AMG-951, RhApo2L/TRAIL); Mapatumumab (HRS-ETR1, CAS 658052-09-6); Lexatumumab (HGS-ETR2, CAS 845816-02-6); Apomab (Apomab®);

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Conatumumab (AMG655, CAS 896731-82-1); and Tigatuzumab (CS1008, CAS 946415-34-5, available from Daiichi Sankyo).

Checkpoint Kinase (CHK) inhibitors include but are not limited to, 7-Hydroxystaurosporine (UCN-01); 6-Bromo-3-(1-methyl-1*H*-pyrazol-4-yl)-5-(3*R*)-3-piperidinyl-pyrazolo[1,5-*a*]pyrimidin-7-amine (SCH900776, CAS 891494-63-6); 5-(3-Fluorophenyl)-3-ureidothiophene-2-carboxylic acid N-[(S)-piperidin-3-yl]amide (AZD7762, CAS 860352-01-8); 4-[(3S)-1-Azabicyclo[2.2.2]oct-3-yl)amino]-3-(1H-benzimidazol-2-yl)-6-chloroquinolin-2(1H)-one (CHIR 124, CAS 405168-58-3); 7-Aminodactinomycin (7-AAD), Isogranulatimide, debromohymenialdisine; N-[5-Bromo-4-methyl-2-[(2S)-2-morpholinylmethoxy]-phenyl]-N'-(5-methyl-2-pyrazinyl)urea (LY2603618, CAS 911222-45-2); Sulforaphane (CAS 4478-93-7, 4-Methylsulfinylbutyl isothiocyanate); 9,10,11,12-Tetrahydro-9,12-epoxy-1*H*-diindolo[1,2,3-*fg*:3',2',1'-*kl*]pyrrolo[3,4-*i*][1,6]benzodiazocine-1,3(2*H*)-dione (SB-218078, CAS 135897-06-2); and TAT-S216A (YGRKKRRQRRRLYRSPAMPENL (SEQ ID NO: 282)), and CBP501 ((d-Bpa)sws(d-Phe-F5)(d-Cha)rrrqrr).

In some embodiments, the present disclosure provides a method of treating or preventing cancer by administering to a subject in need thereof an antibody drug conjugate or formulation of the present disclosure in combination with one or more immunomodulators (*e.g.*, one or more of: an activator of a costimulatory molecule or an inhibitor of an immune checkpoint molecule).

In certain embodiments, the immunomodulator is an activator of a costimulatory molecule. In one embodiment, the agonist of the costimulatory molecule is chosen from an agonist (*e.g.*, an agonistic antibody or antigen-binding fragment thereof, or a soluble fusion) of OX40, CD2, CD27, CDS, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), 4-1BB (CD137), GITR, CD30, CD40, BAFFR, HVEM, CD7, LIGHT, NKG2C, SLAMF7, NKp80, CD160, B7-H3, STING, or CD83 ligand.

In certain embodiments, the immunomodulator is an inhibitor of an immune checkpoint molecule. In one embodiment, the immunomodulator is an inhibitor of PD-1, PD-L1, PD-L2, CTLA4, TIM3, LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4 and/or TGFR beta. In one embodiment, the inhibitor of an immune checkpoint molecule inhibits PD-1, PD-L1, LAG-3, TIM-3 or CTLA4, or any combination thereof. The term "inhibition" or "inhibitor" includes a reduction in a certain parameter, *e.g.*, an activity, of a given molecule, *e.g.*, an immune checkpoint inhibitor. For example, inhibition of an activity, *e.g.*, a PD-1 or PD-L1 activity, of at least 5%, 10%, 20%, 30%, 40%, 50% or more is included by this term. Thus, inhibition need not be 100%.

Inhibition of an inhibitory molecule can be performed at the DNA, RNA or protein level. In some embodiments, an inhibitory nucleic acid (*e.g.*, a dsRNA, siRNA or shRNA), can be used to inhibit expression of an inhibitory molecule. In other embodiments, the inhibitor of an inhibitory signal is a polypeptide *e.g.*, a soluble ligand (*e.g.*, PD-1-Ig or CTLA-4 Ig), or an antibody or antigen-binding fragment thereof, that binds to the inhibitory molecule; *e.g.*, an antibody or fragment thereof

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(also referred to herein as "an antibody molecule") that binds to PD-1, PD-L1, PD-L2, CTLA4, TIM3, LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4 and/or TGFR beta, or a combination thereof.

In some embodiments, the antibody molecule is a full antibody or fragment thereof (*e.g.*, a Fab, $F(ab')_2$, Fv, or a single chain Fv fragment (scFv)). In yet other embodiments, the antibody molecule has a heavy chain constant region (Fc) chosen from, *e.g.*, the heavy chain constant regions of IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD, and IgE; particularly, chosen from, *e.g.*, the heavy chain constant regions of IgG1, IgG2, IgG3, and IgG4, more particularly, the heavy chain constant region of IgG1 or IgG4 (*e.g.*, human IgG1 or IgG4). In one embodiment, the heavy chain constant region is human IgG1 or human IgG4. In one embodiment, the constant region is altered, *e.g.*, mutated, to modify the properties of the antibody molecule (*e.g.*, to increase or decrease one or more of: Fc receptor binding, antibody glycosylation, the number of cysteine residues, effector cell function, or complement function).

In certain embodiments, the antibody molecule is in the form of a bispecific or multispecific antibody molecule. In some embodiments, the bispecific antibody molecule has a first binding specificity to PD-1 or PD-L1 and a second binding specificity, *e.g.*, a second binding specificity to TIM-3, LAG-3, or PD-L2. In some embodiments, the bispecific antibody molecule binds to PD-1 or PD-L1 and TIM-3. In some embodiments, the bispecific antibody molecule binds to PD-1 or PD-L1 and LAG-3. In some embodiments, the bispecific antibody molecule binds to PD-1 and PD-L1. In some embodiments, the bispecific antibody molecule binds to PD-1 and PD-L2. In some embodiments, the bispecific antibody molecule binds to TIM-3 and LAG-3. Any combination of the aforesaid molecules can be made in a multi-specific antibody molecule, *e.g.*, a tri-specific antibody that includes a first binding specificity to PD-1 or PD-1, and a second and third binding specificities to two or more of: TIM-3, LAG-3, or PD-L2.

In certain embodiments, the immunomodulator is an inhibitor of PD-1, *e.g.*, human PD-1. In another embodiment, the immunomodulator is an inhibitor of PD-L1, *e.g.*, human PD-L1. In one embodiment, the inhibitor of PD-1 or PD-L1 is an antibody molecule to PD-1 or PD-L1. The PD-1 or PD-L1 inhibitor can be administered alone, or in combination with other immunomodulators, *e.g.*, in combination with an inhibitor of LAG-3, TIM-3 or CTLA4. In some embodiments, the inhibitor of PD-1 or PD-L1, *e.g.*, the anti-PD-1 or PD-L1 antibody molecule, is administered in combination with a LAG-3 inhibitor, *e.g.*, an anti-LAG-3 antibody molecule. In another embodiment, the inhibitor of PD-1 or PD-L1, *e.g.*, the anti-PD-1 or PD-L1 antibody molecule, is administered in combination with a TIM-3 inhibitor, *e.g.*, an anti-TIM-3 antibody molecule. In some embodiments, the inhibitor of PD-1 or PD-L1, *e.g.*, the anti-PD-1 antibody molecule, is administered in combination with a LAG-3 inhibitor, *e.g.*, an anti-LAG-3 antibody molecule, and a TIM-3 inhibitor, *e.g.*, an anti-TIM-3 antibody molecule. Other combinations of immunomodulators with a PD-1 inhibitor (*e.g.*, one or more of PD-L2, CTLA4, TIM3, LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4 and/or TGFR) are also

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within the present disclosure. Any of the antibody molecules known in the art or disclosed herein can be used in the aforesaid combinations of inhibitors of checkpoint molecule.

In some embodiments, the PD-1 inhibitor is an anti-PD-1 antibody chosen from Nivolumab, Pembrolizumab or Pidilizumab. In some embodiments, the anti-PD-1 antibody is Nivolumab. Alternative names for Nivolumab include MDX- 1106, MDX-1106-04, ONO-4538, or BMS-936558. In some embodiments, the anti-PD- 1 antibody is Nivolumab (CAS Registry Number: 946414-94-4). Nivolumab is a fully human IgG4 monoclonal antibody which specifically blocks PD-1. Nivolumab (clone 5C4) and other human monoclonal antibodies that specifically bind to PD-1 are disclosed in US Pat No. 8,008,449 and PCT Publication No. WO2006/121168.

In some embodiments, the anti-PD-1 antibody is Pembrolizumab. Pembrolizumab (Trade name KEYTRUDA formerly Lambrolizumab, also known as Merck 3745, MK-3475 or SCH-900475) is a humanized IgG4 monoclonal antibody that binds to PD-1. Pembrolizumab is disclosed, *e.g.*, in Hamid, O. *et al.* (2013) *New England Journal of Medicine* 369 (2): 134–44, PCT Publication No. WO2009/114335, and US Patent No. 8,354,509.

In some embodiments, the anti-PD-1 antibody is Pidilizumab. Pidilizumab (CT-011; Cure Tech) is a humanized IgG1k monoclonal antibody that binds to PD-1. Pidilizumab and other humanized anti-PD-1 monoclonal antibodies are disclosed in PCT Publication No. WO2009/101611. Other anti-PD1 antibodies are disclosed in US Patent No. 8,609,089, US Publication No. 2010028330, and/or US Publication No. 20120114649. Other anti-PD-1 antibodies include AMP 514 (Amplimmune).

In some embodiments, the PD-1 inhibitor is PDR001, also known as spartalizumab, or any other anti-PD-1 antibody disclosed in WO2015/112900.

In some embodiments, the PD-1 inhibitor is an immunoadhesin (*e.g.*, an immunoadhesin comprising an extracellular or PD-1 binding portion of PD-L1 or PD-L2 fused to a constant region (*e.g.*, an Fc region of an immunoglobulin sequence). In some embodiments, the PD-1 inhibitor is AMP-224.

In some embodiments, the PD-L1 inhibitor is anti-PD-L1 antibody. In some embodiments, the anti-PD-L1 inhibitor is chosen from YW243.55.S70, MPDL3280A, MEDI-4736, or MDX-1105MSB-0010718C (also referred to as A09-246-2) disclosed in, *e.g.*, WO 2013/0179174, and having a sequence disclosed herein (or a sequence substantially identical or similar thereto, *e.g.*, a sequence at least 85%, 90%, 95% identical or higher to the sequence specified).

In some embodiments, the PD-L1 inhibitor is MDX-1105. MDX-1105, also known as BMS-936559, is an anti-PD-L1 antibody described in PCT Publication No. WO2007/005874.

In some embodiments, the PD-L1 inhibitor is YW243.55.S70. The YW243.55.S70 antibody is an anti-PD-L1 described in PCT Publication No. WO 2010/077634 (heavy and light chain variable region sequences shown in SEQ ID Nos. 20 and 21, respectively).

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In some embodiments, the PD-L1 inhibitor is MDPL3280A (Genentech / Roche). MDPL3280A is a human Fc optimized IgG1 monoclonal antibody that binds to PD-L1. MDPL3280A and other human monoclonal antibodies to PD-L1 are disclosed in U.S. Patent No.: 7,943,743 and U.S Publication No.: 20120039906.

In some embodiments, the PD-L2 inhibitor is AMP-224. AMP-224 is a PD-L2 Fc fusion soluble receptor that blocks the interaction between PD1 and B7-H1 (B7-DCIg; Amplimmune; *e.g.*, disclosed in PCT Publication Nos. WO2010/027827 and WO2011/066342).

In o some embodiments, the LAG-3 inhibitor is an anti-LAG-3 antibody molecule. In one embodiment, the LAG-3 inhibitor is BMS-986016. In one embodiment, the LAG-3 inhibitor is LAG525 or any anti-LAG3 antibody disclosed in WO2015/138920.

In some embodiments, the TIM-3 inhibitor is an anti-TIM3 antibody molecule. In one embodiment, the TIM-3 inhibitor is MBG453 or any anti-TIM3 antibody disclosed in WO2015/117002.

Pharmaceutical Compositions and Formulations

To prepare pharmaceutical or sterile compositions including immunoconjugates (*e.g.*, immunoconjugates made by a process described herein), the immunoconjugates of the disclosure are mixed with a pharmaceutically acceptable carrier or excipient. The compositions can additionally contain one or more other therapeutic agents that are suitable for treating or preventing a PMEL17 expressing cancer (including, but not limited to carcinoma (*e.g.*, hepatocellular carcinoma), sarcoma, leukemia, lymphoma, eye cancer, eye neoplasm, melanoma (*e.g.*, uveal melanoma, non-uveal melanoma, malignant melanoma, ocular melanoma, subcutaneous melanoma, cutaneous melanoma, or mucosal melanoma), or a metastatic cancer thereof.

In certain embodiments, the immunoconjugates or pharmaceutical compositions of the disclosure can be formulated into a formulation (*e.g.,* a dose formulation or dosage form) suitable for administration to a subject as described herein.

In some embodiments, the formulation is a liquid formulation. In other embodiments, the formulation is a reconstituted or dried formulation, *e.g.*, reconstituted or dried from a lyophilized formulation. In other embodiments, the formulation is a lyophilized or dried formulation.

In some embodiments, the formulation comprises an antibody drug conjugate described herein (*e.g.*, an antibody drug conjugate made by a process described herein), a buffering agent, a stabilizing agent, and a surfactant, wherein the formulation has a pH of 4.5 to 6.5.

In some embodiments, the antibody drug conjugate is present at a concentration of about 5 mg/mL to about 30 mg/mL, *e.g.,* about 10 mg to about 30 mg, about 15 mg/mL to about 25 mg/mL, about 18 mg/mL to about 22 mg/mL, about 10 mg/mL to 25 mg/mL, about 10 mg/mL to about 20 mg/mL, about 10 mg/mL to about 15 mg/mL, about 25 mg to about 30 mg/mL, about 20 mg/mL to about 30 mg/mL, about 5 mg/mL to about 15 mg/mL, about 15 mg/mL to about 25 mg/mL, or about

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18 mg/mL to about 22 mg/mL, *e.g.,* about 5 mg/mL, about 6 mg/mL, about 7 mg/mL, about 8 mg/mL, about 9 mg/mL, about 10 mg/mL, about 11 mg/mL, about 12 mg/mL, about 13 mg/mL, about 14 mg/mL, about 15 mg/mL, about 16 mg/mL, about 17 mg/mL, about 18 mg/mL, about 19 mg/mL, about 20 mg/mL, about 21 mg/mL, about 22 mg/mL, about 23 mg/mL, about 24 mg/mL, or about 25 mg/mL. In some embodiments, the antibody drug conjugate is present at a concentration of about 15 mg/mL to about 25 mg/mL, *e.g.*, about 20 mg/mL.

In some embodiments, the buffering agent comprises histidine. In some embodiments, the buffering agent comprises succinate. In some embodiments, the buffering agent is present at a concentration of about 5 mM to about 50 mM, *e.g.*, about 10 mM to about 40 nM, about 15 mM to about 35 mM, about 20 mM to about 30 mM, about 5 mM to about 40 mM, about 5 mM to about 30 mM, about 5 mM to about 20 mM, about 5 mM to about 15 mM, about 5 mM to about 10 mM, about 40 mM to about 50 mM, about 35 mM to about 50 mM, about 30 mM to about 50 mM, about 25 mM to about 50 mM, about 20 mM to about 50 mM, about 15 mM to about 50 mM, about 10 mM to about 50 mM, about 10 mM to about 20 mM, about 15 mM to about 25 mM, about 25 mM to about 35 mM, about 30 mM to about 40 mM, or about 35 mM to about 45 mM, *e.g.*, about 5 mM, about 10 mM, about 15 mM, about 20 mM, about 25 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, or about 50 mM. In some embodiments, the buffering agent is present at a concentration of about 15 mM to about 25 mM, *e.g.*, about 20 mM.

In some embodiments, the stabilizing agent comprises sucrose. In some embodiments, the stabilizing agent comprises trehalose. In some embodiments, the stabilizing agent is present at a concentration of about 100 mM to about 500 mM, *e.g.*, about 150 mM to about 450 mM, about 200 mM to about 400 mM, about 250 mM to about 350 mM, about 100 mM to about 450 mM, about 100 mM to about 400 mM, about 100 mM to about 350 mM, about 100 mM to about 300 mM, about 100 mM to about 250 mM, about 100 mM to about 200 mM, about 100 mM to about 150 mM, about 450 mM to about 500 mM, about 400 mM to about 500 mM, about 350 mM to about 500 mM, about 300 mM to about 500 mM, about 250 mM to about 500 mM, about 200 mM to about 500 mM, about 150 mM to about 500 mM, about 150 mM to about 250 mM, about 200 mM to about 250 mM, about 200 mM to about 300 mM, about 300 mM to about 400 mM, or about 350 mM to about 450 mM, *e.g.*, about 100 mM, about 150 mM, about 200 mM, about 240 mM, about 250 mM, about 300 mM, about 350 mM, about 400 mM, about 450 mM, or about 500 mM. In some embodiments, the stabilizing agent and is present at a concentration of about 200 mM to about 300 mM, *e.g.*, about 240 mM.

In some embodiments, the surfactant comprises polysorbate 20. In some embodiments, the surfactant is present at a concentration of about 0.01% to about 0.06%, *e.g.*, about 0.02% to about 0.05%, about 0.03% to about 0.04%, about 0.01% to about 0.05%, about 0.01% to about 0.04%, about 0.01% to about 0.03%, about 0.01% to about 0.02%, about 0.05% to about 0.06%, about 0.04% to about 0.06%, about 0.03% to about 0.06%, about 0.02% to about 0.06%, about 0.02% to about 0.04%, about 0.03% to about 0.05%, *e.g.*, about 0.01%, about 0.02%, about 0.03%, about 0.04%, about

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0.05%, or about 0.06%. In some embodiments, the surfactant is present at a concentration of about 0.01% to about 0.03%, *e.g.*, about 0.02%.

In some embodiments, the formulation has a pH of about 4.7 to about 5.3, about 5.0 to about 6.0, about 5.2 to about 5.8, about 5.4 to about 5.6, about 5.2 to about 6.0, about 5.4 to about 6.0, about 5.6 to about 6.0, about 5.8 to about 6.0, about 5 to about 5.8, about 5 to about 5.6.0, about 5 to about 5.4, about 5 to about 5.2, about 4.8 to about 5.2, about 5.1 to about 5.3, about 5.2 to about 5.4, about 5.3 to about 5.5, about 5.5 to about 5.7, about 5.6 to about 5.8, about 5.7 to about 5.9, about 4.9 to about 5.5, about 5.5 to about 6.1, or about 5.7 to about 6.3, *e.g.*, about 4.5, about 4.6, about 4.7, about 4.8, about 4.9, about 5, about 5.1, about 5.2, about 5.3, about 5.4, about 5.5, about 5.6, about 5.7, about 5.8, about 5.9, about 6.0, about 6.1, about 6.2, about 6.3, about 6.4, or about 6.5. In some embodiments, the formulation has a pH of about 5.0 to about 5.6, *e.g.*, about 5.3.

In some embodiments, the formulation comprises about 15 mg/mL to about 25 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose, and about 0.01% to about 0.03% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.0 to about 5.6. In some embodiments, the formulation comprises about 20 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine, about 240 mM of a stabilizing agent comprising sucrose, and about 0.02% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.3.

In some embodiments, the formulation comprises about 15 mg/mL to about 25 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose, and about 0.01% to about 0.03% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.0 to about 5.6. In some embodiments, the formulation comprises about 20 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine, about 240 mM of a stabilizing agent comprising sucrose, and about 0.02% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.3.

In some embodiments, the formulation comprises about 15 mg/mL to about 25 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose, and about 0.01% to about 0.03% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 4.7 to about 5.3. In some embodiments, the formulation comprises about 20 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine, about 240 mM of a stabilizing agent comprising sucrose, and about 0.02% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.0.

In some embodiments, the formulation comprises about 15 mg/mL to about 25 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine,

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about 200 mM to about 300 mM of a stabilizing agent comprising sucrose, and about 0.01% to about 0.03% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 4.7 to about 5.3. In some embodiments, the formulation comprises about 20 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine, about 240 mM of a stabilizing agent comprising sucrose, and about 0.02% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.0.

In some embodiments, the formulation comprises about 15 mg/mL to about 25 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose, and about 0.01% to about 0.03% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.2 to about 5.8. In some embodiments, the formulation comprises about 20 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine, about 240 mM of a stabilizing agent comprising sucrose, and about 0.02% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.5.

In some embodiments, the formulation comprises about 15 mg/mL to about 25 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose, and about 0.01% to about 0.03% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.2 to about 5.8. In some embodiments, the formulation comprises about 20 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine, about 240 mM of a stabilizing agent comprising sucrose, and about 0.02% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.5.

In some embodiments, the formulation comprises about 15 mg/mL to about 25 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose, and about 0.03% to about 0.05% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.2 to about 5.8. In some embodiments, the formulation comprises about 20 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine, about 240 mM of a stabilizing agent comprising sucrose, and about 0.04% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.5.

In some embodiments, the formulation comprises about 15 mg/mL to about 25 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose, and about 0.03% to about 0.05% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.2 to about 5.8. In some embodiments, the formulation comprises about 20 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine, about 240 mM of a stabilizing

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agent comprising sucrose, and about 0.04% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.5.

In some embodiments, the formulation comprises about 5 mg/mL to about 15 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose, and about 0.03% to about 0.05% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.2 to about 5.8. In some embodiments, the formulation comprises about 10 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine, about 240 mM of a stabilizing agent comprising sucrose, and about 0.02% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.5.

In some embodiments, the formulation comprises about 5 mg/mL to about 15 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose, and about 0.03% to about 0.05% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.2 to about 5.8. In some embodiments, the formulation comprises about 10 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine, about 240 mM of a stabilizing agent comprising sucrose, and about 0.02% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.5.

In some embodiments, the formulation comprises about 15 mg/mL to about 25 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose, and about 0.01% to about 0.03% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.7 to about 6.3. In some embodiments, the formulation comprises about 20 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine, about 240 mM of a stabilizing agent comprising sucrose, and about 0.02% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 6.0.

In some embodiments, the formulation comprises about 15 mg/mL to about 25 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose, and about 0.01% to about 0.03% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 5.7 to about 6.3. In some embodiments, the formulation comprises about 20 mg/mL of the antibody drug conjugate, about 20 mM of a buffering agent comprising histidine, about 240 mM of a stabilizing agent comprising sucrose, and about 0.02% of a surfactant comprising polysorbate 20, wherein the formulation has a pH of about 6.0.

In some embodiments, the formulation comprises about 15 mg/mL to about 25 mg/mL of the antibody drug conjugate, about 15 mM to about 25 mM of a buffering agent comprising histidine, about 200 mM to about 300 mM of a stabilizing agent comprising sucrose, and about 0.01% to about

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JUMBO APPLICATIONS/PATENTS

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NOTE POUR LE TOME / VOLUME NOTE:

CLAIMS:

1. A process for producing a partially reoxidized antibody or antigen binding fragment thereof, comprising:

providing an antibody or antigen binding fragment thereof comprising one or more capped cysteine residues;

reducing the one or more capped cysteine residues, thereby providing an antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues; and

storing the antibody or antigen binding fragment thereof comprising the one or more decapped cysteine residues under oxidation conditions that allow for re-capping of one or more off-target cysteine residues,

thereby producing the partially reoxidized antibody or antigen binding fragment thereof.

2. The process of claim 1, wherein the one or more capped cysteine residues are located in the constant domain a heavy chain of the antibody or antigen binding fragment thereof.

3. The process of claim 1 or 2, wherein the antibody or antigen binding fragment thereof comprises two or four capped cysteine residues.

4. The process of any one of claims 1-3, wherein the antibody or antigen binding fragment thereof comprises a capped cysteine residue located in a CH1 region of the antibody or antigen binding fragment thereof, optionally wherein the antibody or antigen binding fragment thereof comprises a capped cysteine residue located in each CH1 region of the antibody or antigen binding fragment thereof.

5. The process of any one of claims 1-4, wherein the one or more capped cysteine residues are one or more an engineered surface-exposed cysteine residues.

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6. The process of any one of claims 1-5, wherein the antibody or antigen binding fragment thereof comprises a capped cysteine residue located at residue 152 (EU numbering) in the CH1 region of the antibody or antigen binding fragment thereof.

7. The process of any one of claims 1-6, wherein the one or more capped cysteine residues are capped with a cysteine or a glutathione.

8. The process of any one of claims 1-7, wherein the one or more decapped cysteine residues are located in the constant domain a heavy chain of the antibody or antigen binding fragment thereof.

9. The process of any one of claims 1-8, wherein the antibody or antigen binding fragment thereof comprises two or four decapped cysteine residues.

10. The process of any one of claims 1-9, wherein the antibody or antigen binding fragment thereof comprises a decapped cysteine residue located in a CH1 region of the antibody or antigen binding fragment thereof, optionally wherein the antibody or antigen binding fragment thereof comprises a decapped cysteine residue located in each CH1 region of the antibody or antigen binding fragment thereof.

11. The process of any one of claims 1-10, wherein the one or more decapped cysteine residues are one or more engineered surface-exposed cysteine residues.

12. The process of any one of claims 1-11, wherein the antibody or antigen binding fragment thereof comprises a decapped cysteine residue located at residue 152 (EU numbering) in the CH1 region of the antibody or antigen binding fragment thereof.

13. The process of any one of claims 1-12, wherein the process comprises reducing one or more capped cysteine residues by contacting the antibody or antigen binding fragment thereof comprising one or more capped cysteine residues with a reduction wash buffer, thereby providing an antibody or antigen binding fragment comprising one or more decapped cysteine residue.

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14. The process of any one of claims 1-13, wherein the reduction wash buffer comprises 5 mM to 50 mM cysteine, optionally wherein the reduction wash buffer comprises 10 mM to 40 mM, 20 mM to 30 mM, 5 mM to 15 mM, 5 mM to 25 mM, 5 mM to 35 mM, 5 mM to 45 mM, 40 mM to 50 mM, 30 mM to 50 mM, 20 mM to 50 mM, 10 mM to 50 mM, 10 mM to 20 mM, 15 mM to 25 mM, 25 mM to 35 mM, 30 mM to 40 mM, or 35 mM to 45 mM cysteine, optionally wherein the reduction wash buffer comprises 10 mM, 12 mM, 14 mM, 16 mM, 20 mM, 25 mM, or 30 mM cysteine.

15. The process of any one of claims 1-14, wherein the reduction wash buffer has a pH of 6.0 to 7.5, optionally wherein the pH is 6.5 to 7.2 or 6.8 to 7.0, optionally wherein the pH is 6.9.

16. The process of any one of claims 1-15, wherein contacting the antibody or antigen binding fragment thereof comprising one or more capped cysteine residues with the reduction wash buffer is performed on a column, optionally wherein the column is a cation exchange chromatography column.

17. The process of any one of claims 1-16, wherein the process further comprises collecting the antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues in an eluate after contacting with the reduction wash buffer.

18. The process of any one of claims 1-17, wherein the eluate has a pH of 5.5 to 6.0, optionally wherein the pH is 5.8.

19. The process of any one of claims 1-18, wherein the concentration of the antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues in the eluate is 5 g/L to 25 g/L, optionally wherein the concentration of the antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues in the eluate is 8 g/L to 20 g/L, 10 g/L to 18 g/L, 12 g/L to 15 g/L, 5 g/L to 20 g/L, 5 g/L to 15 g/L, 5 g/L to 10 g/L, 20 g/L to 25 g/L, 15 g/L to 25 g/L, 10 g/L to 25 g/L, 10 g/L to 20 g/L, 13 g/L to 14 g/L, 12 g/L to 15 g/L, optionally wherein the concentration of the antibody or antigen binding fragment thereof comprising one or more decapped

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cysteine residues in the eluate is 5 g/L, 6 g/L, 7 g/L, 8 g/L, 9 g/L, 10 g/L, 11 g/L, 12 g/L, 13 g/L, 13.5 g/L, 14 g/L, 15 g/L, 16 g/L, 17 g/L, 18 g/L, 19 g/L, 20 g/L, 21 g/L, 22 g/L, 23 g/L, 24 g/L, or 25 g/L.

20. The process of any one of claims 1-19, wherein the antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues is stored in the eluate with stirring.

21. The process of any one of claims 1-19, wherein the antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues is stored in the eluate without stirring.

22. The process of any one of claims 1-21, wherein the antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues is stored in a container filled to a maximum of 50% to 70% or 60% volume, optionally wherein the container is filled to a maximum of 60% volume.

23. The process of any one of claims 1-22, wherein the antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues is stored for 12 hours to 96 hours, optionally wherein the antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues is stored for 12 hours to 84 hours, 24 hours to 84 hours, 36 hours to 72 hours, 48 hours to 60 hours, 24 hours to 72 hours, 24 hours to 60 hours, 24 hours to 48 hours, 24 hours to 36 hours, 72 hours to 84 hours, 60 hours to 84 hours, 48 hours to 84 hours, 36 hours to 84 hours, 12 hours to 36 hours, 24 hours to 48 hours, 36 hours to 60 hours, 48 hours to 72 hours, or 72 hours to 96 hours, optionally wherein the antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues is stored for 12 hours, 24 hours, 36 hours, 48 hours, 60 hours, 72 hours, 84 hours, or 96 hours.

24. The process of any one of claims 1-23, wherein the antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues is stored at room temperature, or at 2 °C to 8 °C or 4 °C.

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25. The process of any one of claims 1-24, wherein the antibody or antigen binding fragment thereof comprising one or more decapped cysteine residues is stored with air overlay.

26. The process of any one of claims 1-25, wherein the process further comprises purifying the partially reoxidized antibody or antigen binding fragment thereof.

27. The process of any one of claims 1-26, wherein the antibody or antigen binding fragment thereof comprises a substitution of one or more amino acids with cysteine on its constant region at positions selected from the group consisting of positions 117, 119, 121, 124, 139, 152, 153, 155, 157, 164, 169, 171, 174, 189, 191, 195, 197, 205, 207, 246, 258, 269, 274, 286, 288, 290, 292, 293, 320, 322, 326, 333, 334, 335, 337, 344, 355, 360, 375, 382, 390, 392, 398, 400, and 422 of a heavy chain of the antibody or antigen binding fragment thereof, and wherein the positions are numbered according to the EU system.

28. The process of any one of claims 1-27, wherein the antibody or antigen binding fragment comprises a substitution of one or more amino acids with cysteine on its constant region at positions selected from the group consisting of positions 107, 108, 109, 114, 129, 142, 143, 145, 152, 154, 156, 159, 161, 165, 168, 169, 170, 182, 183, 197, 199, and 203 of a light chain of the antibody or antigen binding fragment thereof, wherein the positions are numbered according to the EU system, and wherein the light chain is a human kappa light chain.

29. The process of any one of claims 1-28, wherein the antibody or antigen binding fragment thereof comprises a substitution of an amino acid with cysteine on its constant region at position 152, position 375, or both positions 152 and 375, of a heavy chain of the antibody or antigen binding fragment thereof, wherein the positions are numbered according to the EU system.

30. A process of producing an antibody drug conjugate, the process comprising conjugating a linker-drug moiety to the partially reoxidized antibody or antigen binding fragment thereof produced by a process of any one of claims 1-29, thereby producing the antibody drug conjugate.

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31. The process of claim 30, further comprising purifying the antibody drug conjugate.

32. The process of claim 30 or 31, wherein the process results in at least 75%, optionally wherein the process results in at least 80%, 85%, 90%, or 95%, on-site coupling, or one linker-drug moiety per heavy chain ("HC1").

33. The process of any one of claims 30-32, wherein the process results in less than 25%, optionally wherein the process results in less than 20%, 15%, 10%, or 5%, off-site coupling, or one linker-drug moiety per light chain ("LC1") and/or two linker-drug moieties per heavy chain ("HC2").

34. The process of any one of claims 1-33, wherein the process results in at least 70% purity, optionally wherein the process results in at least 75%, 80%, 85%, 90%, or 95% purity, optionally wherein the purity is determined by non-reduced CE-SDS.

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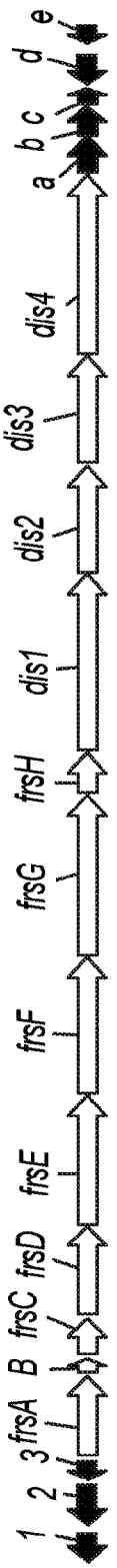

FIG. 1

Date Reçue/Date Received 2024-04-11

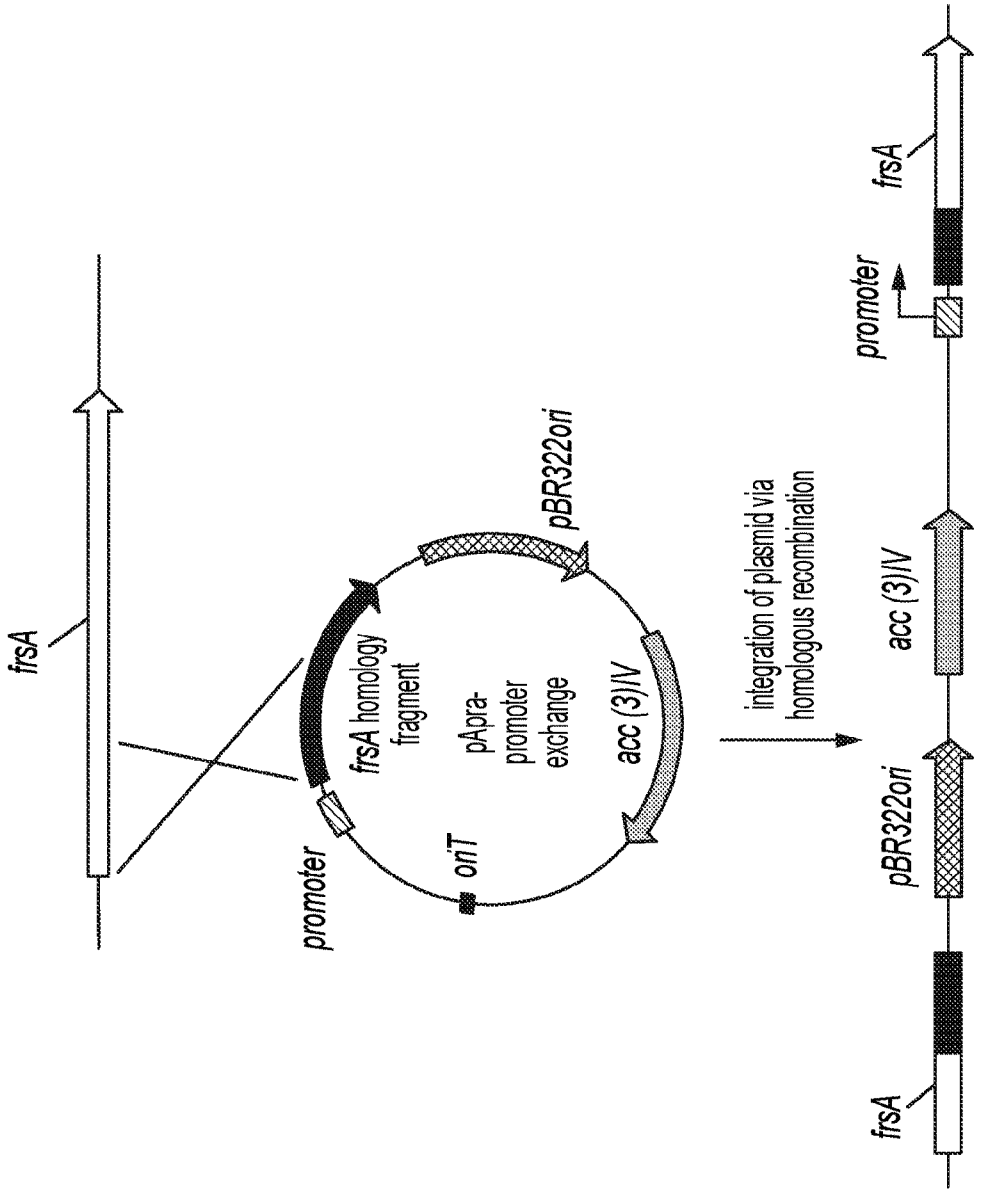

FIG. 2

Date Reçue/Date Received 2024-04-11

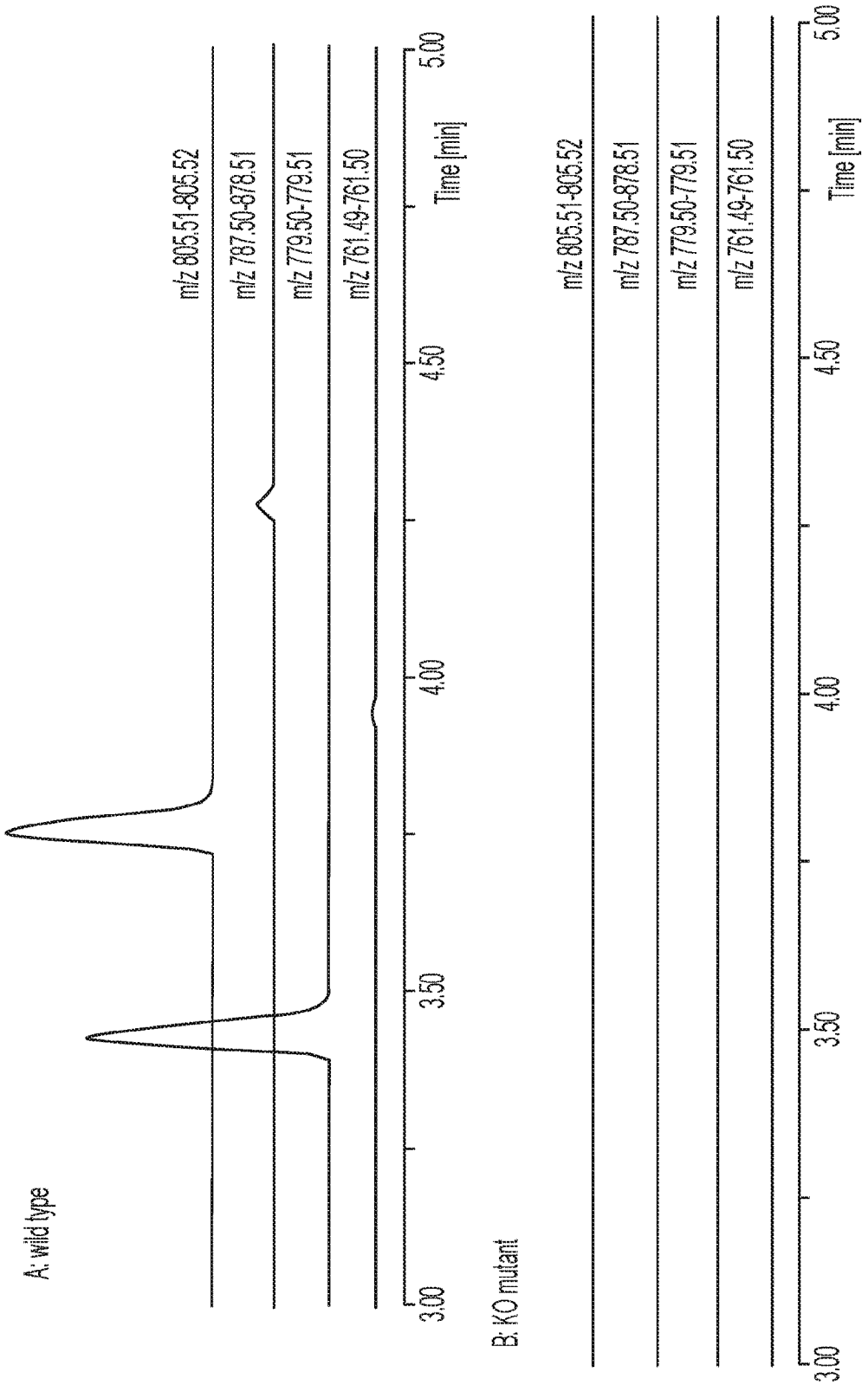

FIG. 3

Date Reçue/Date Received 2024-04-11

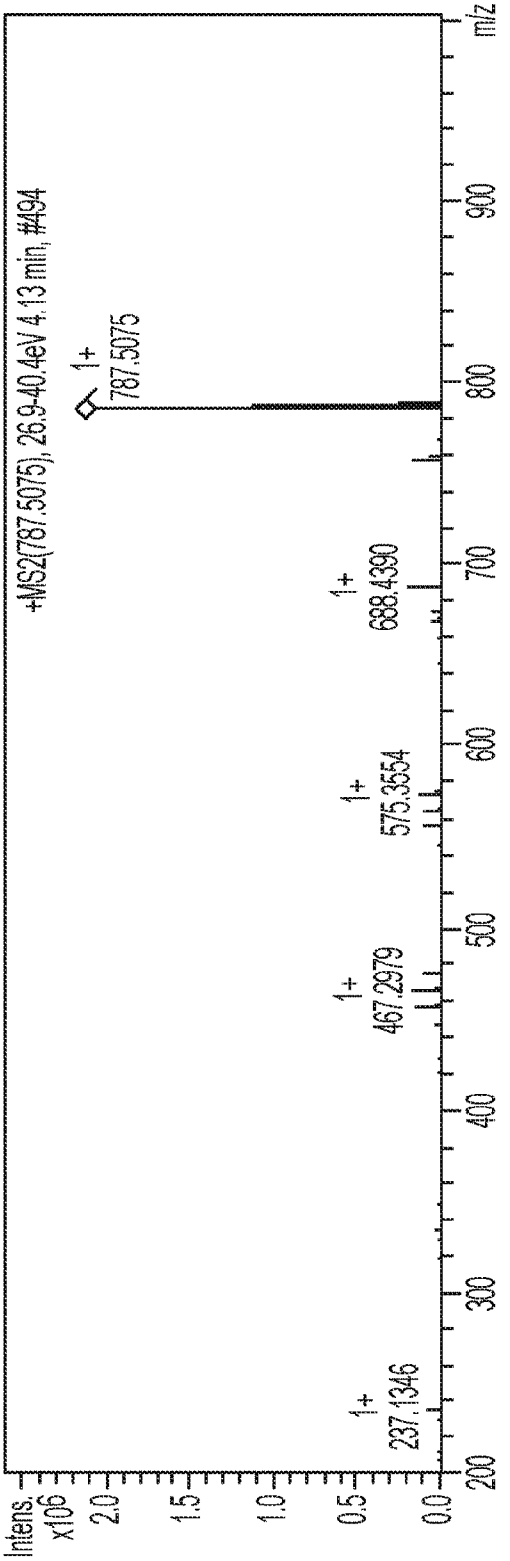

FIG. 4

Date Reçue/Date Received 2024-04-11

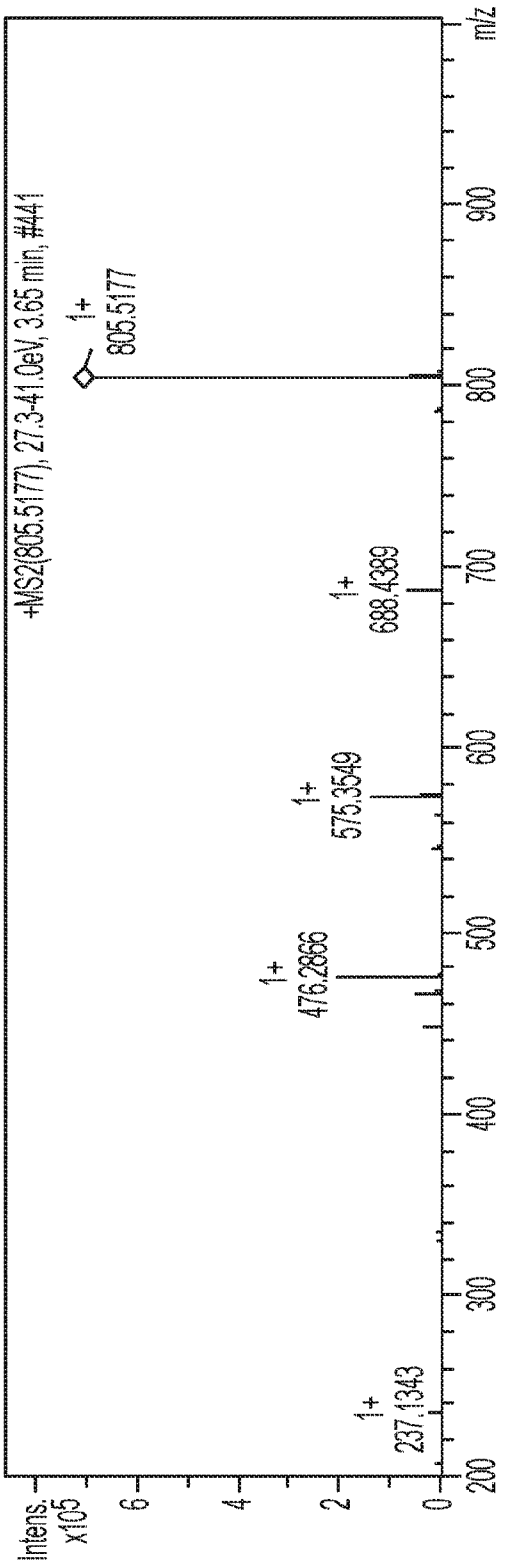

FIG. 5

Date Reçue/Date Received 2024-04-11

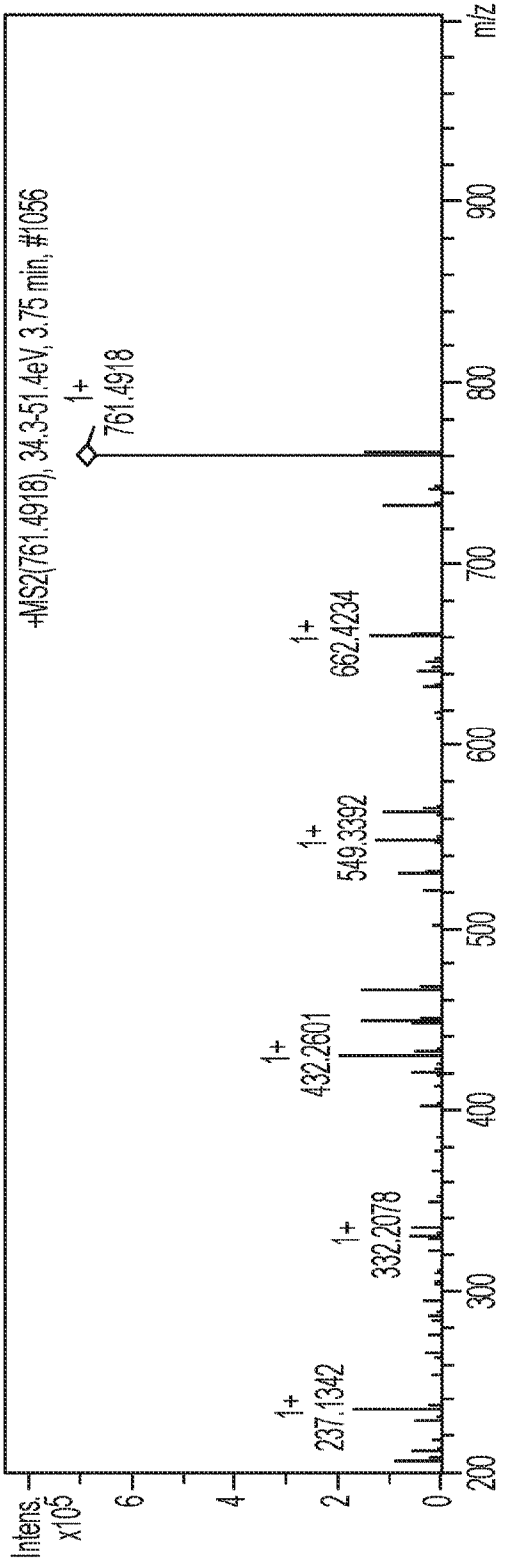

FIG. 6

Date Reçue/Date Received 2024-04-11

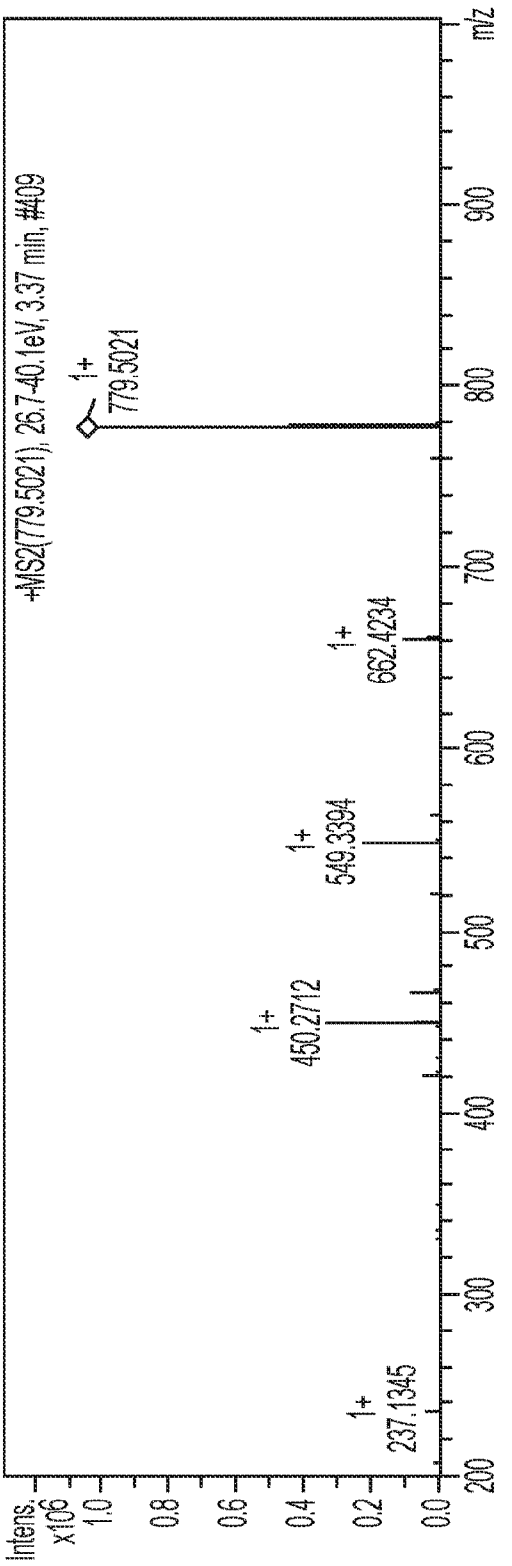

FIG. 7

Date Reçue/Date Received 2024-04-11

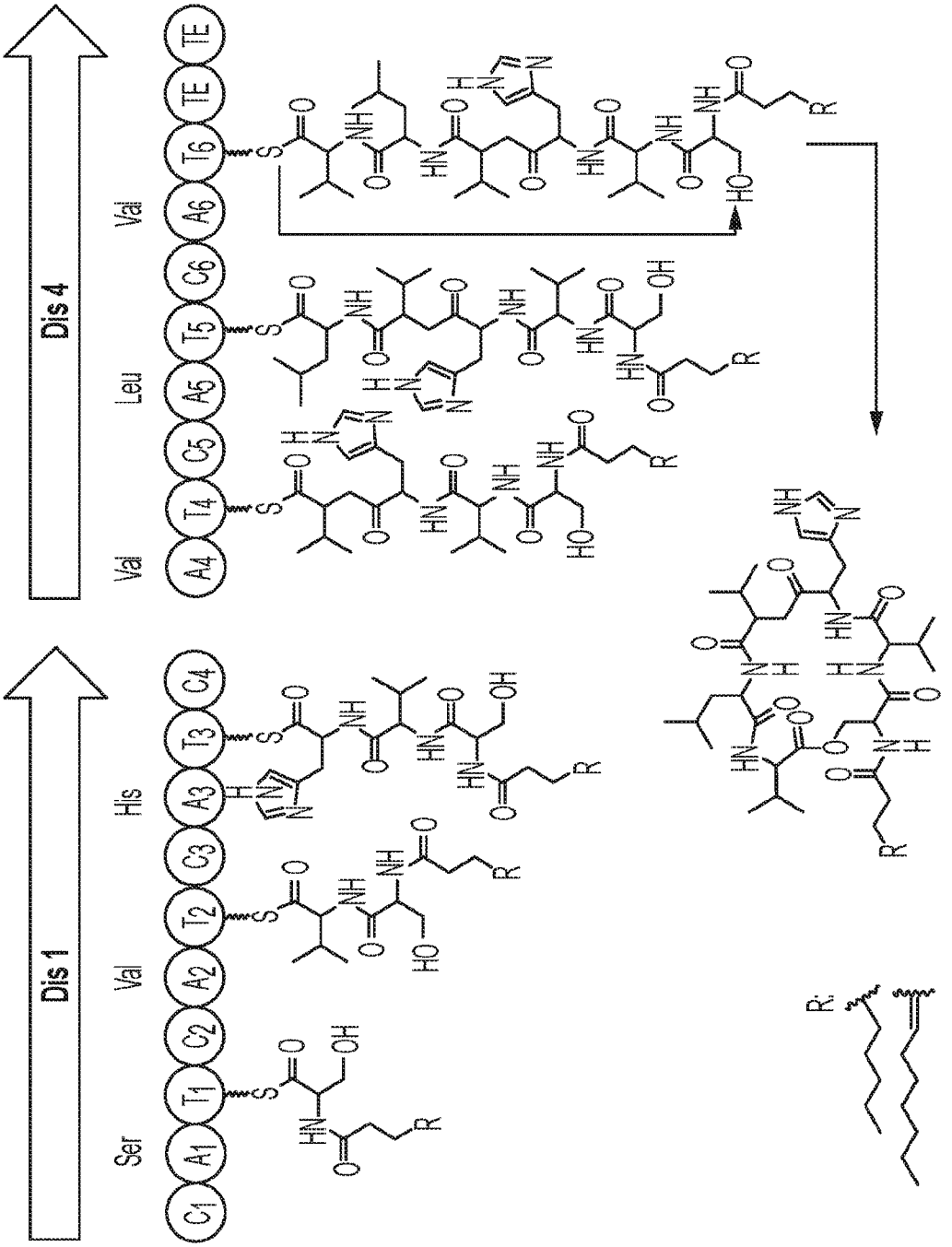

FIG. 8

Date Reçue/Date Received 2024-04-11

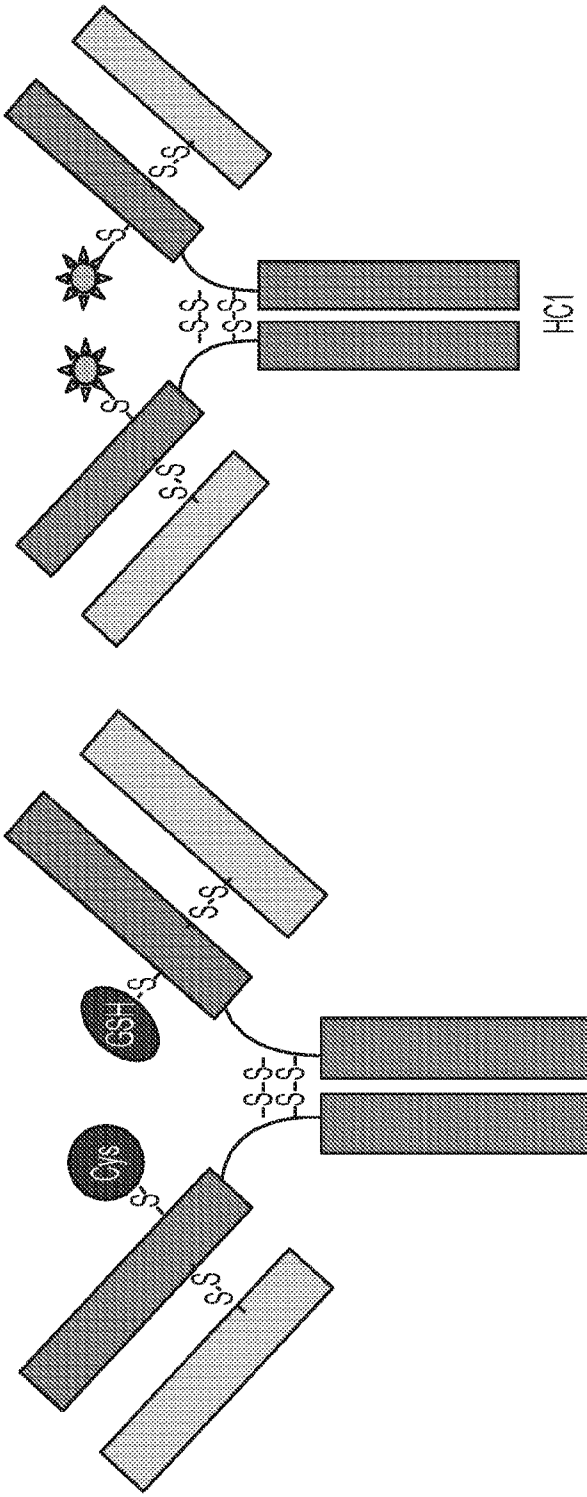

FIG. 9

Date Reçue/Date Received 2024-04-11

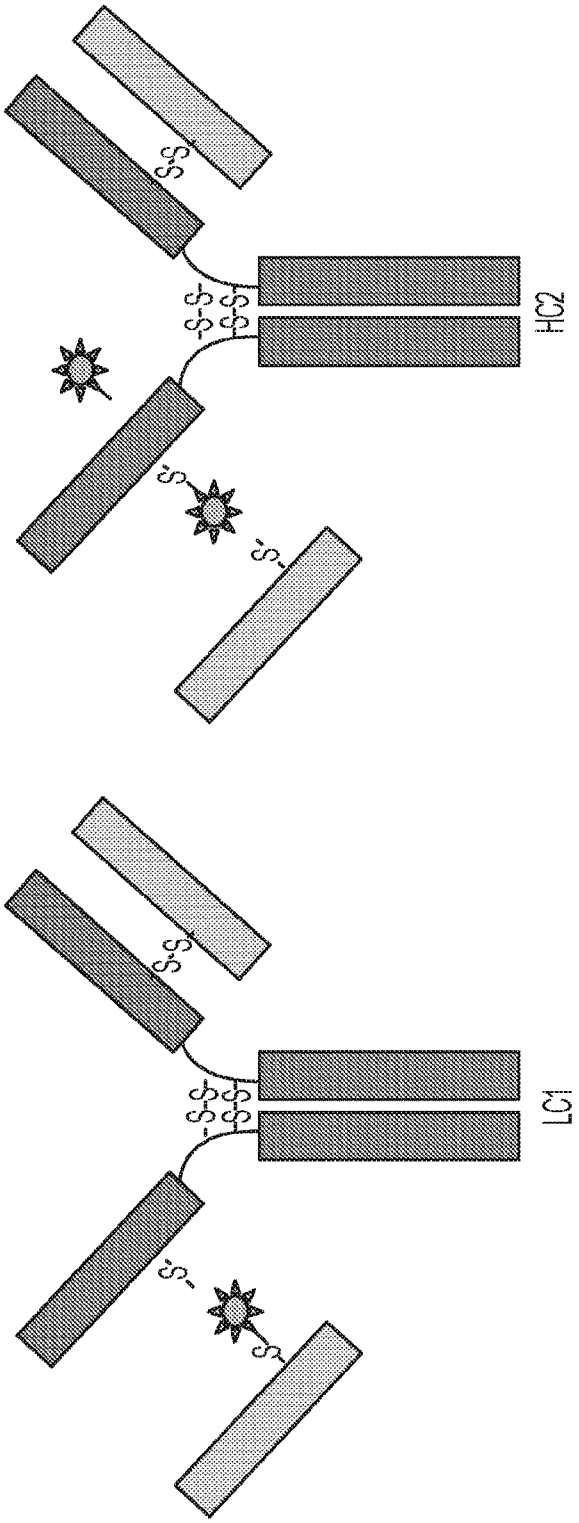

FIG. 9
CONTINUED

Date Reçue/Date Received 2024-04-11

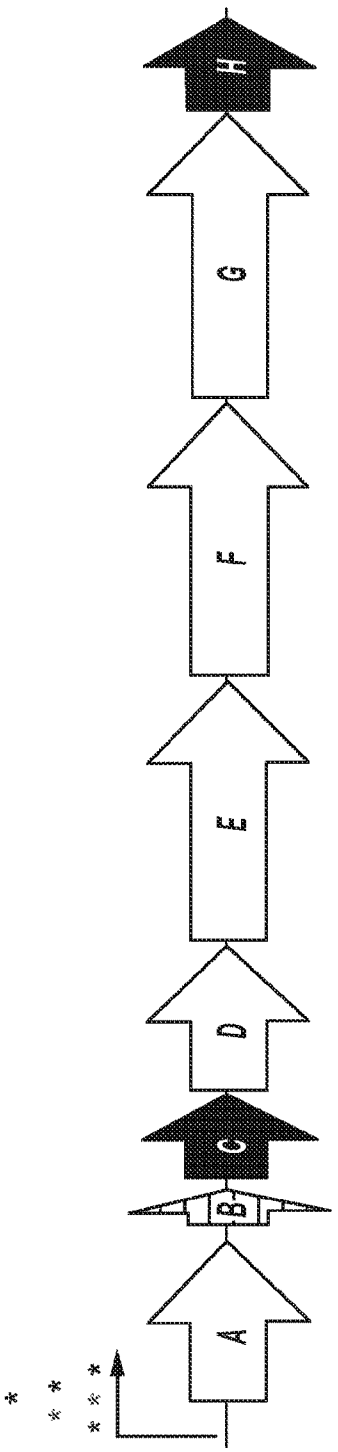

FIG. 10A

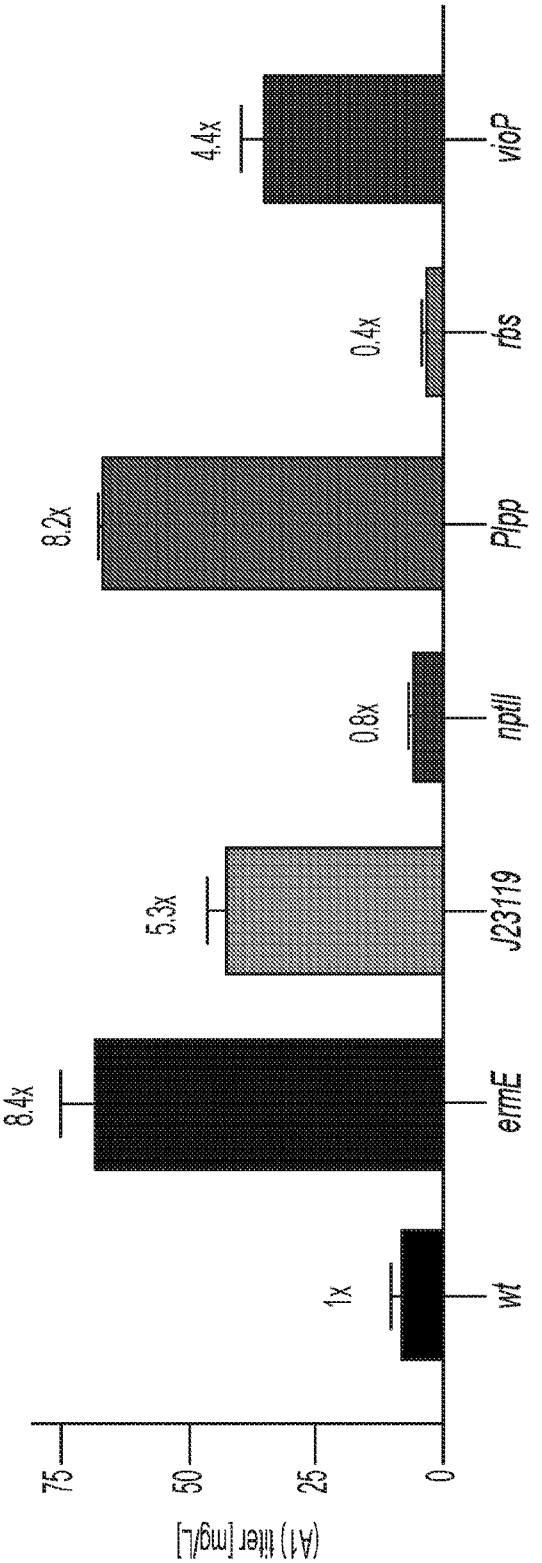

FIG. 10B

Date Reçue/Date Received 2024-04-11

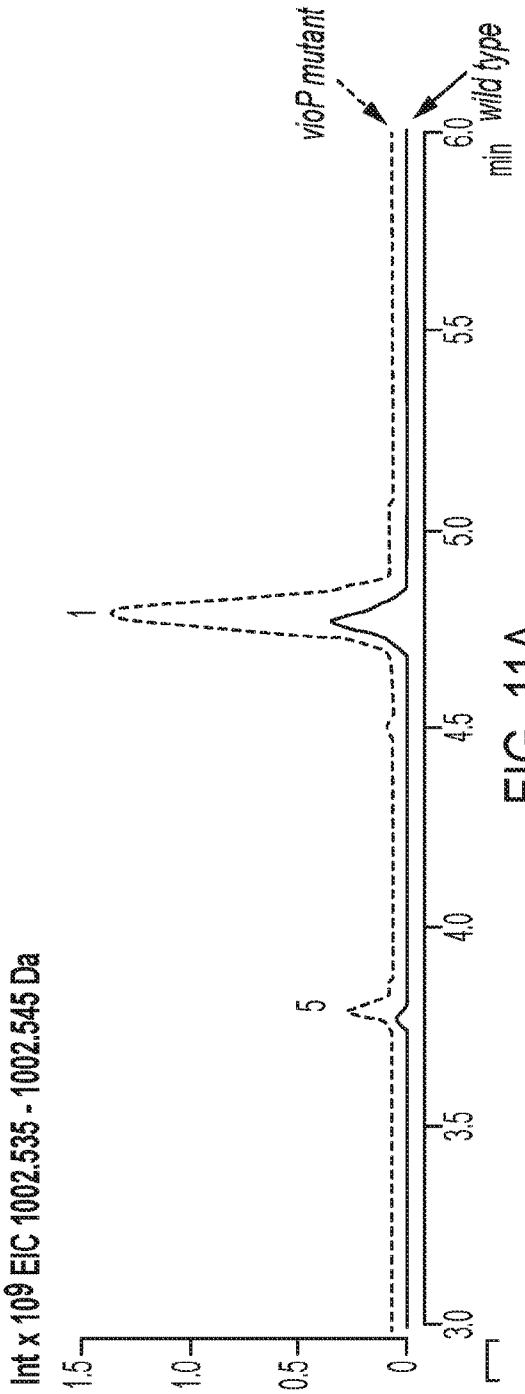

FIG. 11A

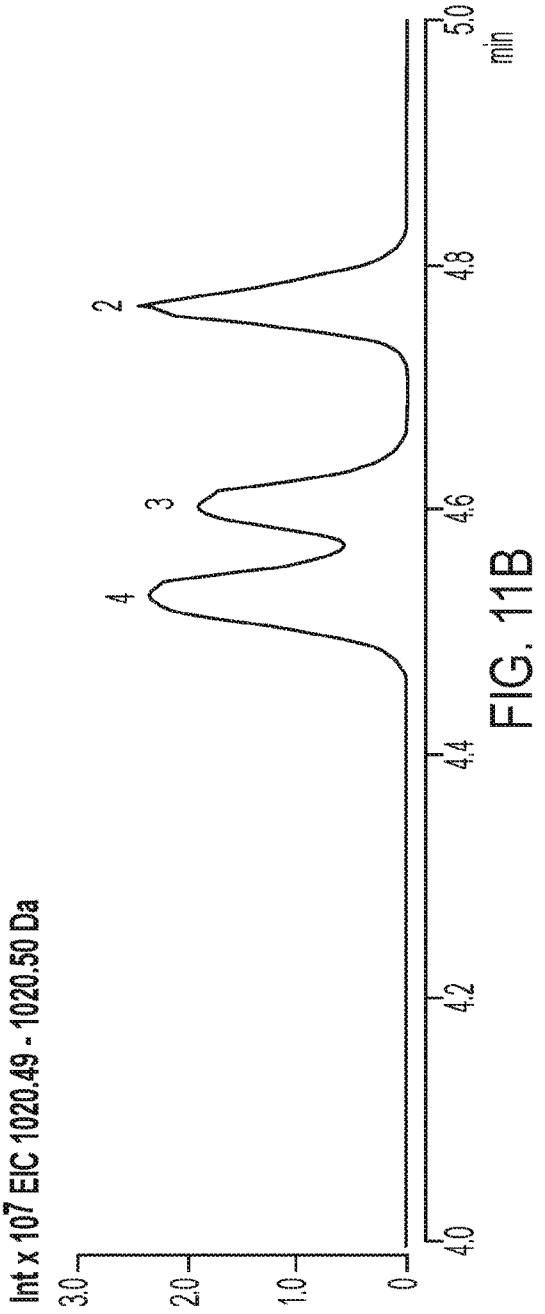

FIG. 11B

Date Reçue/Date Received 2024-04-11

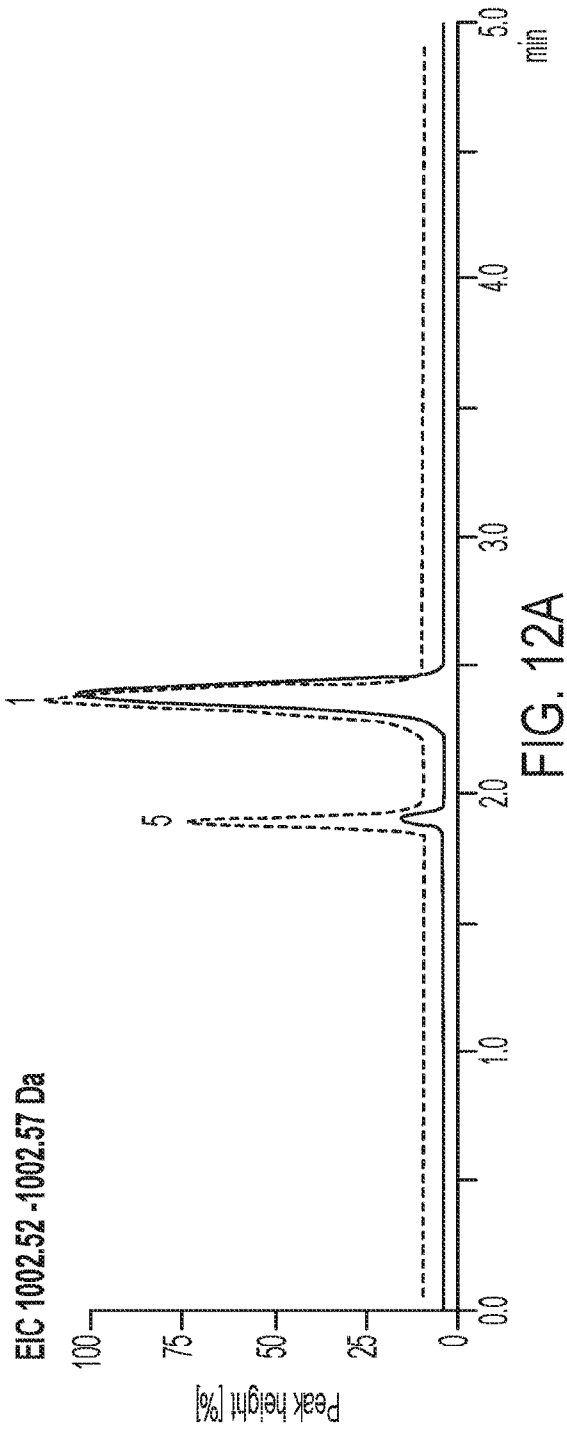

FIG. 12A

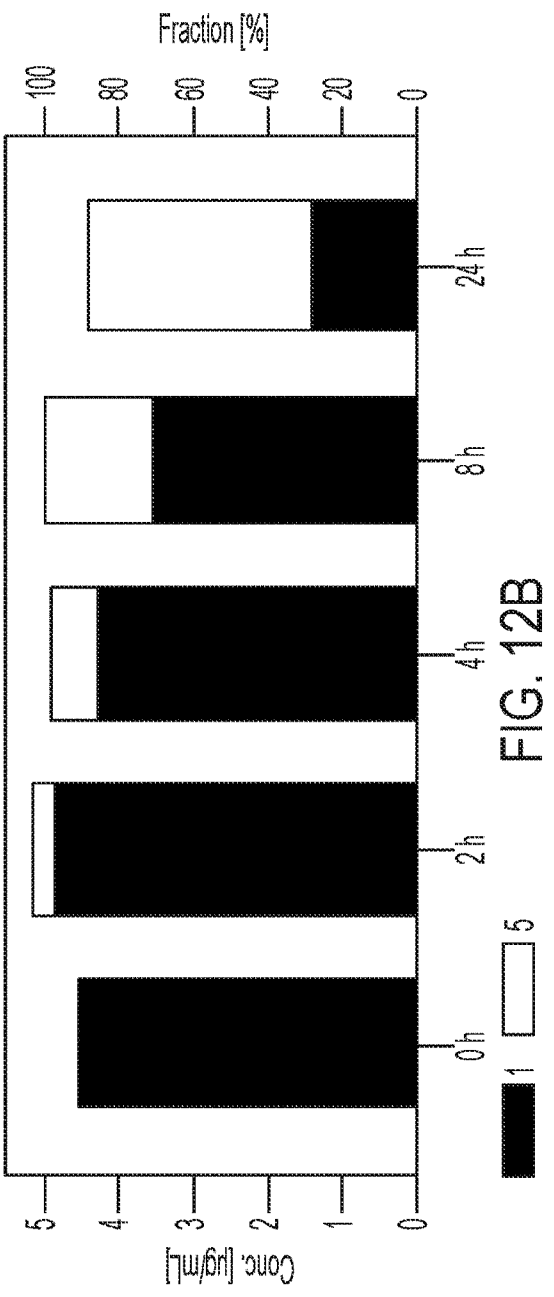

FIG. 12B

Date Reçue/Date Received 2024-04-11

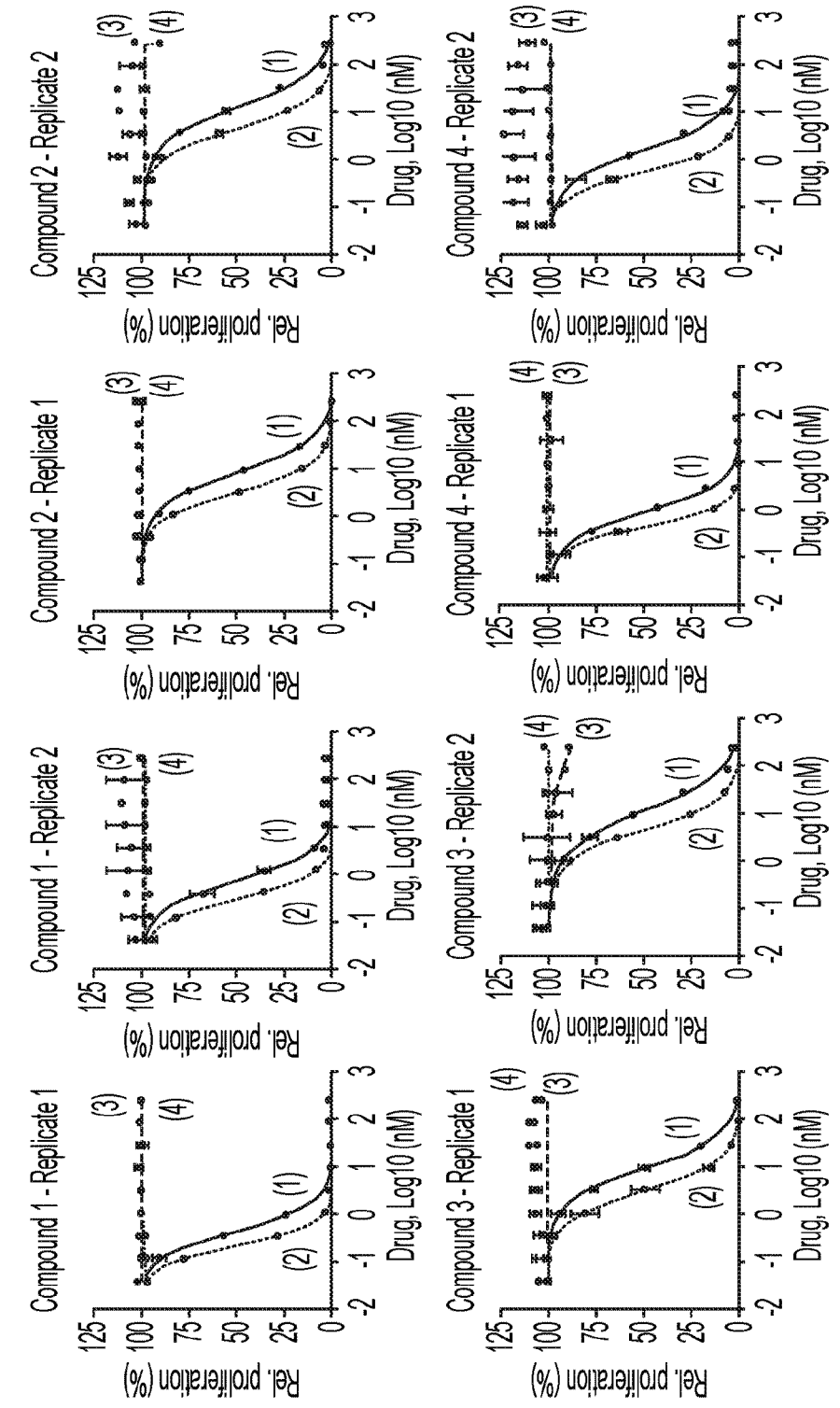

FIG. 13A

Date Reçue/Date Received 2024-04-11

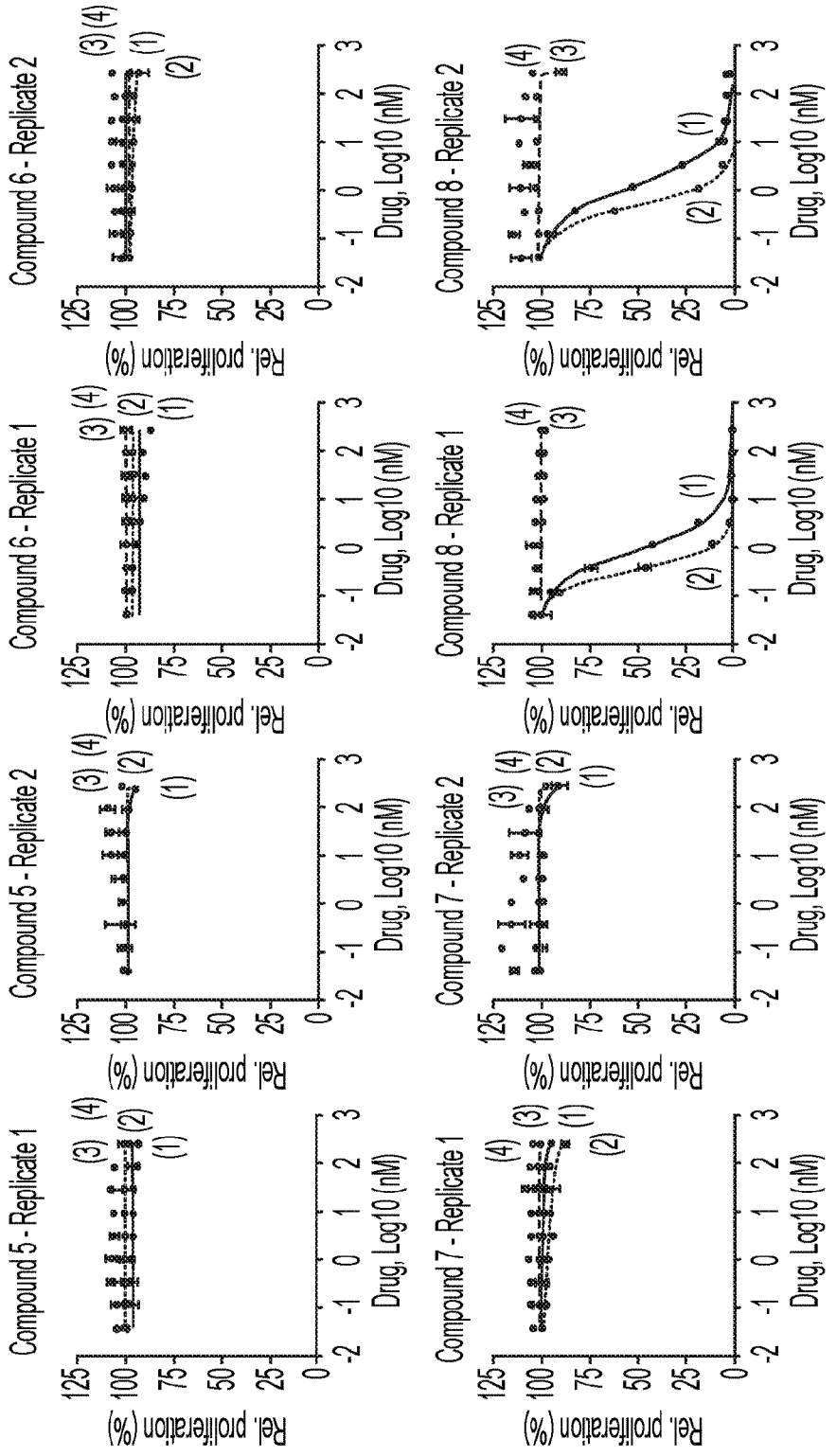

FIG. 13A
CONTINUED

Date Reçue/Date Received 2024-04-11

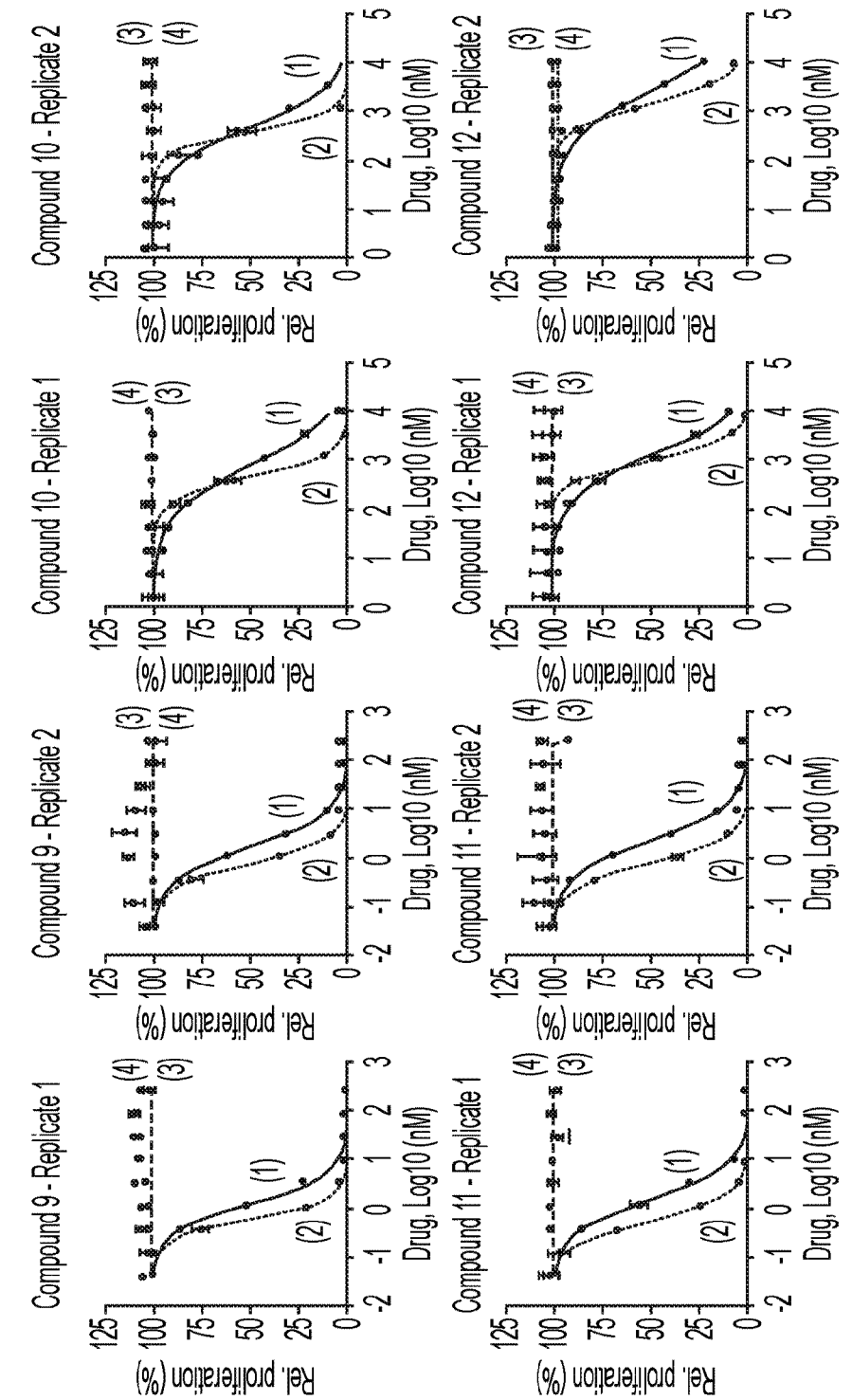

FIG. 13B

Date Reçue/Date Received 2024-04-11

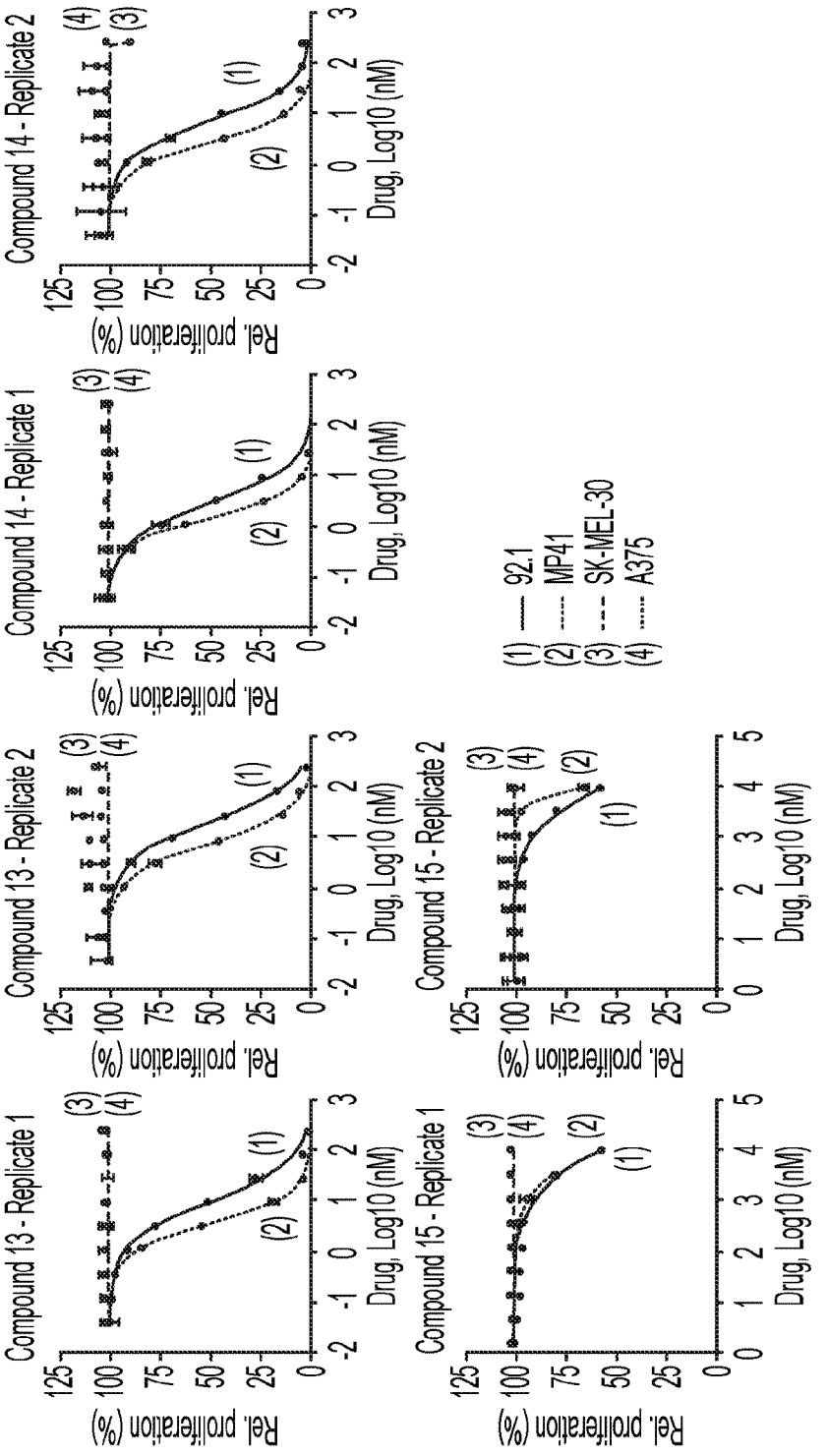

FIG. 13B
CONTINUED

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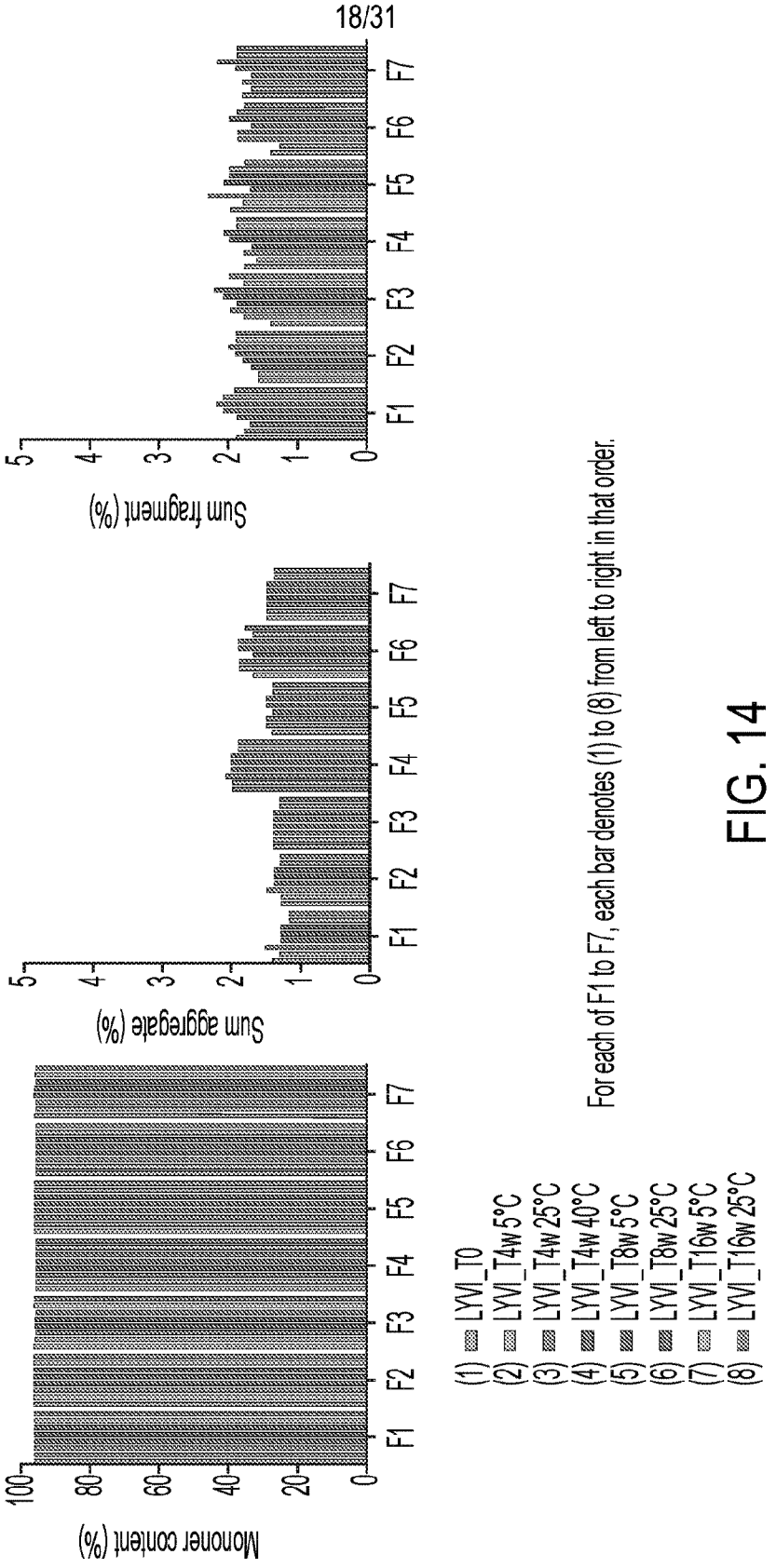

FIG. 14

Date Reçue/Date Received 2024-04-11

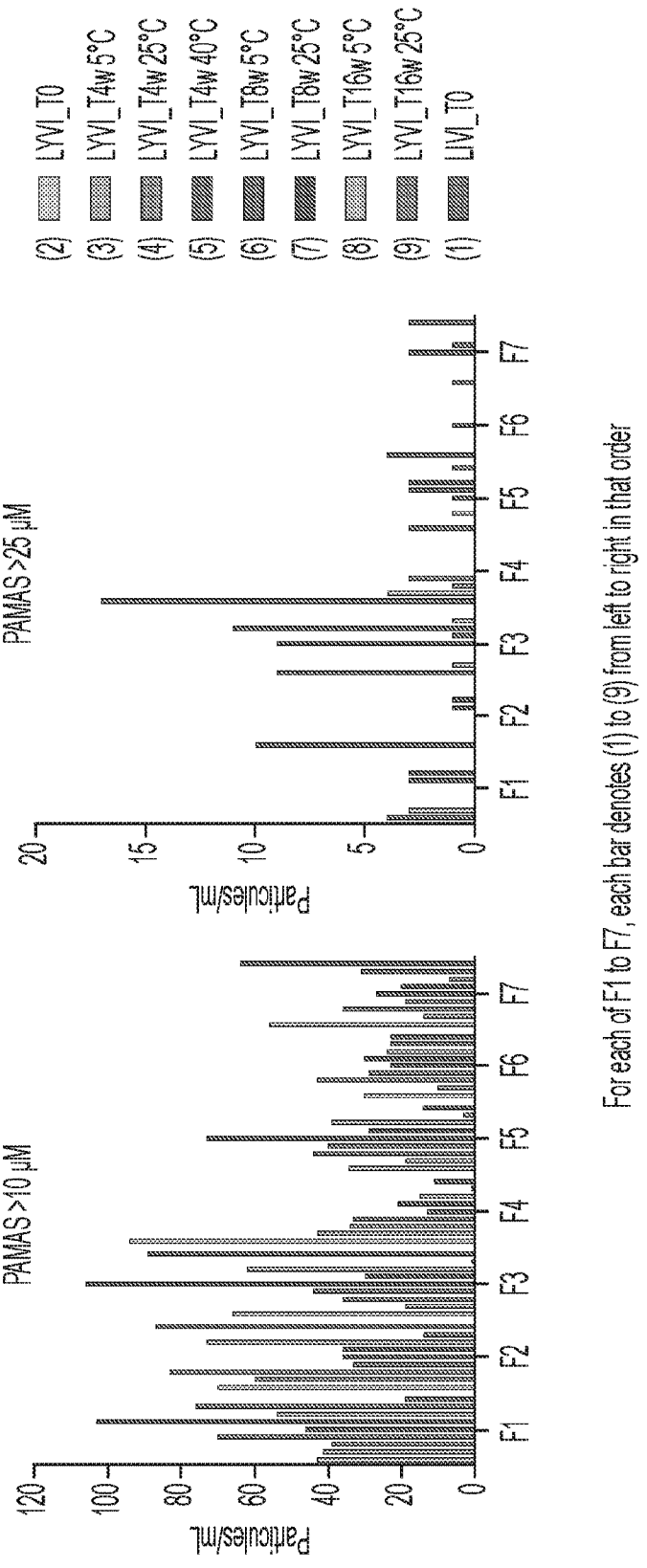

FIG. 15

Date Reçue/Date Received 2024-04-11

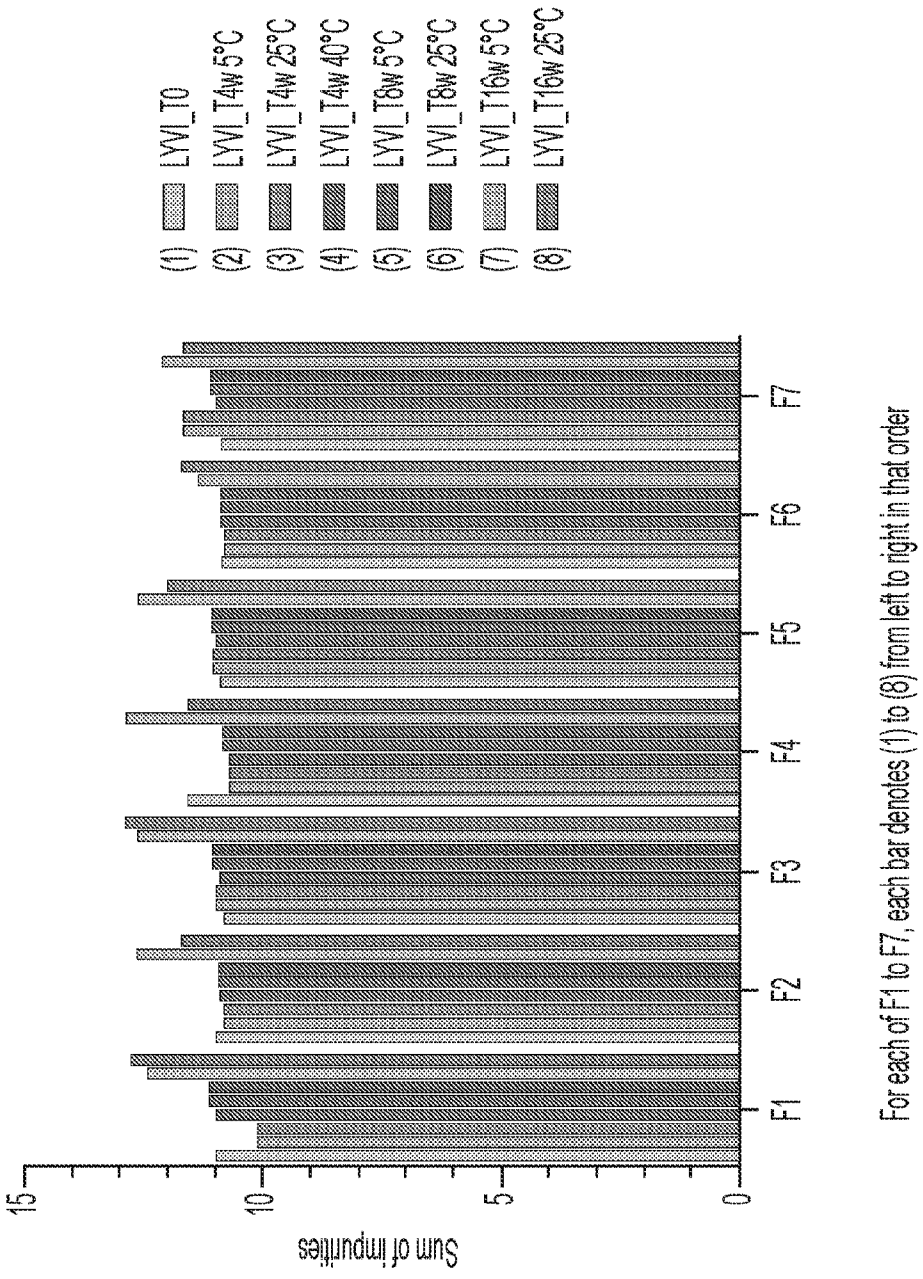

FIG. 16

Date Reçue/Date Received 2024-04-11

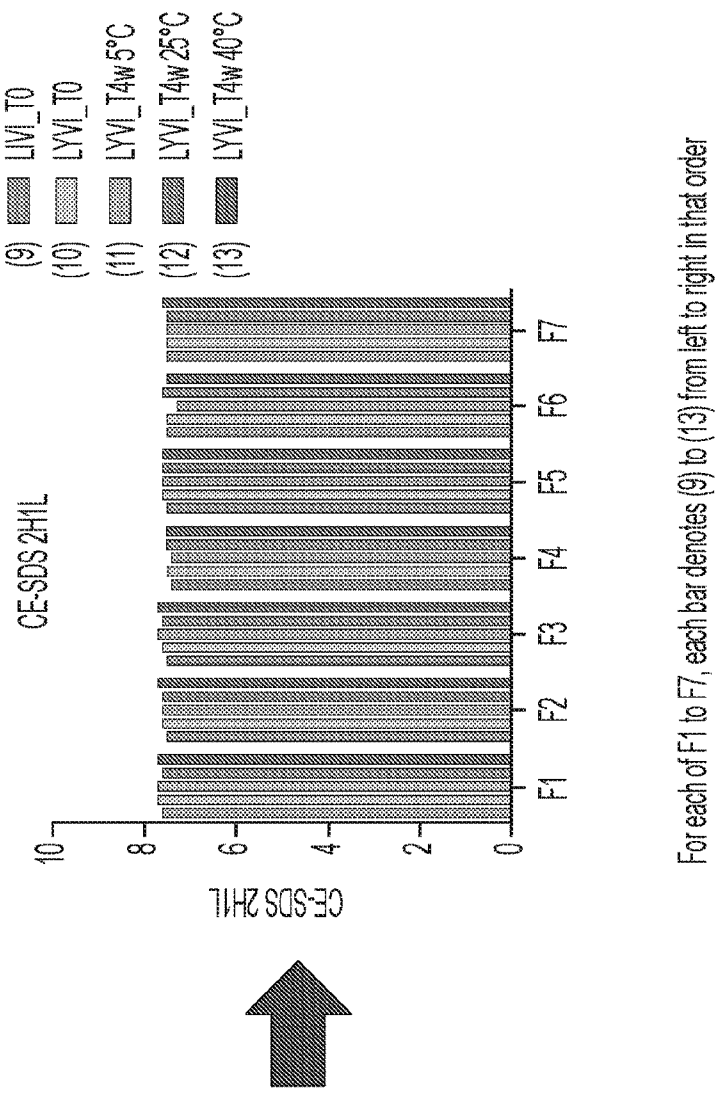

FIG. 16
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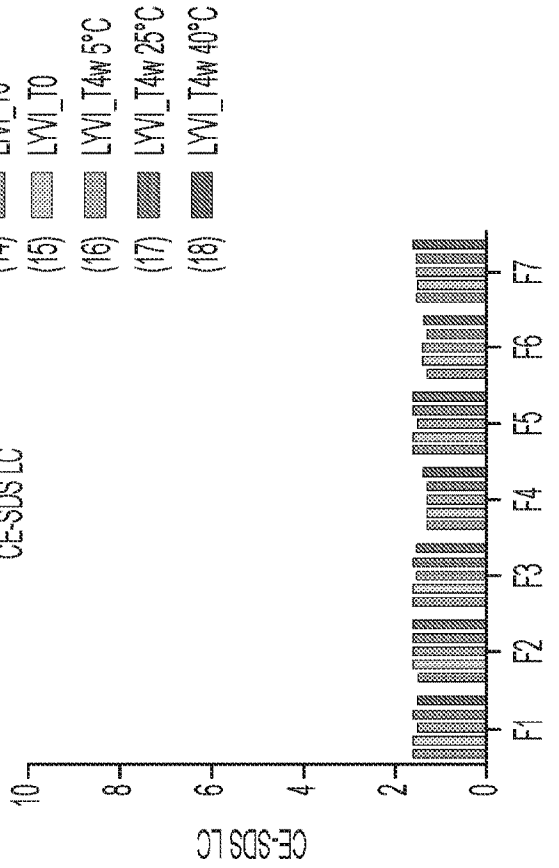
Date Reçue/Date Received 2024-04-11



For each of F1 to F7, each bar denotes (14) to (18) from left to right in that order

FIG. 16
CONTINUED

Date Reçue/Date Received 2024-04-11

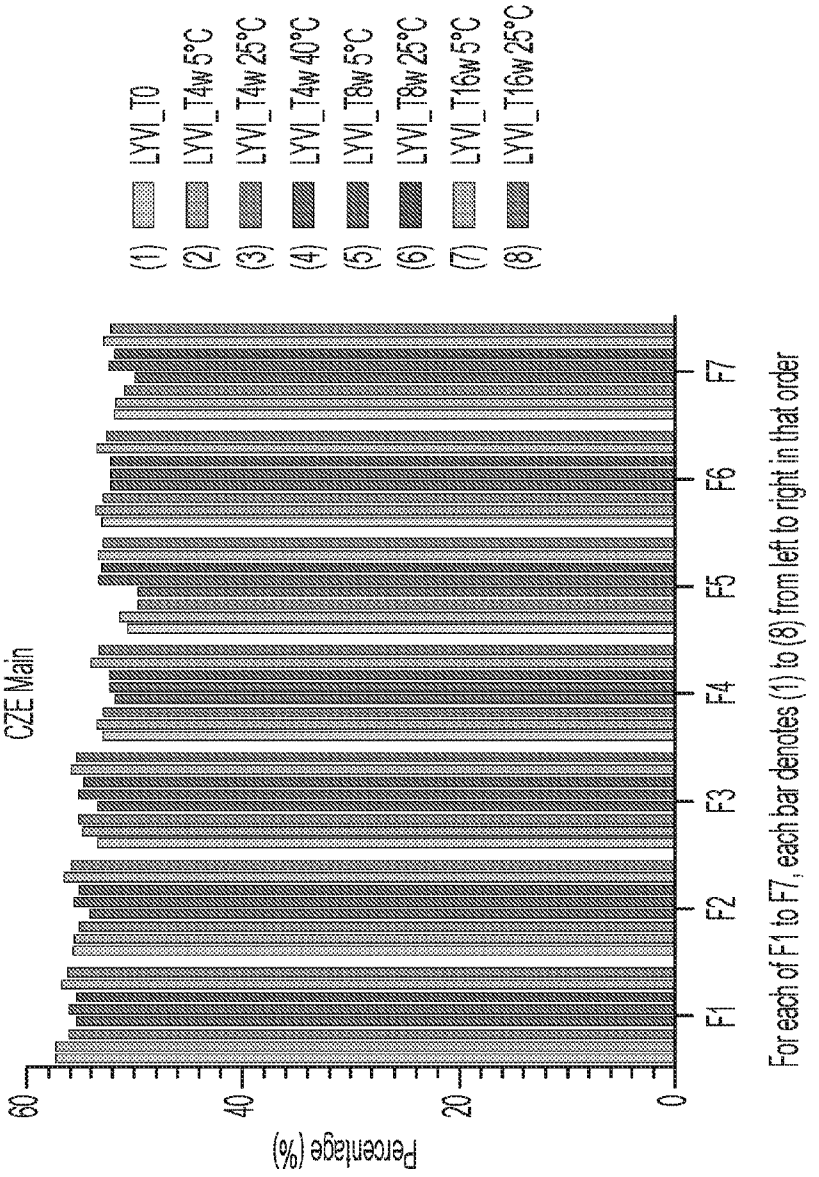


FIG. 17

Date Reçue/Date Received 2024-04-11

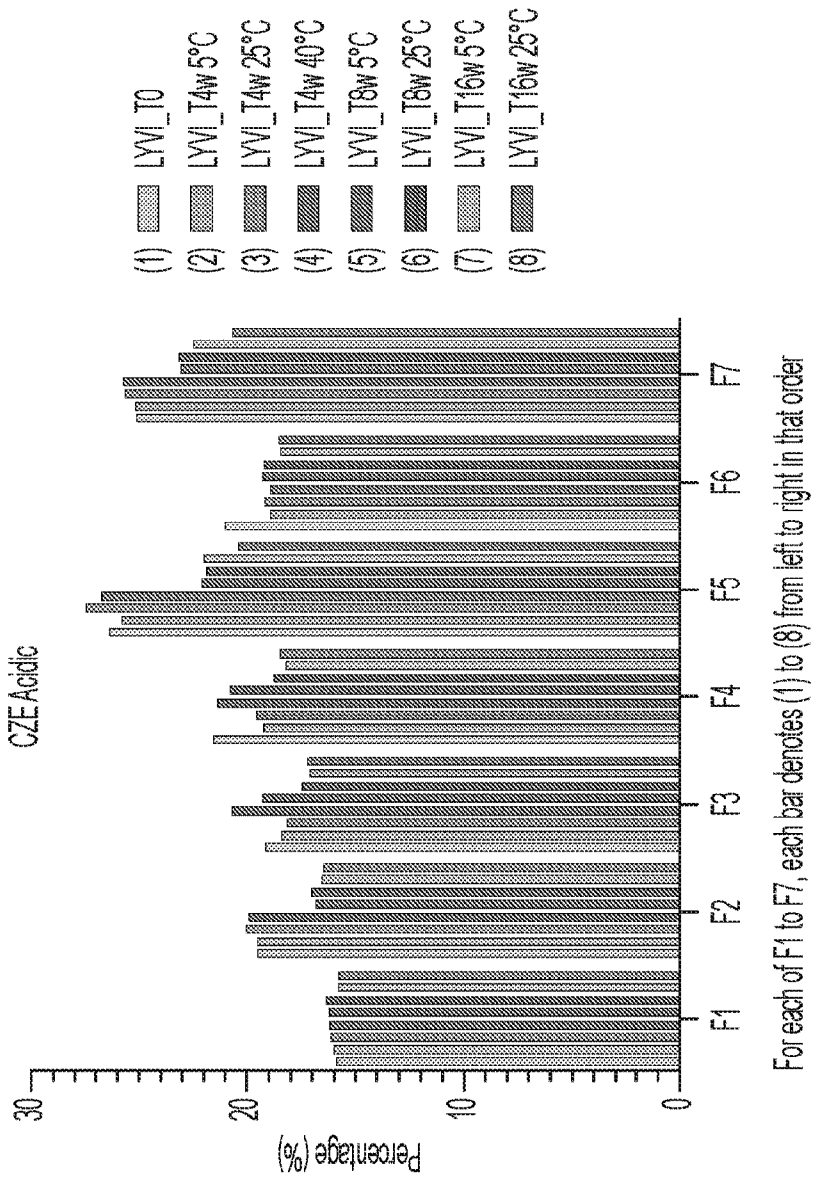


FIG. 17
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Date Reçue/Date Received 2024-04-11

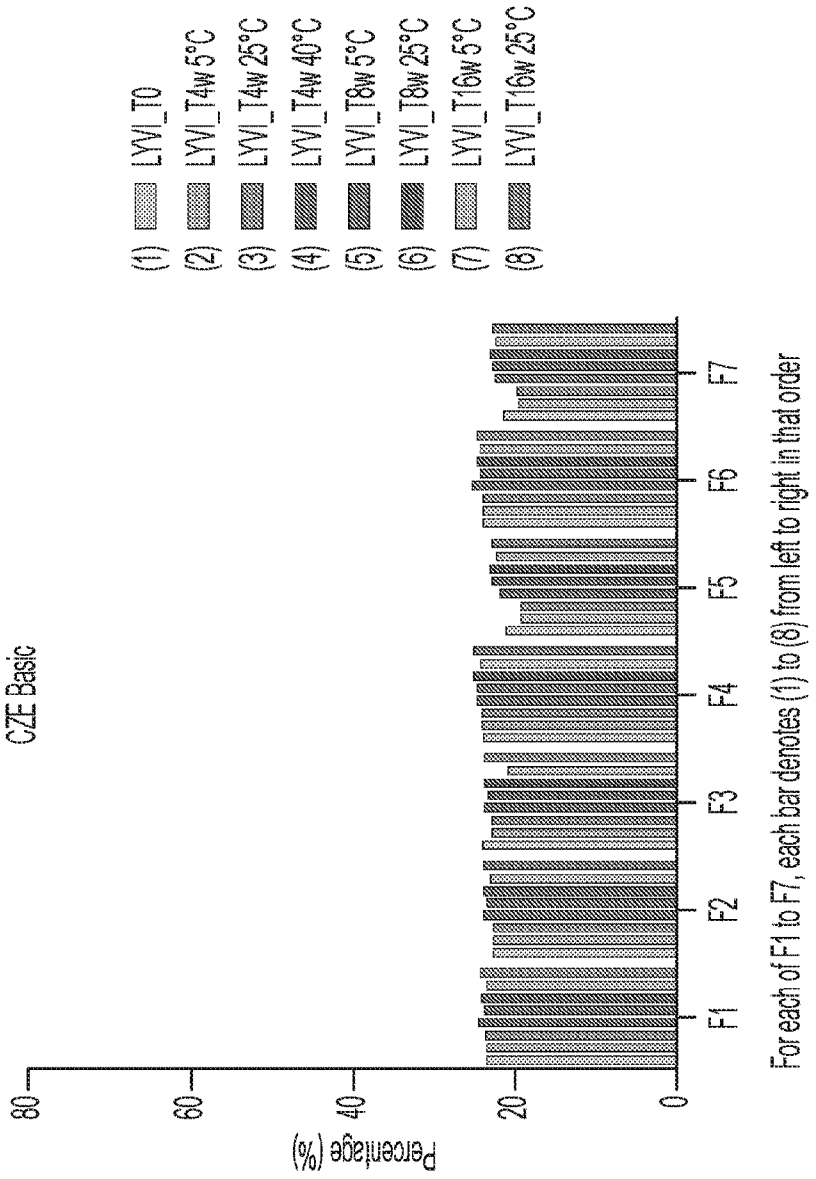

FIG. 17
CONTINUED

Date Reçue/Date Received 2024-04-11

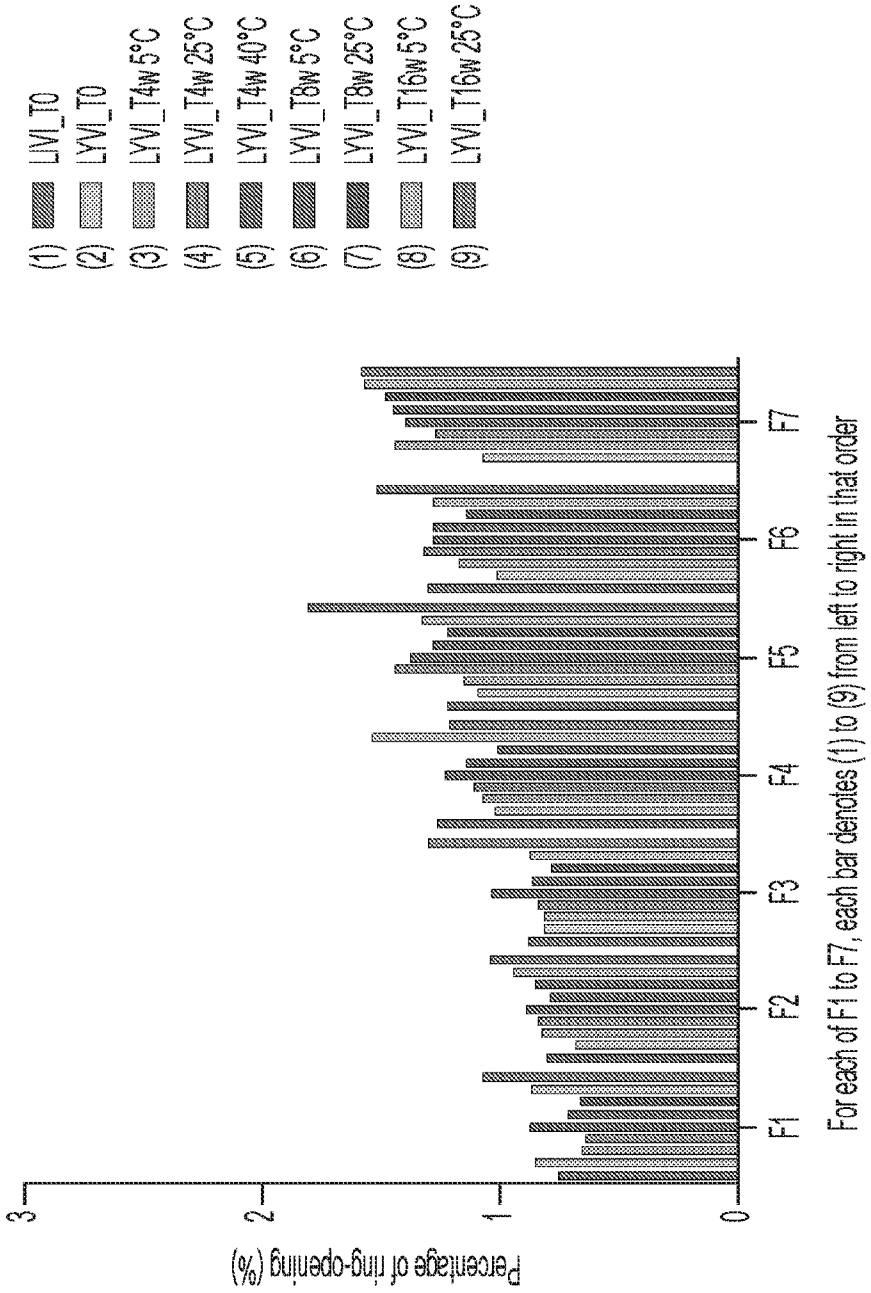

FIG. 18

Date Reçue/Date Received 2024-04-11

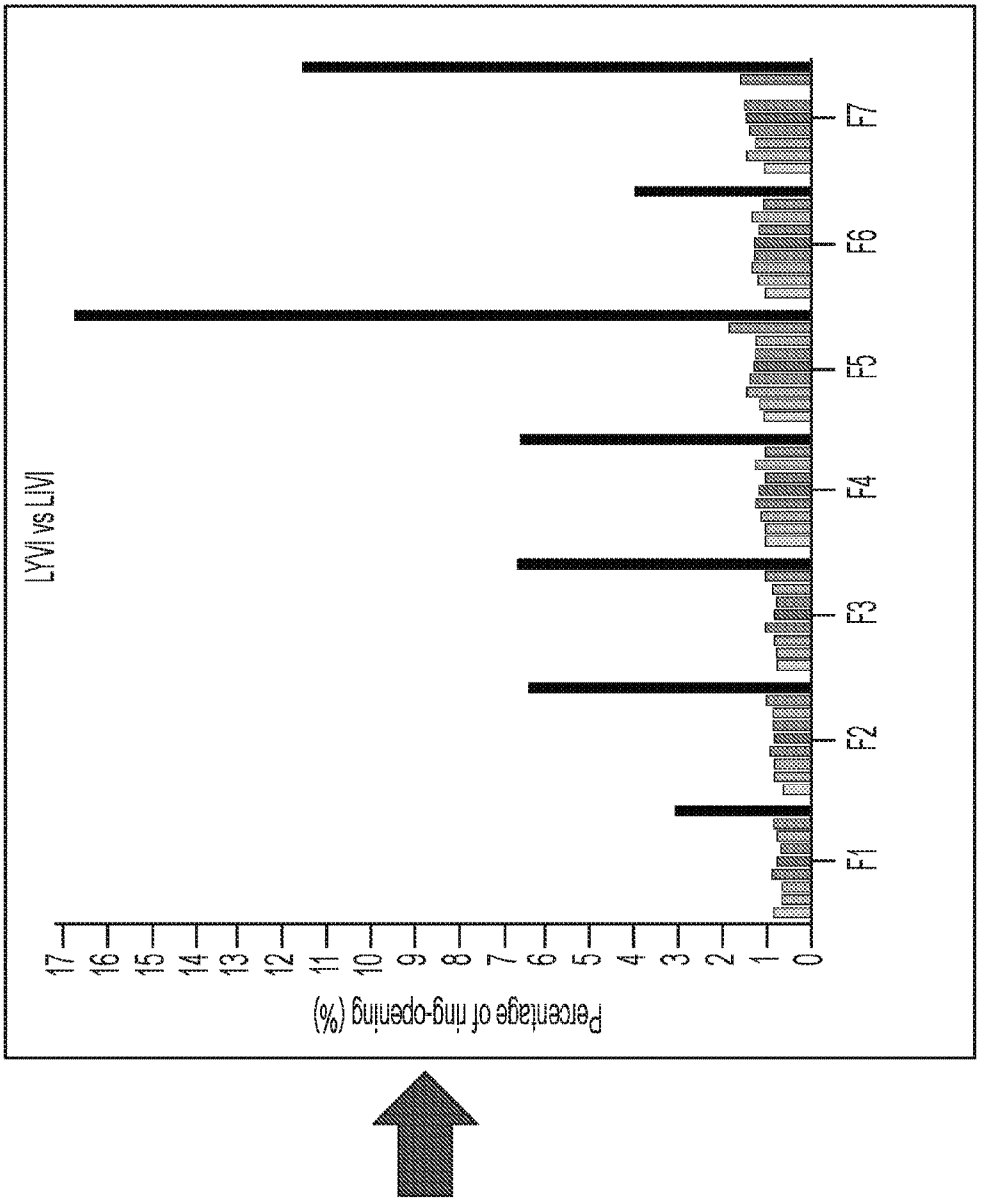

FIG. 18
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Date Reçue/Date Received 2024-04-11

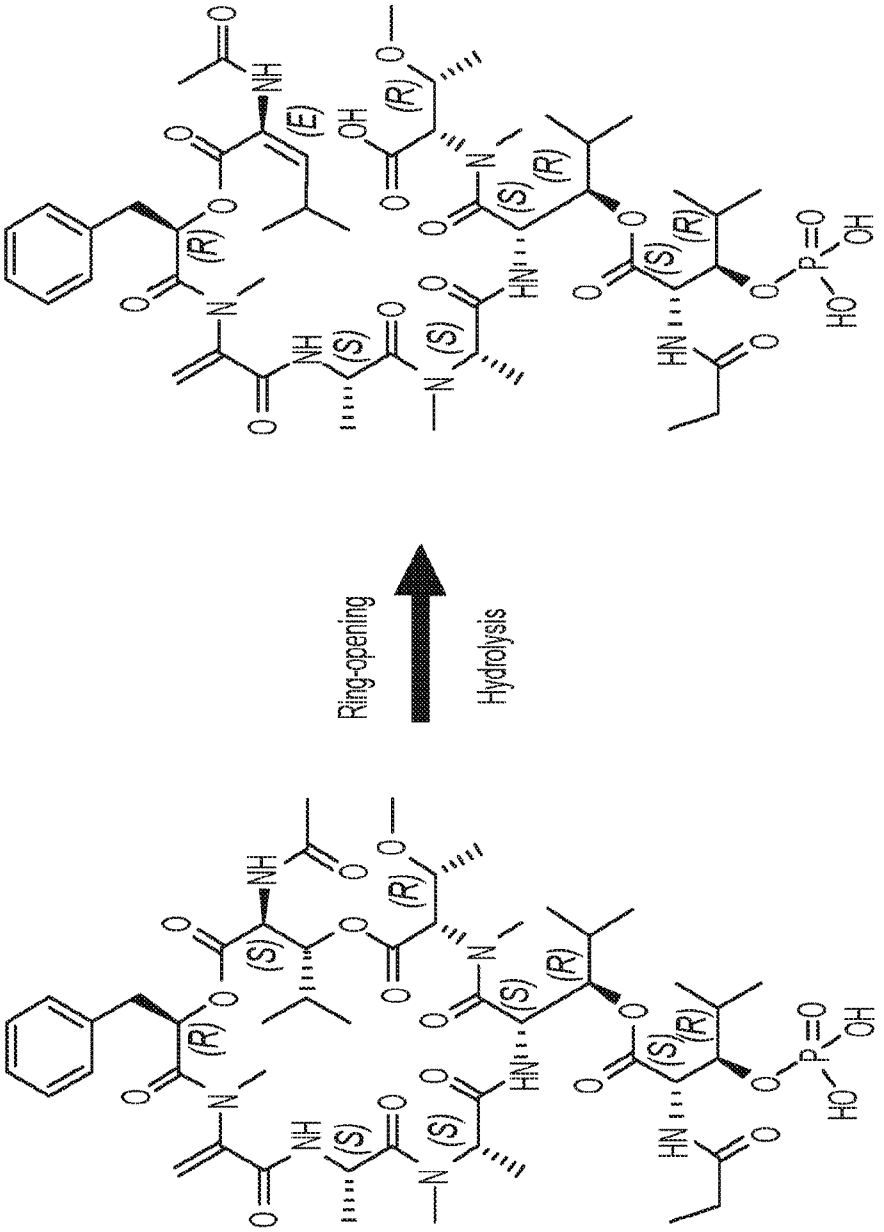

FIG. 18
CONTINUED

Date Reçue/Date Received 2024-04-11

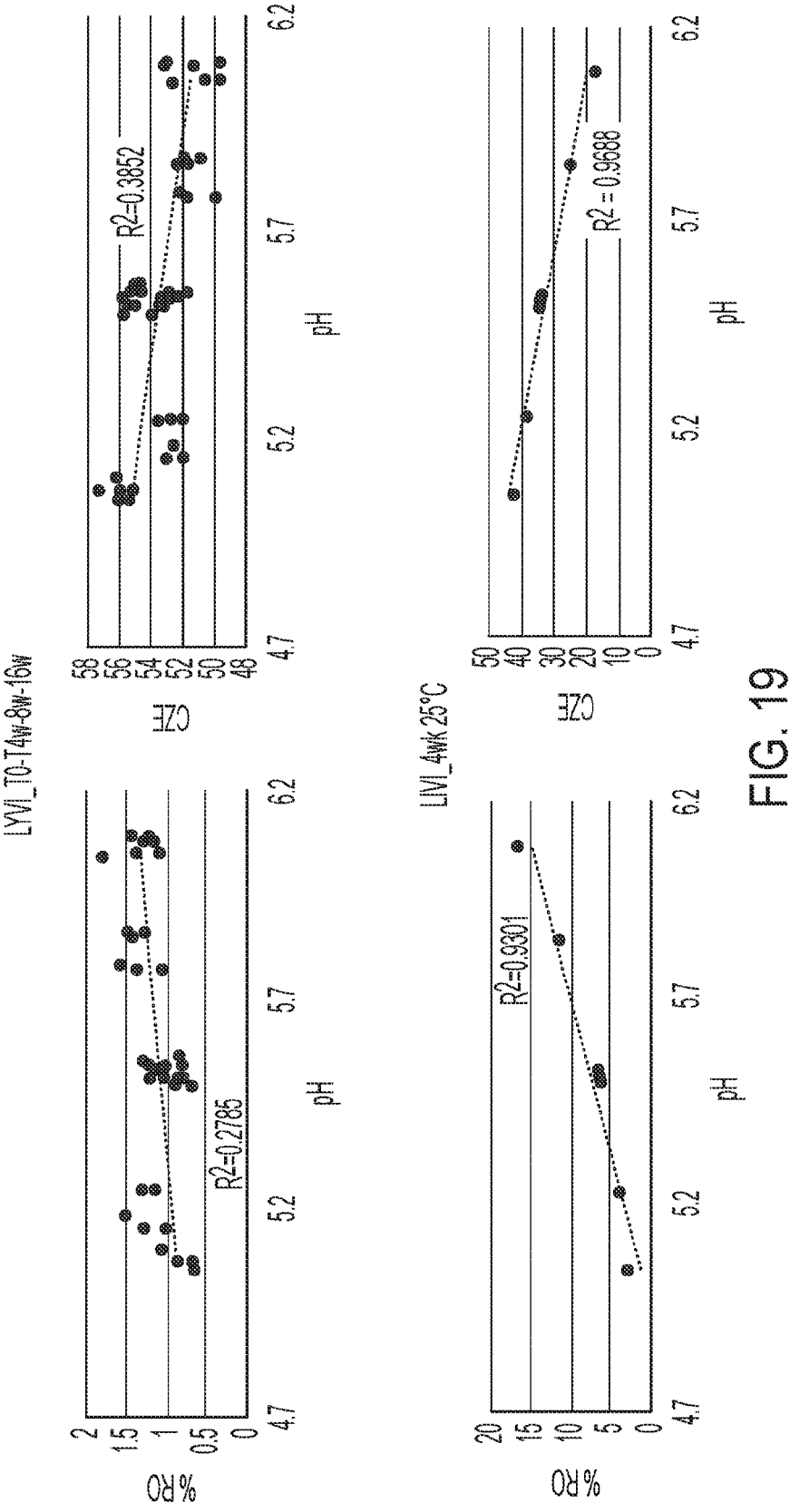

FIG. 19

Date Reçue/Date Received 2024-04-11

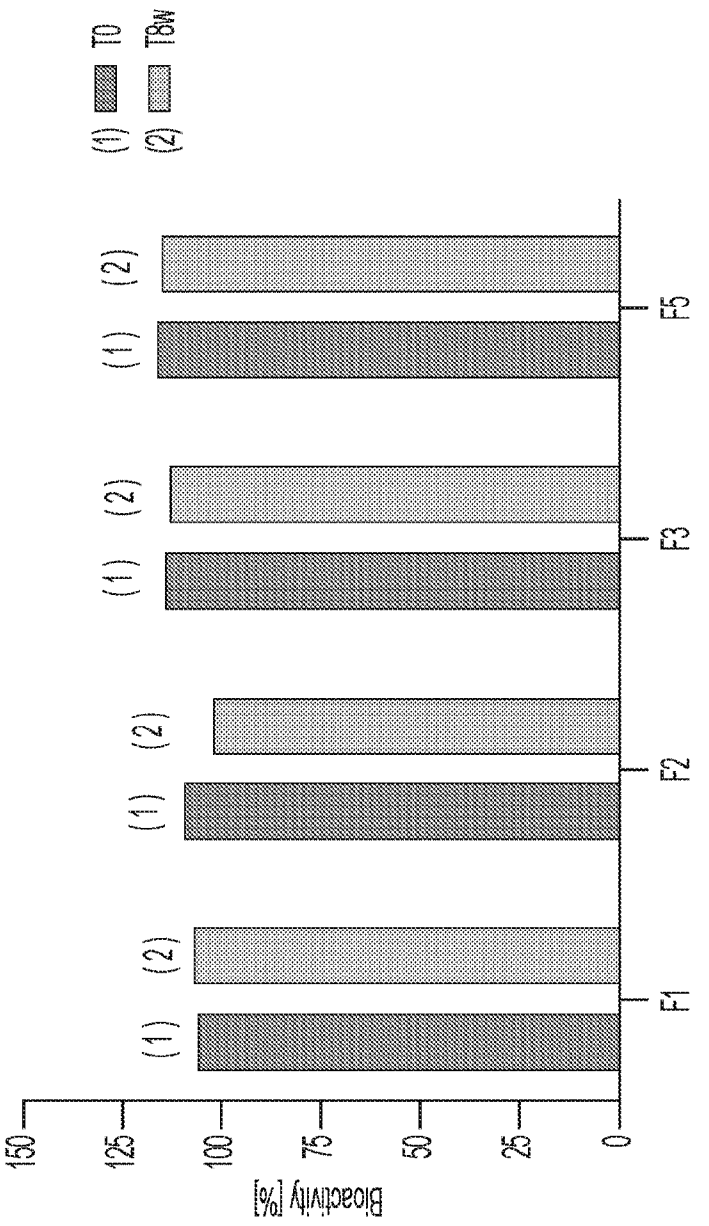

FIG. 20

Date Reçue/Date Received 2024-04-11

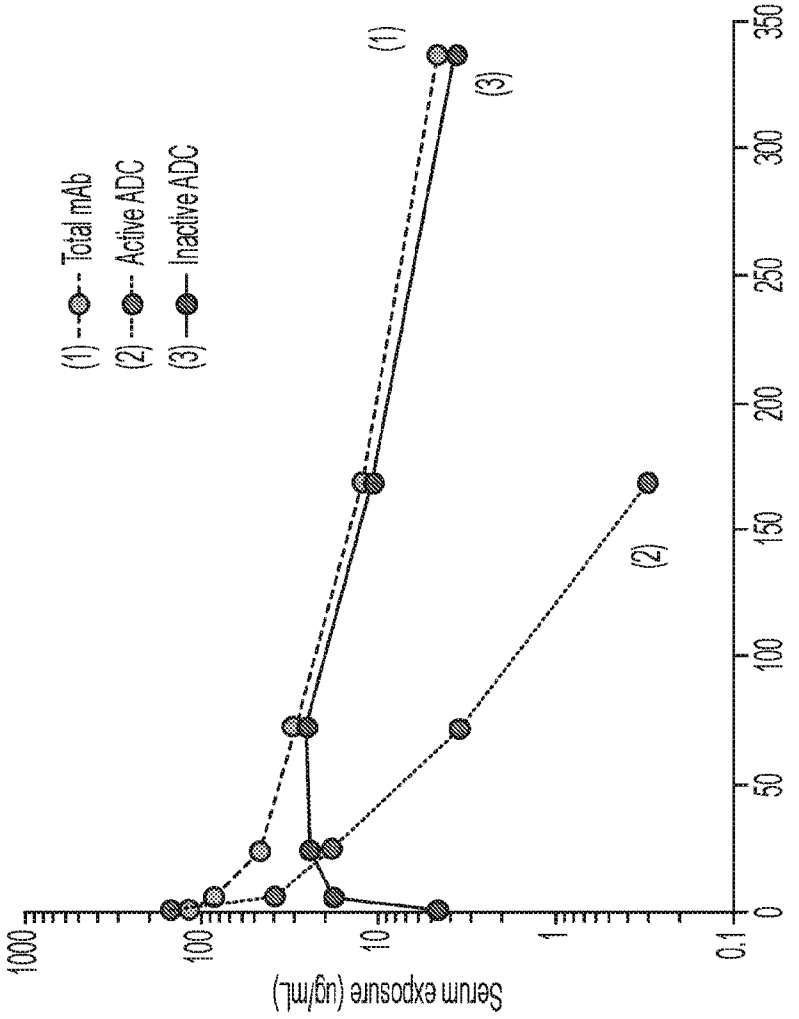

FIG. 21

Date Reçue/Date Received 2024-04-11