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(54) **Titre : ANTICORPS ANTI-GLYCO-MUC4 ET LEURS UTILISATIONS**
(54) **Title: ANTI-GLYCO-MUC4 ANTIBODIES AND THEIR USES**

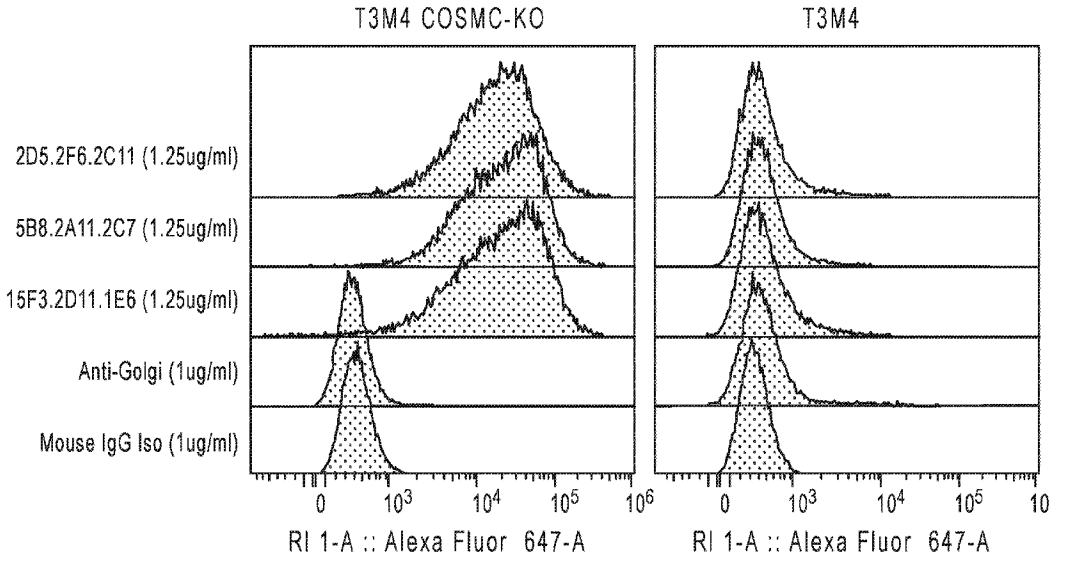

FIG. 1A

(57) **Abrégé/Abstract:**
The present disclosure relates to anti-glyco-MUC4 antibodies and antigen binding fragments thereof that specifically bind to a cancer-specific glycosylation variant of MUC4 and related fusion proteins and antibody-drug conjugates, as well as nucleic acids encoding such biomolecules. The present disclosure further relates to use of the antibodies, antigen-binding fragments, fusion proteins, antibody-drug conjugates and nucleic acids for cancer therapy.

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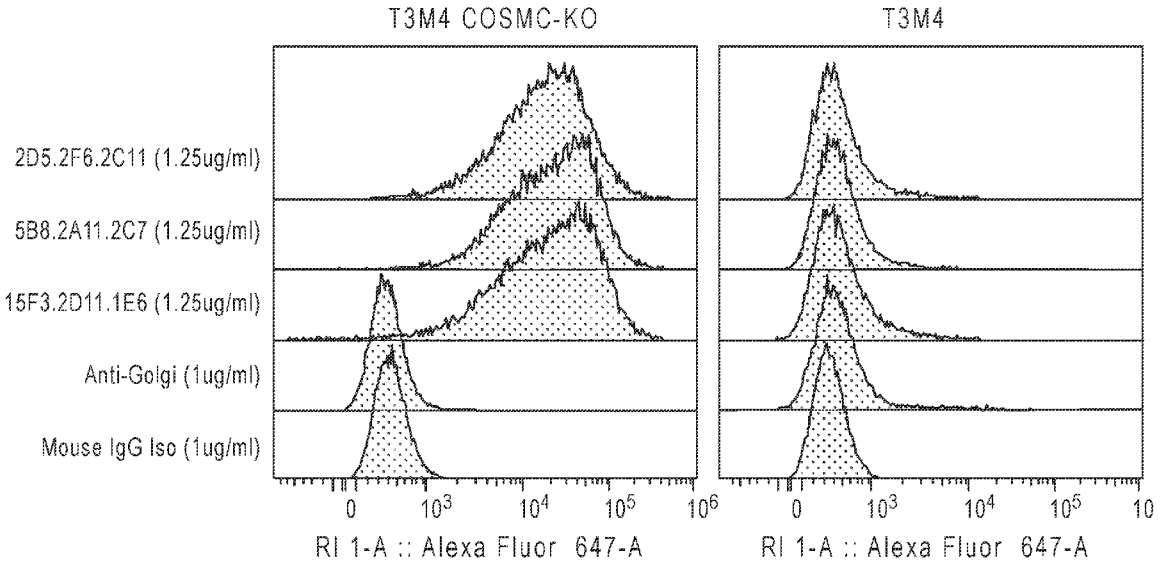

FIG. 1A

(57) Abstract: The present disclosure relates to anti-glyco-MUC4 antibodies and antigen binding fragments thereof that specifically bind to a cancer-specific glycosylation variant of MUC4 and related fusion proteins and antibody-drug conjugates, as well as nucleic acids encoding such biomolecules. The present disclosure further relates to use of the antibodies, antigen-binding fragments, fusion proteins, antibody-drug conjugates and nucleic acids for cancer therapy.

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ANTI-GLYCO-MUC4 ANTIBODIES AND THEIR USES

1. CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of U.S. provisional application nos. 63/229,839, filed August 5, 2021, 63/241,837, filed September 8, 2021, and 63/270,642, filed October 22, 2021, the contents of which are incorporated herein in their entireties by reference thereto.

2. BACKGROUND

[0002] MUC4 is a highly O-glycosylated heterodimeric membrane mucin containing three 125-amino acid repeats and an extensive number of polymorphic mucin repeats of 16 amino acids (Carraway *et al.*, 2010, Future Oncol, 5(10):1631-1640) that form multiple potential sites for O-linked glycosylation. The initiating step by which mucin type O-linked glycosylation occurs involves the addition of N-acetylgalactosamine (GalNAc) to serine or threonine residues present in the mucin backbone to form the Tn-epitope, a step that is catalyzed by a large family of polypeptide GalNAc-transferases (GalNAc-Ts). In healthy cells, the Tn structure is then extended to form more complex structures referred to as Core 1, 2, 3, or 4. Reviewed by Hanson and Hollingsworth, 2016, Biomolecules 6(3):34.

[0003] In a variety of tumor cells, expression of MUC4 and its glycosylation are both deregulated. Elevated expression of MUC4 protein is common in colon adenocarcinoma samples and MUC4 is a putative marker of aggressive pancreatic cancer. MUC4 is therefore promising for targeting in human cancers, *e.g.*, for immunotherapies such as chimeric antigen receptors (CARs), but such strategies have been hampered due to the prominent expression of MUC4 in healthy tissue.

[0004] The glycosylation pathway is deregulated in tumors, and many tumors exhibit aberrantly glycosylated MUC4. The expression of truncated Core 1 based structures, such as T, Tn, or sialyl-Tn (STn), are observed in a majority of human carcinomas but typically absent in healthy tissues. Thus, the identification of MUC4 epitopes that are overexpressed in tumor cells as compared to healthy tissues presents an attractive approach for targeted cancer therapy. Thus, there remains a need for the identification of glyco-MUC4 epitopes that are uniquely or overexpressed in cancer cells as compared to healthy tissues and new therapeutic modalities, such as antibodies and CARs, which target such glyco-MUC4 epitopes with high affinity.

3. SUMMARY

[0005] The disclosure captures the tumor specificity of glycopeptide variants by providing therapeutic and diagnostic agents based on antibodies and antigen binding fragments that are selective for glycosylated MUC4. The antibodies and antigen-binding fragments

advantageously bind to both the MUC4 backbone and its cancer specific O-linked glycans but not MUC4 on healthy tissues.

[0006] Accordingly, the present disclosure provides anti-glyco-MUC4 antibodies and antigen binding fragments thereof that bind to a cancer-specific glycosylation variant of MUC4. The present disclosure further provides fusion proteins and antibody-drug conjugates comprising anti-glyco-MUC4 antibodies and antigen binding fragments, and nucleic acids encoding the anti-glyco-MUC4 antibodies, antigen binding fragments and fusion proteins.

[0007] The present disclosure further provides methods of using the anti-glyco-MUC4 antibodies, antigen-binding fragments, fusion proteins, antibody-drug conjugates and nucleic acids for cancer therapy.

[0008] In certain aspects, the disclosure provides bispecific and other multispecific anti-glyco-MUC4 antibodies and antigen binding fragments that bind to a cancer-specific glycosylation variant of MUC4 and to a second epitope. The second epitope can either be on MUC4 itself, on another protein co-expressed on cancer cells with MUC4, or on another protein presented on a different cell, such as an activated T cell. Further, also disclosed are nucleic acids encoding such antibodies, including nucleic acids comprising codon-optimized coding regions and nucleic acids comprising coding regions that are not codon-optimized for expression in a particular host cell.

[0009] The anti-glyco-MUC4 antibodies and binding fragments can be in the form of fusion proteins containing a fusion partner. The fusion partner can be useful to provide a second function, such as a signaling function of the signaling domain of a T cell signaling protein, a peptide modulator of T cell activation or an enzymatic component of a labeling system. Exemplary T cell signaling proteins include 4-1BB, CD28, CD2, and fusion peptides, *e.g.*, CD28-CD3-zeta, 4-1BB-CD3-zeta, CD2-CD3-zeta, CD28-CD2-CD3-zeta, and 4-1BB CD2-CD3-zeta. 4-1BB, also known as CD137, is a co-stimulatory receptor of T cells; CD2 is a co-stimulatory receptor of T and NK cells; CD3-zeta is a signal-transduction component of the T-cell antigen receptor. The moiety providing a second function can be a modulator of T cell activation, such as IL-15, IL-15Rα, or an IL-15/IL-15Rα fusion, can be an MHC-class I-chain-related (MIC) protein domain useful for making a MicAbody, or it can encode a label or an enzymatic component of a labeling system useful in monitoring the extent and/or location of binding *in vivo* or *in vitro*. Constructs encoding these prophylactically and therapeutically active biomolecules placed in the context of T cells, such as autologous T cells, provide a powerful platform for recruiting adoptively transferred T cells to prevent or treat a variety of cancers in some embodiments of the disclosure.

[0010] In certain aspects, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure comprises heavy and/or light chain CDR sequences (as defined by Kabat, Chothia, IMGT or their combined region of overlap) of the anti-glyco-MUC4 antibodies 2D5.2F6.2C11

(sometimes referred to herein as “2D5”), 5B8.2A11.2C7 (sometimes referred to herein as “5B8”), 15F3.2D11.1E6 (sometimes referred to herein as “15F3”), or humanized counterparts thereof. In some embodiments, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure comprises heavy and/or light chain variable sequences (or encoded by the nucleotide sequences) of the anti-glyco-MUC4 antibodies 2D5, 5B8, 15F3, or humanized counterparts thereof. The CDR and variable sequences (as well as their coding sequences) of the anti-glyco-MUC4 antibodies 2D5, 5B8, and 13F3 are set forth in Tables 1A through 1C, respectively. For clarity, when the term “anti-glyco-MUC4 antibody” is used in this document, it is intended to include monospecific and multi-specific (including bispecific) anti-glyco-MUC4 antibodies, antigen-binding fragments of the monospecific and multi-specific antibodies, and fusion proteins and conjugates containing the antibodies and their antigen-binding fragments, unless the context dictates otherwise. Likewise, when the term “anti-glyco-MUC4 antibody or antigen-binding fragment” is used, it is also intended to include monospecific and multi-specific (including bispecific) anti-glyco-MUC4 antibodies and their antigen-binding fragments, together with fusion proteins and conjugates containing such antibodies and antigen-binding fragments, unless the context dictates otherwise.

[0011] In other aspects, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure comprises heavy and/or light chain CDR sequences (or encoded by the nucleotide sequences) set forth in Tables 1-3. The CDR sequences set forth in Tables 1A and 1B include CDR sequences defined according to the IMGT (Lefranc *et al.*, 2003, Dev Comparat Immunol 27:55-77), Kabat (Kabat *et al.*, 1991, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md.), and Chothia (Al-Lazikani *et al.*, 1997, J. Mol. Biol 273:927-948) schemes for defining CDR boundaries. The CDR sequences set forth in Tables 1D-1F are consensus sequences derived from the CDR sequences set forth in Tables 1A through 1C according to the IMGT, Kabat, and Chothia definitions, respectively. The CDR sequences set forth in Tables 2A through 2C are the combined regions of overlap for the CDR sequences set forth in Tables 1A through 1C, respectively, with the IMGT, Kabat and Chothia sequences shown in underlined bold text. The CDR sequences set forth in Table 2D are the combined regions of overlap for the consensus CDR sequences set forth in Tables 1E-1F. The CDR sequences set forth in Tables 3A-3C are the common regions of overlap for the CDR sequences shown in Tables 1A-1C, respectively. The CDR sequences set forth in Table 3D are the common regions of overlap for the CDR sequences set forth in Tables 1E-1F. The framework sequences for such anti-glyco-MUC4 antibody and antigen-binding fragment can be the native murine framework sequences of the VH and VL sequences set forth in Tables 1A-1C or can be non-native (*e.g.*, humanized or human) framework sequences.

[0012] In other aspects, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure comprises heavy and/or light chain variable sequences of humanized 2D5 set forth in Tables 4A through 4G.

Table 1A
2D5.2F6.2C11 Sequences

Description	Sequence	SEQ ID NO:
VH amino acid sequence (predicted mature)	QVQLQQSDAELVKPGASVRISCKAYGYTFTDHAIHWVK QKPEQGLEWLGYISPGNDDIQYNAKFKGKATLTADKSSS TAYMQLNSLTSDDSAVYFCKRSMANSFDYWGQGTTLTV SS	1
VL amino acid sequence (predicted mature)	NIMLTQSPSSLAVSAGEKVTMSCKSSQSVLYSSDQKNYL AWYQQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTD FTLTISNVQAEDLAVYYCHQYLSSYTFGGGTKLEIK	2
CDR-H1 amino acid sequence (IMGT definition)	GYTFTDHA	3
CDR-H2 amino acid sequence (IMGT definition)	ISPGNDDI	4
CDR-H3 amino acid sequence (IMGT definition)	KRSMANSFDY	5
CDR-L1 amino acid sequence (IMGT definition)	QSVLYSSDQKNY	6
CDR-L2 amino acid sequence (IMGT definition)	WAS	7
CDR-L3 amino acid sequence (IMGT definition)	HQYLSSYT	8
CDR-H1 amino acid sequence (Kabat definition)	DHAIH	9
CDR-H2 amino acid sequence (Kabat definition)	YISPGNDDIQYNAKFKG	10
CDR-H3 amino acid sequence (Kabat definition)	SMANSFDY	11
CDR-L1 amino acid sequence (Kabat definition)	KSSQSVLYSSDQKNYLA	12

Table 1A 2D5.2F6.2C11 Sequences		
Description	**Sequence**	**SEQ ID NO:**
CDR-L2 amino acid sequence (Kabat definition)	WASTRES	13
CDR-L3 amino acid sequence (Kabat definition)	HQYLSSYT	14
CDR-H1 amino acid sequence (Chothia definition)	GYTFTDH	15
CDR-H2 amino acid sequence (Chothia definition)	SPGNDD	16
CDR-H3 amino acid sequence (Chothia definition)	SMANSFDY	17
CDR-L1 amino acid sequence (Chothia definition)	KSSQSVLYSSDQKNYLA	18
CDR-L2 amino acid sequence (Chothia definition)	WASTRES	19
CDR-L3 amino acid sequence (Chothia definition)	HQYLSSYT	20
VH nucleotide sequence (excl. signal sequence)	CAGGTTCAGTTGCAGCAGTCTGACGCTGAGTTGGTGA AACCTGGGGCTTCAGTGAGGATATCCTGCAAGGCTTA TGGCTACACCTTCACTGACCATGCTATTCACTGGGTG AAACAGAAGCCTGAACAGGGCCTGGAATGGCTTGGAT ATATTTCTCCCGGAAATGATGATATTCAGTACAATGCG AAGTTCAAGGGCAAGGCCACACTGACTGCAGACAAAT CCTCCAGCACTGCCTACATGCAGCTCAACAGCCTGAC ATCTGACGATTCTGCAGTGTATTTCTGTAAAAGATCTA TGGCCAACTCCTTTGACTACTGGGGCCAAGGCACCAC TCTCACAGTCTCCTCA	21
VL nucleotide sequence (excl. signal sequence)	AACATTATGCTGACACAGTCGCCATCATCTCTGGCTG TGTCTGCAGGAGAAAAGGTCACTATGAGCTGTAAGTC CAGTCAAAGTGTTTTATACAGTTCAGATCAGAAGAACT ACTTGGCCTGGTACCAGCAGAAGCCAGGGCAGTCTC CTAAACTACTGATCTATTGGGCATCCACTAGGGAATCT GGTGTCCCTGATCGCTTCACAGGCAGTGGATCTGGG ACAGATTTTACTCTTACCATCAGCAATGTACAAGCTGA AGACCTGGCAGTTTATTACTGTCATCAATACCTCTCCT CGTACACGTTCGGAGGGGGGACCAAGTTGGAAATAA AA	22

Table 1B 5B8.2A11.2C7 Sequences		
Description	**Sequence**	**SEQ ID NO:**
VH amino acid sequence (predicted mature)	QVQLQQSDAELVKPGASVKISCKASGYTFTDHAIHWVK QKPEQGLEWIGYFSPGNGDIKYNEKFKGKATLTADRSS STANMHLNSLTSEDSAVYFCKRSMANYFDYWGQGTTLT VSS	23
VL amino acid sequence (predicted mature)	NIMMTQSPSSLVVSAGEKVTMSCKSSHSVLYSSNQKNY LAWYQQKPGQSPKLLIYWASTKNSGVPDRFTGSGSGTD FTLTISSVQAEDLAVYYCHQYLSSYTFGGGTKLEIK	24
CDR-H1 amino acid sequence (IMGT definition)	GYTFTDHA	25
CDR-H2 amino acid sequence (IMGT definition)	FSPGNGDI	26
CDR-H3 amino acid sequence (IMGT definition)	KRSMANYFDY	27
CDR-L1 amino acid sequence (IMGT definition)	HSVLYSSNQKNY	28
CDR-L2 amino acid sequence (IMGT definition)	WAS	29
CDR-L3 amino acid sequence (IMGT definition)	HQYLSSYT	30
CDR-H1 amino acid sequence (Kabat definition)	DHAIH	31
CDR-H2 amino acid sequence (Kabat definition)	YFSPGNGDIKYNEKFKG	32
CDR-H3 amino acid sequence (Kabat definition)	SMANYFDY	33
CDR-L1 amino acid sequence (Kabat definition)	KSSHSVLYSSNQKNYLA	34
CDR-L2 amino acid sequence (Kabat definition)	WASTKNS	35

Table 1B 5B8.2A11.2C7 Sequences		
Description	**Sequence**	**SEQ ID NO:**
CDR-L3 amino acid sequence (Kabat definition)	HQYLSSYT	36
CDR-H1 amino acid sequence (Chothia definition)	GYTFTDH	37
CDR-H2 amino acid sequence (Chothia definition)	SPGNGD	38
CDR-H3 amino acid sequence (Chothia definition)	SMANYFDY	39
CDR-L1 amino acid sequence (Chothia definition)	KSSHSVLYSSNQKNYLA	40
CDR-L2 amino acid sequence (Chothia definition)	WASTKNS	41
CDR-L3 amino acid sequence (Chothia definition)	HQYLSSYT	42
VH nucleotide sequence (excl. signal sequence)	CAGGTTCAGCTGCAGCAGTCTGACGCTGAGTTGGTGA AACCTGGGGCTTCAGTGAAGATATCCTGCAAGGCTTC TGGCTACACCTTCACTGACCATGCTATTCACTGGGTG AAGCAGAAGCCTGAACAGGGCCTGGAATGGATTGGA TATTTTTCTCCCGGAAATGGTGATATTAAATACAATGA GAAGTTCAAGGGCAAGGCCACACTGACTGCAGACAG ATCCTCCAGCACTGCCAACATGCACCTCAACAGCCTG ACATCTGAGGATTCTGCAGTATATTTCTGTAAAAGATC TATGGCCAACTACTTTGACTACTGGGGCCAAGGCACC ACTCTCACAGTCTCCTCA	43
VL nucleotide sequence (excl. signal sequence)	AACATTATGATGACACAGTCGCCATCATCTCTGGTTGT GTCTGCAGGAGAAAAGGTCACTATGAGCTGTAAGTCC AGTCACAGTGTTTTATACAGTTCAAATCAGAAGAACTA CTTGGCCTGGTACCAGCAGAAACCAGGGCAGTCTCCT AAACTACTGATCTACTGGGCATCCACTAAGAACTCTG GTGTCCCTGATCGCTTCACAGGCAGTGGATCTGGGAC AGATTTTACTCTTACCATCAGCAGTGTACAGGCTGAAG ACCTGGCAGTTTATTACTGTCATCAATACCTCTCCTCG TACACGTTCGGAGGGGGGACCAAGCTGGAAATAAAA	44

Table 1C 15F3.2D11.1E6 Sequences		
Description	**Sequence**	**SEQ ID NO:**
VH amino acid sequence (predicted mature)	QVQLQQSDAELVEPGASVKISCKAYGYTFTDHAIHWVK QKPEQGLEWLGYISPGNDDIQYNAKFKGRATLTADKSS STAYMQLNSLTSDDSAVYFCKRSMANSFDFWGQGTTLT VSS	45
VL amino acid sequence (predicted mature)	NIMLTQSPSSLAVSAGEKVTMSCKSSQSVLYSSDQKNYL AWYQQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTD FTLTISNVRAEDLAVYYCHQYLSSYTFGGGTKLEIK	46
CDR-H1 amino acid sequence (IMGT definition)	GYTFTDHA	47
CDR-H2 amino acid sequence (IMGT definition)	ISPGNDDI	48
CDR-H3 amino acid sequence (IMGT definition)	KRSMANSFDF	49
CDR-L1 amino acid sequence (IMGT definition)	QSVLYSSDQKNY	50
CDR-L2 amino acid sequence (IMGT definition)	WAS	51
CDR-L3 amino acid sequence (IMGT definition)	HQYLSSYT	52
CDR-H1 amino acid sequence (Kabat definition)	DHAIH	53
CDR-H2 amino acid sequence (Kabat definition)	YISPGNDDIQYNAKFKG	54
CDR-H3 amino acid sequence (Kabat definition)	SMANSFDF	55
CDR-L1 amino acid sequence (Kabat definition)	KSSQSVLYSSDQKNYLA	56
CDR-L2 amino acid sequence (Kabat definition)	WASTRES	57

Table 1C 15F3.2D11.1E6 Sequences		
Description	**Sequence**	**SEQ ID NO:**
CDR-L3 amino acid sequence (Kabat definition)	HQYLSSYT	58
CDR-H1 amino acid sequence (Chothia definition)	GYTFTDH	59
CDR-H2 amino acid sequence (Chothia definition)	SPGNDD	60
CDR-H3 amino acid sequence (Chothia definition)	SMANSFDF	61
CDR-L1 amino acid sequence (Chothia definition)	KSSQSVLYSSDQKNYLA	62
CDR-L2 amino acid sequence (Chothia definition)	WASTRES	63
CDR-L3 amino acid sequence (Chothia definition)	HQYLSSYT	64
VH nucleotide sequence (excl. signal sequence)	CAGGTTCAGTTGCAGCAATCTGACGCTGAGTTGGTGG AACCTGGGGCTTCAGTGAAGATATCCTGCAAGGCTTA TGGCTACACCTTCACTGACCATGCTATTCACTGGGTG AAGCAGAAGCCTGAACAGGGCCTGGAATGGCTTGGA TATATTTCTCCCGGAAATGATGATATTCAGTACAATGC GAAGTTCAAGGGCAGGGCCACACTGACTGCAGACAA ATCCTCCAGCACTGCCTACATGCAGCTCAACAGCCTG ACATCTGACGATTCTGCAGTGTATTTCTGTAAAAGATC TATGGCCAACTCCTTTGACTTCTGGGGCCAAGGCACC ACTCTCACAGTCTCCTCA	65
VL nucleotide sequence (excl. signal sequence)	AACATTATGTTGACACAGTCGCCATCATCTCTGGCTGT GTCTGCAGGAGAAAAGGTCACTATGAGCTGTAAGTCC AGTCAAAGTGTTTTATACAGTTCAGATCAGAAGAACTA CTTGGCCTGGTACCAGCAGAAGCCAGGGCAGTCTCC TAAACTACTGATCTATTGGGCATCCACTAGGGAATCTG GTGTCCCTGATCGCTTCACAGGCAGTGGATCTGGGAC AGATTTTACTCTTACCATCAGCAATGTACGAGCTGAAG ACCTGGCAGTTTATTACTGTCATCAATACCTCTCCTCG TACACGTTCGGAGGGGGGACCAAGCTGGAAATAAAA	66

Table 1D CDR Consensus sequences – IMGT definition		
Description	**Sequence**	**SEQ ID NO:**
CDR-H1 amino acid sequence (IMGT definition)	GYTFTDHA	67
CDR-H2 amino acid sequence (IMGT definition)	X_1SPGNX_2DI	68
CDR-H3 amino acid sequence (IMGT definition)	KRSMANX_5FDX_6	69
CDR-L1 amino acid sequence (IMGT definition)	X_7SVLYSSX_8QKNY	70
CDR-L2 amino acid sequence (IMGT definition)	WAS	71
CDR-L3 amino acid sequence (IMGT definition)	HQYLSSYT	72
X_1 = I or F; X_2 = D or G; X_5 = S or Y; X_6 = Y or F; X_7 = Q or H; X_8 = D or N		

Table 1E CDR Consensus sequences – Kabat definition		
Description	**Sequence**	**SEQ ID NO:**
CDR-H1 amino acid sequence (Kabat definition)	DHAIH	73
CDR-H2 amino acid sequence (Kabat definition)	YX_1SPGNX_2DIX_3YNX_4KFKG	74
CDR-H3 amino acid sequence (Kabat definition)	SMANX_5FDX_6	75
CDR-L1 amino acid sequence (Kabat definition)	KSSX_7SVLYSSX_8QKNYLA	76
CDR-L2 amino acid sequence (Kabat definition)	WAST$X_9$$X_{10}$S	77
CDR-L3 amino acid sequence (Kabat definition)	HQYLSSYT	78
X_1 = I or F; X_2 = D or G; X_3 = Q or K; X_4 = A or E; X_5 = S or Y; X_6 = Y or F; X_7 = Q or H; X_8 = D or N; X_9 = R or K; X_{10} = E or N		

Table 1F CDR Consensus sequences – Chothia definition		
Description	**Sequence**	**SEQ ID NO:**
CDR-H1 amino acid sequence (Chothia definition)	GYTFTDH	79
CDR-H2 amino acid sequence (Chothia definition)	SPGNX_2D	80
CDR-H3 amino acid sequence (Chothia definition)	SMANX_5FDX_6	81
CDR-L1 amino acid sequence (Chothia definition)	KSSX_7SVLYSSX_8QKNYLA	82
CDR-L2 amino acid sequence (Chothia definition)	WAST$X_9$$X_{10}$S	83
CDR-L3 amino acid sequence (Chothia definition)	HQYLSSYT	84
X_2 = D or G; X_5 = S or Y; X_6 = Y or F; X_7 = Q or H; X_8 = D or N; X_9 = R or K; X_{10} = E or N		

Table 2A 2D5.2F6.2C11 IMGT, Kabat, and Chothia CDR combined overlap sequences		
Description	**Sequence**	**SEQ ID NO:**
CDR-H1 amino acid sequence (combined overlap)	GYTFTDHAIH (IMGT) GYTFTDHAIH (Kabat) GYTFTDHAIH (Chothia)	85
CDR-H2 amino acid sequence (combined overlap)	YISPGNDDIQYNAKFKG (IMGT) YISPGNDDIQYNAKFKG (Kabat) YISPGNDDIQYNAKFKG (Chothia)	86
CDR-H3 amino acid sequence (combined overlap)	KRSMANSFDY (IMGT) KRSMANSFDY (Kabat) KRSMANSFDY (Chothia)	87
CDR-L1 amino acid sequence (combined overlap)	KSSQSVLYSSDQKNYLA (IMGT) KSSQSVLYSSDQKNYLA (Kabat) KSSQSVLYSSDQKNYLA (Chothia)	88
CDR-L2 amino acid sequence (combined overlap)	WASTRES (IMGT) WASTRES (Kabat) WASTRES (Chothia)	89
CDR-L3 amino acid sequence (combined overlap)	HQYLSSYT (IMGT) HQYLSSYT (Kabat) HQYLSSYT (Chothia)	90

Table 2B 5B8.2A11.2C7 IMGT, Kabat, and Chothia CDR combined overlap sequences		
Description	**Sequence**	**SEQ ID NO:**
CDR-H1 amino acid sequence (combined overlap)	GYTFTDHAIH (IMGT) GYTFTDHAIH (Kabat) GYTFTDHAIH (Chothia)	91

Table 2B 5B8.2A11.2C7 IMGT, Kabat, and Chothia CDR combined overlap sequences		
Description	**Sequence**	**SEQ ID NO:**
CDR-H2 amino acid sequence (combined overlap)	Y**FSPGNGDI**KYNEKFKG (IMGT) **YFSPGNGDIKYNEKFKG** (Kabat) YF**SPGNGD**IKYNEKFKG (Chothia)	92
CDR-H3 amino acid sequence (combined overlap)	**KRSMANYFDY** (IMGT) KR**SMANYFDY** (Kabat) KR**SMANYFDY** (Chothia)	93
CDR-L1 amino acid sequence (combined overlap)	KSS**HSVLYSSNQKNY**LA (IMGT) **KSSHSVLYSSNQKNYLA** (Kabat) **KSSHSVLYSSNQKNYLA** (Chothia)	94
CDR-L2 amino acid sequence (combined overlap)	**WAS**TKNS (IMGT) **WASTKNS** (Kabat) **WASTKNS** (Chothia)	95
CDR-L3 amino acid sequence (combined overlap)	**HQYLSSYT** (IMGT) **HQYLSSYT** (Kabat) **HQYLSSYT** (Chothia)	96

Table 2C 15F3.2D11.1E6 IMGT, Kabat, and Chothia CDR combined overlap sequences		
Description	**Sequence**	**SEQ ID NO:**
CDR-H1 amino acid sequence (combined overlap)	**GYTFTDHA**IH (IMGT) GYTFT**DHAIH** (Kabat) **GYTFTDH**AIH (Chothia)	97
CDR-H2 amino acid sequence (combined overlap)	Y**ISPGNDDI**QYNAKFKG (IMGT) **YISPGNDDIQYNAKFKG** (Kabat) YI**SPGNDD**IQYNAKFKG (Chothia)	98
CDR-H3 amino acid sequence (combined overlap)	**KRSMANSFDF** (IMGT) KR**SMANSFDF** (Kabat) KR**SMANSFDF** (Chothia)	99

Table 2C 15F3.2D11.1E6 IMGT, Kabat, and Chothia CDR combined overlap sequences		
Description	**Sequence**	**SEQ ID NO:**
CDR-L1 amino acid sequence (combined overlap)	KSSQSVLYSSDQKNYLA (IMGT) KSSQSVLYSSDQKNYLA (Kabat) KSSQSVLYSSDQKNYLA (Chothia)	100
CDR-L2 amino acid sequence (combined overlap)	WASTRES (IMGT) WASTRES (Kabat) WASTRES (Chothia)	101
CDR-L3 amino acid sequence (combined overlap)	HQYLSSYT (IMGT) HQYLSSYT (Kabat) HQYLSSYT (Chothia)	102

Table 2D Consensus IMGT, Kabat, and Chothia CDR combined overlap sequences		
Description	**Sequence**	**SEQ ID NO:**
CDR-H1 amino acid sequence (combined overlap)	GYTFTDHAIH	103
CDR-H2 amino acid sequence (combined overlap)	$YX_1SPGNX_2DIX_3YNX_4KFKG$	104
CDR-H3 amino acid sequence (combined overlap)	$KRSMANX_5FDX_6$	105
CDR-L1 amino acid sequence (combined overlap)	$KSSX_7SVLYSSX_8QKNYLA$	106
CDR-L2 amino acid sequence (combined overlap)	$WASTX_9X_{10}S$	107
CDR-L3 amino acid sequence (combined overlap)	HQYLSSYT	108
X_1 = I or F; X_2 = D or G; X_3 = Q or K; X_4 = A or E; X_5 = S or Y; X_6 = Y or F; X_7 = Q or H; X_8 = D or N; X_9 = R or K; X_{10} = E or N		

Table 3A 2D5.2F6.2C11 IMGT, Kabat, and Chothia CDR common sequences		
Description	**Sequence**	**SEQ ID NO:**
CDR-H1 amino acid sequence (common sequence)	DH	109
CDR-H2 amino acid sequence (common sequence)	SPGNDD	110
CDR-H3 amino acid sequence (common sequence)	SMANSFDY	111
CDR-L1 amino acid sequence (common sequence)	QSVLYSSDQKNY	112
CDR-L2 amino acid sequence (common sequence)	WAS	113
CDR-L3 amino acid sequence (common sequence)	HQYLSSYT	114

Table 3B 5B8.2A11.2C7 IMGT, Kabat, and Chothia CDR common sequences		
Description	**Sequence**	**SEQ ID NO:**
CDR-H1 amino acid sequence (common sequence)	DH	115
CDR-H2 amino acid sequence (common sequence)	SPGNGD	116
CDR-H3 amino acid sequence (common sequence)	SMANYFDY	117
CDR-L1 amino acid sequence (common sequence)	HSVLYSSNQKNY	118
CDR-L2 amino acid sequence (common sequence)	WAS	119
CDR-L3 amino acid sequence (common sequence)	HQYLSSYT	120

Table 3C 15F3.2D11.1E6 IMGT, Kabat, and Chothia CDR common sequences		
Description	**Sequence**	**SEQ ID NO:**
CDR-H1 amino acid sequence (common sequence)	DH	121
CDR-H2 amino acid sequence (common sequence)	SPGNDD	122
CDR-H3 amino acid sequence (common sequence)	SMANSFDF	123
CDR-L1 amino acid sequence (common sequence)	QSVLYSSDQKNY	124
CDR-L2 amino acid sequence (common sequence)	WAS	125
CDR-L3 amino acid sequence (common sequence)	HQYLSSYT	126

Table 3D Consensus CDR common sequences		
Description	**Sequence**	**SEQ ID NO:**
CDR-H1 amino acid sequence (common sequence)	DH	127
CDR-H2 amino acid sequence (common sequence)	SPGNX_2	128
CDR-H3 amino acid sequence (common sequence)	SMANX_5FDX_6	129
CDR-L1 amino acid sequence (common sequence)	X_7SVLYSSX_8QKNY	130
CDR-L2 amino acid sequence (common sequence)	WAS	131
CDR-L3 amino acid sequence (common sequence)	HQYLSSYT	132
X_2 = D or G; X_5 = S or Y; X_6 = Y or F; X_7 = Q or H; X_8 = D or N		

Table 4A Humanized 2D5 Heavy Chain Sequences – Germline 4-1		
Description	**Sequence**	**SEQ ID NO:**
2D5-HV7-4-1-A	QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWV RQAPGQGLEWLGYISPGNDDIQYNAKFKGRAVLSADK SVSTAYLQISSLKAEDTAVYFCKRSMANSFDYWGQGT LVTVSS	133
2D5-HV7-4-1-B	QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWV RQAPGQGLEWLGYISTGNDDIQYNQKFTGRAVLSLDK SVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGT LVTVSS	134

Table 4A Humanized 2D5 Heavy Chain Sequences – Germline 4-1		
Description	**Sequence**	**SEQ ID NO:**
2D5-HV7-4-1-C	QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWV RQAPGQGLEWLGYISTGNANITYAQGFTGRAVLSLDK SVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGT LVTVSS	135

Table 4B Humanized 2D5 Heavy Chain Sequences – Germline 78		
Description	**Sequence**	**SEQ ID NO:**
2D5-HV5-78-A	EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVR QMPGKELEWLGYISPGNDDIQYNAKFKGHATLSADKS SSTAYLQWSSLKASDAAMYFCKRSMANSFDYWGQGT LVTVSS	136
2D5-HV5-78-B	EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVR QMPGKELEWLGYISPGNDDIRYNAKFKGHVTISADKSS STAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTL VTVSS	137
2D5-HV5-78-C	EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVR QMPGKELEWLGYISPGNADTRYSASFQGHVTISADKS SSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGT LVTVSS	138

Table 4C Humanized 2D5 Heavy Chain Sequences – Germline 69		
Description	**Sequence**	**SEQ ID NO:**
2D5-HV1-69-A	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWV RQAPGQGLEWLGYISPGNDDIQYNAKFKGRATLTADK STSTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGT LVTVSS	139
2D5-HV1-69-B	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWV RQAPGQGLEWLGYISPGNDDIQYNQKFKGRVTITADK STSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQG TLVTVSS	140
2D5-HV1-69-C	QVQLVQSGAEVKKPGSSVKVSCKASGYTFSDHAIHWV RQAPGQGLEWLGYISPGNADINYAQKFQGRVTITADKS TSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTL VTVSS	141

Table 4D Humanized 2D5 Heavy Chain Sequences – Germline 3		
Description	**Sequence**	**SEQ ID NO:**
2D5-HV1-3-A	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWV RQAPGQRLEWLGYISPGNDDIQYNAKFKGRATLTADK SASTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQG TLVTVSS	142
2D5-HV1-3-B	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWV RQAPGQRLEWLGYISPGNDDIQYSQKFKGRVTITADKS ASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTL VTVSS	143
2D5-HV1-3-C	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWV RQAPGQRLEWLGYISPGNADTQYSQKFQGRVTITADK	144

Table 4D Humanized 2D5 Heavy Chain Sequences – Germline 3		
Description	**Sequence**	**SEQ ID NO:**
	SASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQG TLVTVSS	

Table 4E Humanized 2D5 Light Chain Sequences – Germline 1		
Description	**Sequence**	**SEQ ID NO:**
2D5-KV4-1-A	DIVLTQSPDSLAVSLGERATINCKSSQSVLYSSDQKNYL AWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGT DFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK	145
2D5-KV4-1-B	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNLRNY LAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGT DFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK	146
2D5-KV4-1-C	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNERNY LAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGT DFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK	147

Table 4F Humanized 2D5 Light Chain Sequences – Germline 20		
Description	**Sequence**	**SEQ ID NO:**
2D5-KV3-20-A	EIVLTQSPGTLSLSPGERATLSCKSSQSVLYSSDQKNY LAWYQQKPGQAPRLLIYWASTRESGIPDRFSGSGSGT DFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK	148
2D5-KV3-20-B	EIVLTQSPGTLSLSPGERATLSCRSSQSVLYSSDQKSY LAWYQQKPGQAPRLLIYWASTRATGIPDRFSGSGSGT DFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK	149
2D5-KV3-20-C	EIVLTQSPGTLSLSPGERATLSCRASQSVSYSSDQKSY LAWYQQKPGQAPRLLIYWASSRATGIPDRFSGSGSGT DFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK	150

Table 4G Humanized 2D5 Light Chain Sequences – Germline 40		
Description	**Sequence**	**SEQ ID NO:**
2D5-KV2-40-A	DIVLTQTPLSLPVTPGEPASISCKSSQSVLYSSDQKNYL AWYLQKPGQSPQLLIYWASTRESGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK	151
2D5-KV2-40-B	DIVMTQTPLSLPVTPGEPASISCRSSQSVLYSSDEKTYL AWYLQKPGQSPQLLIYWASTRESGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK	152
2D5-KV2-40-C	DIVMTQTPLSLPVTPGEPASISCRSSQSLLYSSDERTYL AWYLQKPGQSPQLLIYWASTRASGVPDRFSGSGSGT DFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK	153

[0013] In certain aspects, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure comprises CDRs comprising the amino acid sequences of any of the CDR combinations set forth in Tables 1-3. In certain embodiments, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:127, a CDR-H2 comprising the amino acid sequence of SEQ ID

NO:128, a CDR-H3 comprising the amino acid sequence of SEQ ID NO:129, a CDR-L1 comprising the amino acid sequence of SEQ ID NO:130, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:131, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:132. In some embodiments, CDR-H1 comprises the amino acid sequence of SEQ ID NO:127. In some embodiments, CDR-H2 comprises the amino acid sequence of SEQ ID NO:128. In some embodiments, CDR-H3 comprises the amino acid sequence of SEQ ID NO:129. In some embodiments, CDR-L1 comprises the amino acid sequence of SEQ ID NO:130. In some embodiments, CDR-L2 comprises the amino acid sequence of SEQ ID NO:131. In some embodiments, CDR-L3 comprises the amino acid sequence of SEQ ID NO:132.

[0014] In other aspects, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure comprises heavy chain CDRs of SEQ ID NOS:3-5 and light chain CDRs of SEQ ID NOS:6-8. In other aspects, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure comprises heavy chain CDRs of SEQ ID NOS:9-11 and light chain CDRs of SEQ ID NOS:12-14. In other aspects, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure comprises heavy chain CDRs of SEQ ID NOS:15-17 and light chain CDRs of SEQ ID NOS:18-20. In other aspects, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure comprises heavy chain CDRs of SEQ ID NOS:85-87 and light chain CDRs of SEQ ID NOS:88-90.

[0015] In other aspects, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure comprises heavy chain CDRs of SEQ ID NOS:25-27 and light chain CDRs of SEQ ID NOS:28-30. In other aspects, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure comprises heavy chain CDRs of SEQ ID NOS:31-33 and light chain CDRs of SEQ ID NOS:32-34. In other aspects, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure comprises heavy chain CDRs of SEQ ID NOS:35-37 and light chain CDRs of SEQ ID NOS:38-40. In other aspects, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure comprises heavy chain CDRs of SEQ ID NOS:91-93 and light chain CDRs of SEQ ID NOS:94-96.

[0016] In other aspects, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure comprises heavy chain CDRs of SEQ ID NOS:47-49 and light chain CDRs of SEQ ID NOS:50-52. In other aspects, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure comprises heavy chain CDRs of SEQ ID NOS:53-55 and light chain CDRs of SEQ ID NOS:56-58. In other aspects, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure comprises heavy chain CDRs of SEQ ID NOS:59-61 and light chain CDRs of SEQ ID NOS:62-64. In other aspects, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure comprises heavy chain CDRs of SEQ ID NOS:97-99 and light chain CDRs of SEQ ID NOS:100-102.

[0017] In other aspects, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure comprises heavy chain CDRs of SEQ ID NOS:67-69 and light chain CDRs of SEQ ID NOS:70-72. In other aspects, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure comprises heavy chain CDRs of SEQ ID NOS:73-75 and light chain CDRs of SEQ ID NOS:76-78. In other aspects, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure comprises heavy chain CDRs of SEQ ID NOS:79-81 and light chain CDRs of SEQ ID NOS:82-84. In other aspects, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure comprises heavy chain CDRs of SEQ ID NOS:103-105 and light chain CDRs of SEQ ID NOS:106-108.

[0018] In certain embodiments, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO:67, 73, 79, 85, 91, 97, 103, 109, 115, 121, or 127; a CDR-H2 comprising the amino acid sequence of SEQ ID NO:68, 74, 80, 86, 92, 98, 104, 110, 116, 122, or 128; a CDR-H3 comprising the amino acid sequence of SEQ ID NO:69, 75, 81, 87, 93, 99, 105, 111, 117, 123, or 129; a CDR-L1 comprising the amino acid sequence of SEQ ID NO:70, 76, 82, 88, 94, 100, 106, 112, 118, 124, or 130; a CDR-L2 comprising the amino acid sequence of SEQ ID NO:71, 77, 83, 89, 95, 101, 107, 113, 119, 125, or 131; and a CDR-L3 comprising the amino acid sequence of SEQ ID NO:72, 78, 84, 90, 96, 102, 108, 114, 120, 126, or 132.

[0019] The antibodies and antigen-binding fragments of the disclosure can be murine, chimeric, humanized or human.

[0020] In further aspects, an anti-glyco-MUC4 antibody or antigen binding fragment of the disclosure competes with an antibody or antigen binding fragment comprising heavy and light chain variable regions of SEQ ID NOS:1 and 2, respectively. In yet other aspects, the disclosure provides an anti-MUC4 antibody or antigen binding fragment having heavy and light chain variable regions having at least 95%, 98%, 99%, or 99.5% sequence identity of SEQ ID NOS:1 and 2, respectively.

[0021] In yet other aspects, an anti-glyco-MUC4 antibody or antigen binding fragment of the disclosure competes with an antibody or antigen binding fragment comprising heavy and light chain variable regions of SEQ ID NOS:23 and 24, respectively. In yet other aspects, the disclosure provides an anti-MUC4 antibody or antigen binding fragment having heavy and light chain variable regions having at least 95%, 98%, 99%, or 99.5% sequence identity of SEQ ID NOS:23 and 24, respectively.

[0022] In yet other aspects, an anti-glyco-MUC4 antibody or antigen binding fragment of the disclosure competes with an antibody or antigen binding fragment comprising heavy and light chain variable regions of SEQ ID NOS:45 and 46, respectively. In yet other aspects, the disclosure provides an anti-MUC4 antibody or antigen binding fragment having heavy and light

chain variable regions having at least 95%, 98%, 99%, or 99.5% sequence identity of SEQ ID NOS:45 and 46, respectively.

[0023] In yet other aspects, an anti-glyco-MUC4 antibody or antigen binding fragment of the disclosure competes with an antibody or antigen binding fragment comprising a heavy chain variable region of any one of SEQ ID NOS:133-144 and a light chain variable region of any one of SEQ ID NOS:145-153. In yet other aspects, the disclosure provides an anti-MUC4 antibody or antigen binding fragment having a heavy variable region having at least 95%, 98%, 99%, or 99.5% sequence identity of any one of SEQ ID NOS:133-134 and a light variable region having at least 95%, 98%, 99%, or 99.5% sequence identity of any one of SEQ ID NOS:145-153.

[0024] In yet other aspects, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure is a single-chain variable fragment (scFv). An exemplary scFv comprises the heavy chain variable fragment N-terminal to the light chain variable fragment. In some embodiments, the scFv heavy chain variable fragment and light chain variable fragment are covalently bound to a linker sequence of 4-15 amino acids. The scFv can be in the form of a bi-specific T-cell engager or within a chimeric antigen receptor (CAR).

[0025] The anti-glyco-MUC4 antibodies and antigen-binding fragments can be in the form of a multimer of a single-chain variable fragment, a bispecific single-chain variable fragment and a multimer of a bispecific single-chain variable fragment. In some embodiments, the multimer of a single chain variable fragment is selected a divalent single-chain variable fragment, a tribody or a tetrabody. In some of these embodiments, the multimer of a bispecific single-chain variable fragment is a bispecific T-cell engager.

[0026] Other aspects of the disclosure are drawn to nucleic acids encoding the anti-glyco-MUC4 antibodies and antibody-binding fragments of the disclosure. In some embodiments, the portion of the nucleic acid nucleic acid encoding an anti-glyco-MUC4 antibody or antigen-binding fragment is codon-optimized for expression in a human cell. In certain aspects, the disclosure provides an anti-glyco-MUC4 antibody or antigen binding fragment having heavy and light chain variable regions encoded by a heavy chain nucleotide sequence having at least 95%, 98%, 99%, or 99.5% sequence identity to SEQ ID NO:21, 43, or 65 and a light chain nucleotide sequence having at least 95%, 98%, 99%, or 99.5% sequence identity to SEQ ID NO:22, 44 or 66. Vectors (*e.g.*, a viral vector such as a lentiviral vector) and host cells comprising the nucleic acids are also within the scope of the disclosure. The heavy and light chains coding sequences can be present on a single vector or on separate vectors.

[0027] Yet another aspect of the disclosure is a pharmaceutical composition comprising an anti-glyco-MUC4 antibody, antigen-binding fragment, nucleic acid (or pair of nucleic acids), vector (or pair of vectors) or host cell according to the disclosure, and a physiologically suitable buffer, adjuvant or diluent.

[0028] Still another aspect of the disclosure is a method of making a chimeric antigen receptor comprising incubating a cell comprising a nucleic acid or a vector according to the disclosure, under conditions suitable for expression of the coding region and collecting the chimeric antigen receptor.

[0029] Another aspect of the disclosure is a method of detecting cancer comprising contacting a biological sample (*e.g.*, a cell, tissue sample, or extracellular vesicle) with an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure and detecting whether the antibody is bound to the biological sample (*e.g.*, cell, tissue sample, or extracellular vesicle).

[0030] Yet another aspect of the disclosure is an anti-glyco-MUC4 antibody or antigen-binding fragment according to the disclosure of the disclosure for use in detecting cancer.

[0031] Yet another aspect of the disclosure is a method of treating cancer comprising administering a prophylactically or therapeutically effective amount of an anti-glyco-MUC4 antibody, antigen-binding fragment, nucleic acid, vector, host cell or pharmaceutical composition according to the disclosure to a subject in need thereof.

[0032] Yet another aspect of the disclosure is an anti-glyco-MUC4 antibody, antigen-binding fragment, nucleic acid, vector, host cell or pharmaceutical composition according to the disclosure for use in the treatment of cancer.

[0033] Yet another aspect of the disclosure is use of an anti-glyco-MUC4 antibody, antigen-binding fragment, nucleic acid, vector, host cell or pharmaceutical composition according to the disclosure for the manufacture of a medicament for the treatment of cancer.

[0034] Glyco-MUC4 peptides are also provided herein. The peptides can be 13-30 amino acids in length and comprise amino acids 4-16 of SEQ ID NO:154 (CTIPSTAMHTR**ST**AAPIPILP), glycosylated with GalNAc on the serine and threonine residues shown in bold underlined text). The glyco-MUC4 peptides are describe in Section 5.10 and numbered embodiments 653 to 665. The peptides can be included in a composition, as described in Section 5.10.1 and numbered embodiments 666 and 667. The glyco-MUC4 peptides can be used in methods for producing antibodies in an animal and/or eliciting an immune response in an animal. Methods for using the glyco-MUC4 peptides are described in Section 5.10.2 and numbered embodiments 668 to 671.

4. BRIEF DESCRIPTION OF THE FIGURES

[0035] FIGS. 1A-1E: Flow cytometry analysis of MUC4 mouse antibodies on T3M4 COSMC-KO and T3M4 cells. FIG. 1A shows representative histograms for staining of 2D5.2F6.2C11, 5B8.2A11.2C7, 15F3.2D11.1E6, anti-Golgi, and mouse IgG isotype control on T3M4 COSMC-KO and T3M4 cells. FIG. 1B-D shows results of titration of 2D5.2F6.2C11 (FIG. 1C), 5B8.2A11.2C7 (FIG. 1D), and 15F3.2D11.1E6 (FIG 1E) on cell surface antigens found on T3M4 COSMC-KO and T3M4 cells. FIG. 1B shows an overlay of FIGS. 1C-1E.

[0036] FIG. 2: Immunofluorescence staining of 2D5.2F6.2C11, 5B8.2A11.2C7, 15F3.2D11.1E6, anti-MUC4 monoclonal antibody 1G8 (ThermoFisher Scientific) that indiscriminately binds MUC4 regardless of glycosylation status, and an anti-Tn antibody on T3M4 COSMC-KO and T3M4 cells.

[0037] FIGS. 3A-3G: Immunohistochemistry of MUC4 mouse antibodies. FIG. 3A shows staining of 2D5.2F6.2C11, 5B8.2A11.2C7, and 15F3.2D11.1E6 antibodies on pancreatic cancer and normal tissues. FIG. 3B shows statistics of positive and negative stained tissues. FIGS. 3C-3E shows staining of 2D5.2F6.2C11 antibody on FDA normal tissue microarray. FIGS. 3F-3G shows staining of 2D5.2F6.2C11 on multiple cancer microarray. Rectum, ovarian and pancreatic cancer tissues were positive.

[0038] FIGS. 4A-4C: Exemplary MUC4 CART constructs. FIG. 4A: 2D5-CART; FIG. 4B:15F3-CART; FIG. 4C:5B8-CART. Testing of the constructs is described in Example 5.

[0039] FIGS. 5A-5B: Cell killing assay of MUC4 CARTs on T3M4 COSMC-KO and T3M4 cells, showing killing by MUC4 CARTs (2D5.2F6.2C11 (FIG. 5A) and 5B8.2A11.2C7 (FIG. 5B)) on T3M4 COSMC-KO and T3M4 target cells with a titration of ratios of T cells to target cells (1, 5, and 10).

[0040] FIG. 6: In vivo activity of 2D5-CART in solid tumor mouse models. T3M4 COSMC-KO solid tumor model established by flank injection in an immunocompromised mouse (cell line derived tumor xenograft (CDX)) model. The tumor volume at injection was 200 mm3 and Mice were treated with 2nd generation 2D5-CAR-T by IV injection (2 doses at 10^7 cells). Tumor volume was measured by caliper.

[0041] FIGS. 7: Exemplary MUC4 TCB (CrossMab) constructs. Testing of the constructs is described in Example 6.

[0042] FIG. 8: Cytotoxicity of CrossMab (2x1) TCB-2D5.2F6.2C11 on HaCaTs (CrossMAb 2x1).

[0043] FIGS. 9A-9B: Cytotoxicity of CrossMab (2x1) TCB-2D5.2F6.2C11 on MCF7 (FIG. 9A) and HCT116 (FIG. 9B).

[0044] FIG. 10: *In vivo* activity of 2D5-TCB in solid tumor mouse models. Lung cancer solid tumor model (patient derived tumor xenograft mouse model (PDX) established by flank injection (Champions model CTG-2823). The tumor volume at TCB injection was 200 mm3 and TCB was delivered by IV injection. PBMCs were injected at day 0 and TCB dosed at Day 0, 1, 2, 3, 4. PBMCs were also injected at day 17 and TCB dosed at day 20, Day 22. Tumor volume was measured by caliper.

5. DETAILED DESCRIPTION

5.1 Antibodies

[0045] The disclosure provides novel antibodies that are directed to a glycoform of MUC4 present on tumor cells. These are exemplified by the antibodies 2D5.2F6.2C11 (hereinafter, "2D5"), 5B8.2A11.2C7 (hereinafter, "5B8"), and 15F3.2D11.1E6 (hereinafter, "15F3"). 2D5, 5B8, and 15F3 were identified in a screen for antibodies that bind to a glycosylated peptide present in MUC4 CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154), glycosylated with GalNAc on the serine and threonine residues shown in bold underlined text so as to mimic the glycosylation pattern of MUC4 present on tumor cells.

[0046] The anti-glyco-MUC4 antibodies of the disclosure, exemplified by antibodies 2D5, 5B8, and 15F3, are useful as tools in cancer diagnosis and therapy.

[0047] Thus, in certain aspects, the disclosure provides antibodies and antigen binding fragments that bind to a glycoform of MUC4 present on tumor cells (referred to herein as "glyco-MUC4"), and preferably to the peptide CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) glycosylated with GalNAc on the serine and threonine residues shown in bold underlined text.

[0048] The anti-glyco-MUC4 antibodies of the disclosure may be polyclonal, monoclonal, genetically engineered, and/or otherwise modified in nature, including but not limited to chimeric antibodies, humanized antibodies, human antibodies, primatized antibodies, single chain antibodies, bispecific antibodies, dual-variable domain antibodies, *etc.* In various embodiments, the antibodies comprise all or a portion of a constant region of an antibody. In some embodiments, the constant region is an isotype selected from: IgA (*e.g.*, IgA_1 or IgA_2), IgD, IgE, IgG (*e.g.*, IgG_1, IgG_2, IgG_3 or IgG_4), and IgM. In specific embodiments, the anti-glyco-MUC4 antibodies of the disclosure comprise an IgG_1 constant region isotype.

[0049] The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. A monoclonal antibody is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, by any means available or known in the art. Monoclonal antibodies useful with the present disclosure can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. In many uses of the present disclosure, including *in vivo* use of the anti-glyco-MUC4 antibodies in humans, chimeric, primatized, humanized, or human antibodies can suitably be used.

[0050] The term "chimeric" antibody as used herein refers to an antibody having variable sequences derived from a non-human immunoglobulin, such as a rat or a mouse antibody, and human immunoglobulin constant regions, typically chosen from a human immunoglobulin template. Methods for producing chimeric antibodies are known in the art. See, *e.g.*, Morrison, 1985, Science 229(4719):1202-7; Oi *et al.*, 1986, BioTechniques 4:214-221; Gillies *et al.*, 1985,

J. Immunol. Methods 125:191-202; U.S. Pat. Nos. 5,807,715; 4,816,567; and 4,816397, which are incorporated herein by reference in their entireties.

[0051] "Humanized" forms of non-human (*e.g.*, murine) antibodies are chimeric immunoglobulins that contain minimal sequences derived from non-human immunoglobulin. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody can also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin consensus sequence. Methods of antibody humanization are known in the art. See, *e.g.*, Riechmann *et al.*, 1988, Nature 332:323-7; U.S. Pat. Nos. 5,530,101; 5,585,089; 5,693,761; 5,693,762; and 6,180,370 to Queen *et al.*; EP239400; PCT publication WO 91/09967; U.S. Pat. No. 5,225,539; EP592106; EP519596; Padlan, 1991, Mol. Immunol., 28:489-498; Studnicka *et al.*, 1994, Prot. Eng. 7:805-814; Roguska *et al.*, 1994, Proc. Natl. Acad. Sci. 91:969-973; and U.S. Pat. No. 5,565,332, all of which are hereby incorporated by reference in their entireties.

[0052] Exemplary humanized sequences are described in numbered embodiments 5 to 112. The variable region sequences for humanized antibodies and antigen-binding fragments thereof are set forth in Tables 4A-4G.

[0053] "Human antibodies" include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins. Human antibodies can be made by a variety of methods known in the art including phage display methods using antibody libraries derived from human immunoglobulin sequences. See U.S. Pat. Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645; WO 98/50433; WO 98/24893; WO 98/16654; WO 96/34096; WO 96/33735; and WO 91/10741, each of which is incorporated herein by reference in its entirety. Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. See, *e.g.*, PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; U.S. Pat. Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entireties. Fully human antibodies that recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach, a selected non-human monoclonal antibody, *e.g.*, a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope (see, Jespers *et al.*, 1988, Biotechnology 12:899-903).

[0054] "Primatized antibodies" comprise monkey variable regions and human constant regions. Methods for producing primatized antibodies are known in the art. See, *e.g.*, U.S. Pat. Nos. 5,658,570; 5,681,722; and 5,693,780, which are incorporated herein by reference in their entireties.

[0055] Anti-glyco-MUC4 antibodies of the disclosure include both full-length (intact) antibody molecules, as well as antigen-binding fragments that are capable of binding glyco-MUC4. Examples of antigen-binding fragments include by way of example and not limitation, Fab, Fab', F $(ab')_2$, Fv fragments, single chain Fv fragments and single domain fragments.

[0056] A Fab fragment contains the constant domain of the light chain (CL) and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. F(ab') fragments are produced by cleavage of the disulfide bond at the hinge cysteines of the $F(ab')_2$ pepsin digestion product. Additional chemical couplings of antibody fragments are known to those of ordinary skill in the art. Fab and $F(ab')_1$ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation of animals, and may have less non-specific tissue binding than an intact antibody (see, *e.g.*, Wahl *et al.*, 1983, J. Nucl. Med. 24:316).

[0057] An "Fv" fragment is the minimum fragment of an antibody that contains a complete target recognition and binding site. This region consists of a dimer of one heavy and one light chain variable domain in a tight, non-covalent association (V_H-V_L dimer). It is in this configuration that the three CDRs of each variable domain interact to define a target binding site on the surface of the V_H-V_L dimer. Often, the six CDRs confer target binding specificity to the antibody. However, in some instances even a single variable domain (or half of an Fv comprising only three CDRs specific for a target) can have the ability to recognize and bind target, although at a lower affinity than the entire binding site.

[0058] "Single-chain Fv" or "scFv" antigen-binding fragments comprise the V_H and V_L domains of an antibody, where these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the scFv to form the desired structure for target binding.

[0059] "Single domain antibodies" are composed of single V_H or V_L domains which exhibit sufficient affinity to glyco-MUC4. In a specific embodiment, the single domain antibody is a camelized antibody (see, *e.g.*, Riechmann, 1999, Journal of Immunological Methods 231:25-38).

[0060] The anti-glyco-MUC4 antibodies of the disclosure may also be bispecific and other multiple specific antibodies. Bispecific antibodies are monoclonal, often human or humanized, antibodies that have binding specificities for two different epitopes on the same or different

antigen. In the present disclosure, one of the binding specificities can be directed towards glyco-MUC4, the other can be for any other antigen, *e.g.*, for a cell-surface protein, receptor, receptor subunit, tissue-specific antigen, virally derived protein, virally encoded envelope protein, bacterially derived protein, or bacterial surface protein, *etc.* In certain embodiments, the bispecific and other multispecific anti-glyco-MUC4 antibodies and antigen binding fragments specifically bind to a second MUC4 epitope, an epitope on another protein co-expressed on cancer cells with MUC4, or an epitope on another protein presented on a different cell, such as an activated T cell. Bispecific antibodies of the disclosure include IgG format bispecific antibodies and single chain-based bispecific antibodies.

[0061] IgG format bispecific antibodies of the disclosure can be any of the various types of IgG format bispecific antibodies known in the art, such as quadroma bispecific antibodies, "knobs-in-holes" bispecific antibodies, CrossMab bispecific antibodies (*i.e.*, bispecific domain-exchanged antibodies), charge paired bispecific antibodies, common light chain bispecific antibodies, one-arm single-chain Fab-immunoglobulin gamma bispecific antibodies, disulfide stabilized Fv bispecific antibodies, DuetMabs, controlled Fab-arm exchange bispecific antibodies, strand-exchange engineered domain body bispecific antibodies, two-arm leucine zipper heterodimeric monoclonal bispecific antibodies, κλ-body bispecific antibodies, dual variable domain bispecific antibodies, and cross-over dual variable domain bispecific antibodies. See, *e.g.*, Köhler and Milstein, 1975, Nature 256:495-497; Milstein and Cuello, 1983, Nature 305:537-40; Ridgway *et al.*, 1996, Protein Eng. 9:617-621; Schaefer *et al.*, 2011, Proc Natl Acad Sci USA 108:11187-92; Gunasekaran et al., 2010, J Biol Chem 285:19637-46; Fischer *et al.*, 2015 Nature Commun 6:6113; Schanzer *et al.*, 2014, J Biol Chem 289:18693–706; Metz *et al.*, 2012 Protein Eng Des Sel 25:571–80; Mazor *et al.*, 2015 MAbs 7:377–89; Labrijn *et al.*, 2013 Proc Natl Acad Sci USA 110:5145–50; Davis *et al.*, 2010 Protein Eng Des Sel 23:195–202; Wranik *et al.*, 2012, J Biol Chem 287:43331–9; Gu et al., 2015, PLoS One 10(5):e0124135; Steinmetz et al., 2016, MAbs 8(5):867-78; Klein *et al.*, 2016, mAbs, 8(6):1010-1020; Liu *et al.*, 2017, Front. Immunol. 8:38; and Yang *et al.*, 2017, Int. J. Mol. Sci. 18:48, which are incorporated herein by reference in their entireties.

[0062] In some embodiments, the bispecific antibodies of the disclosure are domain exchanged antibodies referred to in the scientific and patent literature as CrossMabs. See, *e.g.*, Schaefer *et al.*, 2011, Proc Natl Acad Sci USA 108:11187-92. The CrossMab technology is described in detail in WO 2009/080251, WO 2009/080252, WO 2009/080253, WO 2009/080254, WO 2013/026833, WO 2016/020309, and Schaefer *et al.*, 2011, Proc Natl Acad Sci USA 108:11187-92, which are incorporated herein by reference in their entireties. Briefly, the CrossMab technology is based on a domain crossover between heavy and light chains within one Fab-arm of a bispecific IgG, which promotes correct chain association. A CrossMab bispecific antibody of the disclosure can be a "CrossMab^{FAB}" antibody, in which the heavy and

light chains of the Fab portion of one arm of a bispecific IgG antibody are exchanged. In other embodiments, a CrossMab bispecific antibody of the disclosure can be a "CrossMab^{VH-VL}" antibody, in which the only the variable domains of the heavy and light chains of the Fab portion of one arm of a bispecific IgG antibody are exchanged. In yet other embodiments, a CrossMab bispecific antibody of the disclosure can be a "CrossMab^{CH1-CL}" antibody, in which only the constant domains of the heavy and light chains of the Fab portion of one arm of a bispecific IgG antibody are exchanged. CrossMab^{CH1-CL} antibodies, in contrast to CrossMab^{FAB} and CrossMab^{VH-VL}, do not have predicted side products and, therefore, in some embodiments CrossMab^{CH1-CL} bispecific antibodies are preferred. See, Klein *et al.*, 2016, mAbs, 8(6):1010-1020.

[0063] In some embodiments, the bispecific antibodies of the disclosure are controlled Fab-arm exchange bispecific antibodies. Methods for making Fab-arm exchange bispecific antibodies are described in PCT Publication No. WO2011/131746 and Labrijn *et al.*, 2014 Nat Protoc. 9(10):2450-63, incorporated herein by reference in their entireties. Briefly, controlled Fab-arm exchange bispecific antibodies can be made by separately expressing two parental IgG1s containing single matching point mutations in the CH3 domain, mixing the parental IgG1s under redox conditions *in vitro* to enable recombination of half-molecules, and removing the reductant to allow reoxidation of interchain disulfide bonds, thereby forming the bispecific antibodies.

[0064] In some embodiments, the bispecific antibodies of the disclosure are "bottle opener," "mAb-Fv," "mAb-scFv," "central-scFv," "central-Fv," "one-armed central-scFv" or "dual scFv" format bispecific antibodies. Bispecific antibodies of these formats are described in PCT Publication No. WO 2016/182751, the contents of which are incorporated herein by reference in their entireties. Each of these formats relies on the self-assembling nature of Fc domains of antibody heavy chains, whereby two Fc subunit containing "monomers" assemble into a Fc domain containing "dimer."

[0065] In the bottle opener format, the first monomer comprises a scFv covalently linked to the N-terminus of a Fc subunit, optionally via a linker, and the second monomer comprises a heavy chain (comprising a VH, CH1, and second Fc subunit). A bottle opener format bispecific antibody further comprises a light chain capable of pairing with the second monomer to form a Fab.

[0066] The mAb-Fv bispecific antibody format relies upon an "extra" VH domain attached to the C-terminus of one heavy chain monomer and an "extra" VL domain attached to the other heavy chain monomer, forming a third antigen binding domain. In some embodiments, a mAb-Fv bispecific antibody comprises a first monomer comprising a first VH domain, CH1 domain and a first Fc subunit, with a VL domain covalently attached to the C-terminus. The second monomer comprises a VH domain, a CH1 domain a second Fc subunit, and a VH covalently attached to the C-terminus of the second monomer. The two C-terminally attached variable domains make

up a Fv. The mAb-Fv further comprises two light chains, which when associated with the first and second monomers form Fabs.

[0067] The mAb-scFv bispecific format relies on the use of a C-terminal attachment of a scFv to one of the monomers of a mAb, thus forming a third antigen binding domain. Thus, the first monomer comprises a first heavy chain (comprising a VH, CH1 and a first Fc subunit), with a C-terminally covalently attached scFv. mAb-scFv bispecific antibodies further comprise a second monomer (comprising a VH, CH1, and first Fc subunit) and two light chains, which when associated with the first and second monomers form Fabs.

[0068] The central-scFv bispecific format relies on the use of an inserted scFv domain in a mAb, thus forming a third antigen binding domain. The scFv domain is inserted between the Fc subunit and the CH1 domain of one of the monomers, thus providing a third antigen binding domain. Thus, the first monomer can comprise a VH domain, a CH1 domain (and optional hinge) and a first Fc subunit, with a scFv covalently attached between the C-terminus of the CH1 domain and the N-terminus of the first Fc subunit using optional domain linkers. The other monomer can be a standard Fab side monomer. Central-scFv bispecific antibodies further comprise two light chains, which when associated with the first and second monomers form Fabs.

[0069] The central-Fv bispecific format relies on the use of an inserted Fv domain thus forming a third antigen binding domain. Each monomer can contain a component of the Fv (*e.g.*, one monomer comprises a variable heavy domain and the other a variable light domain). Thus, one monomer can comprise a VH domain, a CH1 domain, a first Fc subunit and a VL domain covalently attached between the C-terminus of the CH1 domain and the N-terminus of the first Fc subunit, optionally using domain linkers. The other monomer can comprise a VH domain, a CH1 domain, a second Fc subunit and an additional VH domain covalently attached between the C-terminus of the CH1 domain and the N-terminus of the second Fc domain, optionally using domain linkers. Central-Fv bispecific antibodies further comprise two light chains, which when associated with the first and second monomers form Fabs.

[0070] The one-armed central-scFv bispecific format comprises one monomer comprising just a Fc subunit, while the other monomer comprises an inserted scFv domain thus forming a second antigen binding domain. Thus, one monomer can comprise a VH domain, a CH1 domain and a first Fc subunit, with a scFv covalently attached between the C-terminus of the CH1 domain and the N-terminus of the first Fc subunit, optionally using domain linkers. The second monomer can comprise an Fc domain. This embodiment further utilizes a light chain comprising a variable light domain and a constant light domain, that associates with the first monomer to form a Fab.

[0071] The dual scFv bispecific format comprises a first monomer comprising a scFv covalently attached to the N-terminus of a first Fc subunit, optionally via a linker, and second monomer

comprising a scFv covalently attached to the N-terminus of a second Fc subunit, optionally via a linker.

[0072] Bispecific antibodies of the disclosure can comprise an Fc domain composed of a first and a second subunit. In one embodiment, the Fc domain is an IgG Fc domain. In a particular embodiment, the Fc domain is an IgG_1 Fc domain. In another embodiment the Fc domain is an IgG_4 Fc domain. In a more specific embodiment, the Fc domain is an IgG_4 Fc domain comprising an amino acid substitution at position S228 (Kabat EU index numbering), particularly the amino acid substitution S228P. Unless otherwise specified herein, numbering of amino acid residues in an Fc domain or constant region is according to the EU numbering system, also called the EU index, as described in Kabat *et al.*, 1991, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. This amino acid substitution reduces *in vivo* Fab arm exchange of IgG_4 antibodies (see Stubenrauch *et al.*, 2010, Drug Metabolism and Disposition 38:84-91). In a further particular embodiment, the Fc domain is a human Fc domain. In an even more particular embodiment, the Fc domain is a human IgG_1 Fc domain. An exemplary sequence of a human IgG_1 Fc region is:

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP Q (SEQ ID NO:156).

[0073] In particular embodiments, the Fc domain comprises a modification promoting the association of the first and the second subunit of the Fc domain. The site of most extensive protein-protein interaction between the two subunits of a human IgG Fc domain is in the CH3 domain. Thus, in one embodiment said modification is in the CH3 domain of the Fc domain.

[0074] In a specific embodiment said modification promoting the association of the first and the second subunit of the Fc domain is a so-called “knob-into-hole” modification, comprising a “knob” modification in one of the two subunits of the Fc domain and a “hole” modification in the other one of the two subunits of the Fc domain. The knob-into-hole technology is described *e.g.*, in US 5,731,168; US 7,695,936; Ridgway *et al.*, 1996, Prot Eng 9:617-621, and Carter, J, 2001, Immunol Meth 248:7-15. Generally, the method involves introducing a protuberance (“knob”) at the interface of a first polypeptide and a corresponding cavity (“hole”) in the interface of a second polypeptide, such that the protuberance can be positioned in the cavity so as to promote heterodimer formation and hinder homodimer formation. Protuberances are constructed by replacing small amino acid side chains from the interface of the first polypeptide with larger side chains (*e.g.*, tyrosine or tryptophan). Compensatory cavities of identical or similar size to the protuberances are created in the interface of the second polypeptide by replacing large amino acid side chains with smaller ones (*e.g.*, alanine or threonine).

[0075] Accordingly, in some embodiments, an amino acid residue in the CH3 domain of the first subunit of the Fc domain is replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance within the CH3 domain of the first subunit which is positionable in a cavity within the CH3 domain of the second subunit, and an amino acid residue in the CH3 domain of the second subunit of the Fc domain is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the CH3 domain of the second subunit within which the protuberance within the CH3 domain of the first subunit is positionable. Preferably said amino acid residue having a larger side chain volume is selected from the group consisting of arginine (R), phenylalanine (F), tyrosine (Y), and tryptophan (W). Preferably said amino acid residue having a smaller side chain volume is selected from the group consisting of alanine (A), serine (S), threonine (T), and valine (V). The protuberance and cavity can be made by altering the nucleic acid encoding the polypeptides, *e.g.*, by site-specific mutagenesis, or by peptide synthesis.

[0076] In a specific such embodiment, in the first subunit of the Fc domain the threonine residue at position 366 is replaced with a tryptophan residue (T366W), and in the second subunit of the Fc domain the tyrosine residue at position 407 is replaced with a valine residue (Y407V) and optionally the threonine residue at position 366 is replaced with a serine residue (T366S) and the leucine residue at position 368 is replaced with an alanine residue (L368A) (numbering according to Kabat EU index). In a further embodiment, in the first subunit of the Fc domain additionally the serine residue at position 354 is replaced with a cysteine residue (S354C) or the glutamic acid residue at position 356 is replaced with a cysteine residue (E356C) (particularly the serine residue at position 354 is replaced with a cysteine residue), and in the second subunit of the Fc domain additionally the tyrosine residue at position 349 is replaced by a cysteine residue (Y349C) (numbering according to Kabat EU index). In a particular embodiment, the first subunit of the Fc domain comprises the amino acid substitutions S354C and T366W, and the second subunit of the Fc domain comprises the amino acid substitutions Y349C, T366S, L368A and Y407V (numbering according to Kabat EU index).

[0077] In some embodiments, electrostatic steering (*e.g.*, as described in Gunasekaran *et al.*, 2010, J Biol Chem 285(25):19637-46) can be used to promote the association of the first and the second subunit of the Fc domain.

[0078] In some embodiments, the Fc domain comprises one or more amino acid substitutions that reduces binding to an Fc receptor and/or effector function.

[0079] In a particular embodiment the Fc receptor is an Fcγ receptor. In one embodiment the Fc receptor is a human Fc receptor. In one embodiment the Fc receptor is an activating Fc receptor. In a specific embodiment the Fc receptor is an activating human Fcγ receptor, more specifically human FcγRIIIa, FcγRI or FcγRIIa, most specifically human FcγRIIIa. In one

embodiment the effector function is one or more selected from the group of complement dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and cytokine secretion. In a particular embodiment, the effector function is ADCC.

[0080] Typically, the same one or more amino acid substitution is present in each of the two subunits of the Fc domain. In one embodiment, the one or more amino acid substitution reduces the binding affinity of the Fc domain to an Fc receptor. In one embodiment, the one or more amino acid substitution reduces the binding affinity of the Fc domain to an Fc receptor by at least 2-fold, at least 5-fold, or at least 10-fold.

[0081] In one embodiment, the Fc domain comprises an amino acid substitution at a position selected from the group of E233, L234, L235, N297, P331 and P329 (numberings according to Kabat EU index). In a more specific embodiment, the Fc domain comprises an amino acid substitution at a position selected from the group of L234, L235 and P329 (numberings according to Kabat EU index). In some embodiments, the Fc domain comprises the amino acid substitutions L234A and L235A (numberings according to Kabat EU index). In one such embodiment, the Fc domain is an IgG_1 Fc domain, particularly a human IgG_1 Fc domain. In one embodiment, the Fc domain comprises an amino acid substitution at position P329. In a more specific embodiment, the amino acid substitution is P329A or P329G, particularly P329G (numberings according to Kabat EU index). In one embodiment, the Fc domain comprises an amino acid substitution at position P329 and a further amino acid substitution at a position selected from E233, L234, L235, N297 and P331 (numberings according to Kabat EU index). In a more specific embodiment, the further amino acid substitution is E233P, L234A, L235A, L235E, N297A, N297D or P331S. In particular embodiments, the Fc domain comprises amino acid substitutions at positions P329, L234 and L235 (numberings according to Kabat EU index). In more particular embodiments, the Fc domain comprises the amino acid mutations L234A, L235A and P329G (which can be referred to using the shorthand terms “P329G LALA”, “PGLALA” or “LALAPG”). Specifically, in particular embodiments, each subunit of the Fc domain comprises the amino acid substitutions L234A, L235A and P329G (Kabat EU index numbering), *i.e.* in each of the first and the second subunit of the Fc domain the leucine residue at position 234 is replaced with an alanine residue (L234A), the leucine residue at position 235 is replaced with an alanine residue (L235A) and the proline residue at position 329 is replaced by a glycine residue (P329G) (numbering according to Kabat EU index). In one such embodiment, the Fc domain is an IgG_1 Fc domain, particularly a human IgG_1 Fc domain.

[0082] Single chain-based bispecific antibodies of the disclosure can be any of the various types of single chain-based bispecific antibodies known in the art, such as bispecific T-cell engagers (BiTEs), diabodies, tandem diabodies (tandabs), dual-affinity retargeting molecules (DARTs), and bispecific killer cell engagers. See, *e.g.*, Löffler *et al.*, 2000, Blood 95:2098–103;

Holliger *et al.*, 1993, Proc Natl Acad Sci USA, 90:6444–8; Kipriyanov *et al.*, 1999, Mol Biol 293:41–56; Johnson *et al.*, 2010, Mol Biol 399:436–49; Wiernik et al., 2013, Clin Cancer Res 19:3844–55; Liu *et al.*, 2017, Front. Immunol. 8:38; and Yang *et al.*, 2017, Int. J. Mol. Sci. 18:48, which are incorporated herein by reference in their entireties.

[0083] In some embodiments, the bispecific antibodies of the disclosure are bispecific T-cell engagers (BiTEs). BiTEs are single polypeptide chain molecules having two antigen-binding domains, one of which binds to a T-cell antigen and the second of which binds to an antigen present on the surface of a target (see, PCT Publication WO 05/061547; Baeuerle *et al.*, 2008, Drugs of the Future 33: 137-147; Bargou, *et al.*, 2008, Science 321:974-977, incorporated herein by reference in their entireties). Thus, the BiTEs of the disclosure have an antigen binding domain that binds to a T-cell antigen, and a second antigen binding domain that is directed towards glyco-MUC4.

[0084] In some embodiments, the bispecific antibodies of the disclosure are dual-affinity retargeting molecules (DARTs). DARTs comprise at least two polypeptide chains that associate (especially through a covalent interaction) to form at least two epitope binding sites, which may recognize the same or different epitopes. Each of the polypeptide chains of a DART comprise an immunoglobulin light chain variable region and an immunoglobulin heavy chain variable region, but these regions do not interact to form an epitope binding site. Rather, the immunoglobulin heavy chain variable region of one (*e.g.*, the first) of the DART polypeptide chains interacts with the immunoglobulin light chain variable region of a different (*e.g.*, the second) DART™ polypeptide chain to form an epitope binding site. Similarly, the immunoglobulin light chain variable region of one (*e.g.*, the first) of the DART polypeptide chains interacts with the immunoglobulin heavy chain variable region of a different (*e.g.*, the second) DART polypeptide chain to form an epitope binding site. DARTs may be monospecific, bispecific, trispecific, etc., thus being able to simultaneously bind one, two, three or more different epitopes (which may be of the same or of different antigens). DARTs may additionally be monovalent, bivalent, trivalent, tetravalent, pentavalent, hexavalent, etc., thus being able to simultaneously bind one, two, three, four, five, six or more molecules. These two attributes of DARTs (*i.e.*, degree of specificity and valency may be combined, for example to produce bispecific antibodies (*i.e.*, capable of binding two epitopes) that are tetravalent (*i.e.*, capable of binding four sets of epitopes), *etc.* DART molecules are disclosed in PCT Publications WO 2006/113665, WO 2008/157379, and WO 2010/080538, which are incorporated herein by reference in their entireties.

[0085] In some embodiments of the bispecific antibodies of the disclosure, one of the binding specificities is directed towards glyco-MUC4, and the other is directed to an antigen expressed on immune effector cells. The term “immune effector cell” or “effector cell” as used herein refers to a cell within the natural repertoire of cells in the mammalian immune system which can be

activated to affect the viability of a target cell. Immune effector cells include cells of the lymphoid lineage such as natural killer (NK) cells, T cells including cytotoxic T cells, or B cells, but also cells of the myeloid lineage can be regarded as immune effector cells, such as monocytes or macrophages, dendritic cells and neutrophilic granulocytes. Hence, said effector cell is preferably an NK cell, a T cell, a B cell, a monocyte, a macrophage, a dendritic cell or a neutrophilic granulocyte. Recruitment of effector cells to aberrant cells means that immune effector cells are brought in close vicinity to the aberrant target cells such that the effector cells can directly kill, or indirectly initiate the killing of the aberrant cells that they are recruited to. In order to avoid nonspecific interactions, it is preferred that the bispecific antibodies of the disclosure specifically recognize antigens on immune effector cells that are at least over-expressed by these immune effector cells compared to other cells in the body. Target antigens present on immune effector cells may include CD3, CD8, CD16, CD25, CD28, CD64, CD89, NKG2D and NKp46. Preferably, the antigen on immune effector cells is CD3 expressed on T cells.

[0086] As used herein, "CD3" refers to any native CD3 from any vertebrate source, including mammals such as primates (*e.g.*, humans), non-human primates (*e.g.*, cynomolgus monkeys) and rodents (*e.g.*, mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed CD3 as well as any form of CD3 that results from processing in the cell. The term also encompasses naturally occurring variants of CD3, *e.g.*, splice variants or allelic variants. The most preferred antigen on an immune effector cell is the CD3 epsilon chain. This antigen has been shown to be very effective in recruiting T cells to aberrant cells. Hence, a bispecific antibody of the disclosure preferably specifically recognizes CD3 epsilon. The amino acid sequence of human CD3 epsilon is shown in UniProt (uniprot.org) accession no. P07766 (version 144), or NCBI (ncbi.nlm.nih.gov/) RefSeq NP_000724.1. The amino acid sequence of cynomolgus (*Macaca fascicularis*) CD3 epsilon is shown in NCBI GenBank no. BAB71849.1. For human therapeutic use, bispecific antibodies in which the CD3-binding domain specifically binds to human CD3 (*e.g.*, the human CD3 epsilon chain) are used. For preclinical testing in non-human animals and cell lines, bispecific antibodies in which the CD3-binding domain specifically binds to the CD3 in the species utilized for the preclinical testing (*e.g.*, cynomolgus CD3 for primate testing) can be used.

[0087] As used herein, a binding domain that "specifically binds to" or "specifically recognizes" a target antigen from a particular species does not preclude the binding to or recognition of the antigen from other species, and thus encompasses antibodies in which one or more of the binding domains have inter-species cross-reactivity. For example, a CD3-binding domain that "specifically binds to" or "specifically recognizes" human CD3 may also bind to or recognize cynomolgus CD3, and vice versa.

[0088] In some embodiments, a bispecific antibody of the disclosure can compete with monoclonal antibody H2C (described in PCT publication no. WO2008/119567) for binding an epitope of CD3. In other embodiments, a bispecific antibody of the disclosure can compete with monoclonal antibody V9 (described in Rodrigues *et al.*, 1992, Int J Cancer Suppl 7:45-50 and U.S. Pat. No. 6,054,297) for binding an epitope of CD3. In yet other embodiments, a bispecific antibody of the disclosure can compete with monoclonal antibody FN18 (described in Nooij *et al.*, 1986, Eur J Immunol 19:981-984) for binding an epitope of CD3. In yet other embodiments, a bispecific antibody of the disclosure can compete with monoclonal antibody SP34 (described in Pessano *et al.*, 1985, EMBO J 4:337-340) for binding an epitope of CD3.

[0089] In some embodiments, a bispecific antibody of the disclosure can compete with monoclonal antibody mAb1 (described in U.S. Pat. No. 10,730,944) for binding an epitope of CD8. In other embodiments, a bispecific antibody of the disclosure can compete with monoclonal antibody YTS169 (described in US2015/ 0191543) for binding an epitope of CD8. In other embodiments, a bispecific antibody of the disclosure can compete with monoclonal antibodies 4C9 5F4 (described in WO1987/005912) for binding an epitope of CD8.

[0090] In some embodiments, a bispecific antibody of the disclosure can compete with monoclonal antibody 3G8_(described in WO2006/064136) for binding an epitope of CD16. In some embodiments, a bispecific antibody of the disclosure can compete with monoclonal antibody VEP13 (described in Ziegler-Heitbrock *et al.*, 1984, Clin.Exp. Immunol. 58:470-477) for binding an epitope of CD16. In some embodiments, a bispecific antibody of the disclosure can compete with monoclonal antibody B73.1 (described in Perussia *et al.*, 1983, J. Immunol.130(5):2142-2148) for binding an epitope of CD16.

[0091] In some embodiments, a bispecific antibody of the disclosure can compete with monoclonal antibody daclizumab and its variants (described in WO2014/145000) for binding an epitope of CD25. In some embodiments, a bispecific antibody of the disclosure can compete with monoclonal antibodies AB1, AB7, AB11, or AB12 (described in WO2004/045512) for binding an epitope of CD25. In some embodiments, a bispecific antibody of the disclosure can compete with monoclonal antibodies ALD25H1, ALD25H2, or ALD25H4 (described in WO2020/234399) for binding an epitope of CD25.

[0092] In some embodiments, a bispecific antibody of the disclosure can compete with monoclonal antibody FR104 (described in WO2017/103003) for binding an epitope of CD28. In some embodiments, a bispecific antibody of the disclosure can compete with monoclonal antibody hCD28.3 (described in WO2011/101791) for binding an epitope of CD28.

[0093] In some embodiments, a bispecific antibody of the disclosure can compete with monoclonal antibodies MS or 21 F2 (described in WO2009/077483) for binding an epitope of NKG2D. In some embodiments, a bispecific antibody of the disclosure can compete with monoclonal antibodies 5C5, 320, 230, 013, 296 or 395 (described in WO2021/009146) for

binding an epitope of NKG2D. In some embodiments, a bispecific antibody of the disclosure can compete with monoclonal antibody KYK-2.0 (described in WO2010/017103) for binding an epitope of NKG2D.

[0094] The anti-glyco-MUC4 antibodies of the disclosure include derivatized antibodies. For example, but not by way of limitation, derivatized antibodies are typically modified by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein. Any of numerous chemical modifications can be carried out by known techniques, including, but not limited to, specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, *etc.* Additionally, the derivative can contain one or more non-natural amino acids, *e.g.*, using ambrx technology (see, *e.g.*, Wolfson, 2006, Chem. Biol. 13(10):1011-2).

[0095] The anti-glyco-MUC4 antibodies or binding fragments may be antibodies or fragments whose sequences have been modified to alter at least one constant region-mediated biological effector function. For example, in some embodiments, an anti-glyco-MUC4 antibody may be modified to reduce at least one constant region-mediated biological effector function relative to the unmodified antibody, *e.g.*, reduced binding to the Fc receptor (FcγR). FcγR binding can be reduced by mutating the immunoglobulin constant region segment of the antibody at particular regions necessary for FcγR interactions (see, *e.g.*, Canfield and Morrison, 1991, J. Exp. Med. 173:1483-1491; and Lund *et al.*, 1991, J. Immunol. 147:2657-2662). Reduction in FcγR binding ability of the antibody can also reduce other effector functions which rely on FcγR interactions, such as opsonization, phagocytosis and antigen-dependent cellular cytotoxicity ("ADCC").

[0096] The anti-glyco-MUC4 antibody or binding fragments described herein include antibodies and/or binding fragments that have been modified to acquire or improve at least one constant region-mediated biological effector function relative to an unmodified antibody, *e.g.*, to enhance FcγR interactions (see, *e.g.*, US 2006/0134709). For example, an anti-glyco-MUC4 antibody of the disclosure can have a constant region that binds FcγRIIA, FcγRIIB and/or FcγRIIIA with greater affinity than the corresponding wild type constant region.

[0097] Thus, antibodies of the disclosure may have alterations in biological activity that result in increased or decreased opsonization, phagocytosis, or ADCC. Such alterations are known in the art. For example, modifications in antibodies that reduce ADCC activity are described in U.S. Pat. No. 5,834,597. An exemplary ADCC lowering variant corresponds to “mutant 3” (shown in FIG. 4 of U.S. Pat. No. 5,834,597) in which residue 236 is deleted and residues 234, 235 and 237 (using EU numbering) are substituted with alanines. Another exemplary ADCC lowering variant comprises amino acid mutations L234A, L235A and P329G (which can be referred to using the shorthand term “P329G LALA”). The “P329G LALA” combination of amino acid substitutions almost completely abolishes Fcγ receptor (as well as complement) binding of a human IgG_1 Fc domain, as described in PCT publication no. WO 2012/130831, incorporated

herein by reference in its entirety. WO 2012/130831 also describes methods of preparing such mutant Fc domains and methods for determining its properties such as Fc receptor binding or effector functions.

[0098] In some embodiments, the anti-glyco-MUC4 antibodies of the disclosure have low levels of, or lack, fucose. Antibodies lacking fucose have been correlated with enhanced ADCC activity, especially at low doses of antibody. See Shields *et al.*, 2002, J. Biol. Chem. 277:26733-26740; Shinkawa *et al.*, 2003, J. Biol. Chem. 278:3466-73. Methods of preparing fucose-less antibodies include growth in rat myeloma YB2/0 cells (ATCC CRL 1662). YB2/0 cells express low levels of FUT8 mRNA, which encodes α-1,6-fucosyltransferase, an enzyme necessary for fucosylation of polypeptides.

[0099] In some embodiments, the anti-glyco-MUC4 antibodies or binding fragments include bisected oligosaccharides, *e.g.*, in which a biantennary oligosaccharide attached to an Fc domain is bisected by GlcNAc. Such variants may have reduced fucosylation and/or improved ADCC function as described above. Examples of such antibody variants are described, *e.g.*, in Umana *et al.*, 1999, Nat Biotechnol 17:176-180; Ferrara *et al.*, 2006, Biotechn Bioeng 93: 851-861; WO 99/54342; WO 2004/065540; and WO 2003/011878.

[0100] In yet another aspect, the anti-glyco-MUC4 antibodies or binding fragments include modifications that increase or decrease their binding affinities to the fetal Fc receptor, FcRn, for example, by mutating the immunoglobulin constant region segment at particular regions involved in FcRn interactions (see, *e.g.*, WO 2005/123780). In particular embodiments, an anti-glyco-MUC4 antibody of the IgG class is mutated such that at least one of amino acid residues 250, 314, and 428 of the heavy chain constant region is substituted alone, or in any combinations thereof, such as at positions 250 and 428, or at positions 250 and 314, or at positions 314 and 428, or at positions 250, 314, and 428, with positions 250 and 428 a specific combination. For position 250, the substituting amino acid residue can be any amino acid residue other than threonine, including, but not limited to, alanine, cysteine, aspartic acid, glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, leucine, methionine, asparagine, proline, glutamine, arginine, serine, valine, tryptophan, or tyrosine. For position 314, the substituting amino acid residue can be any amino acid residue other than leucine, including, but not limited to, alanine, cysteine, aspartic acid, glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, methionine, asparagine, proline, glutamine, arginine, serine, threonine, valine, tryptophan, or tyrosine. For position 428, the substituting amino acid residues can be any amino acid residue other than methionine, including, but not limited to, alanine, cysteine, aspartic acid, glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, leucine, asparagine, proline, glutamine, arginine, serine, threonine, valine, tryptophan, or tyrosine. Specific combinations of suitable amino acid substitutions are identified in Table 1 of

U.S. Pat. No. 7,217,797, which is incorporated herein by reference. Such mutations increase binding to FcRn, which protects the antibody from degradation and increases its half-life.

[0101] In yet other aspects, an anti-glyco-MUC4 antibody of antigen-binding fragment of the disclosure has one or more amino acids inserted into one or more of its hypervariable regions, for example as described in Jung and Pluckthun, 1997, Protein Engineering 10:9, 959-966; Yazaki *et al.*, 2004, Protein Eng. Des Sel. 17(5):481-9. Epub 2004 Aug. 17; and U.S. Pat. App. No. 2007/0280931.

[0102] In yet other aspects, particularly useful for diagnostic applications, an anti-glyco-MUC4 antibody of antigen-binding fragment of the disclosure is attached to a detectable moiety. Detectable moieties include a radioactive moiety, a colorimetric molecule, a fluorescent moiety, a chemiluminescent moiety, an antigen, an enzyme, a detectable bead (such as a magnetic or electrodense (*e.g.*, gold) bead), or a molecule that binds to another molecule (*e.g.*, biotin or streptavidin)).

[0103] Radioisotopes or radionuclides may include ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I.

[0104] Fluorescent labels may include rhodamine, lanthanide phosphors, fluorescein and its derivatives, fluorochrome, GFP (GFP for “Green Fluorescent Protein”), dansyl, umbelliferone, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde, and fluorescamine.

[0105] Enzymatic labels may include horseradish peroxidase, β galactosidase, luciferase, alkaline phosphatase, glucose-6-phosphate dehydrogenase (“G6PDH”), alpha-D-galactosidase, glucose oxidase, glucose amylase, carbonic anhydrase, acetylcholinesterase, lysozyme, malate dehydrogenase and peroxidase.

[0106] Chemiluminescent labels or chemiluminescers, such as isoluminol, luminol and the dioxetanes.

[0107] Other detectable moieties include molecules such as biotin, digoxygenin or 5-bromodeoxyuridine.

[0108] In yet other aspects, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure may be used in a detection system to detect a biomarker in a sample, such as, *e.g.*, a patient-derived biological sample. The biomarker may be a protein biomarker (*e.g.*, a tumor-associated glycoform of MUC4, for example a glycoform of MUC4 comprising the amino acid sequence CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) glycosylated with GalNAc on the serine and threonine residues shown in bold underlined text present on the surface of or within, *e.g.*, a cancer cell (*e.g.*, from a tissue biopsy or a circulating tumor cell) or a cancer-derived extracellular vesicle).

[0109] Extracellular vesicles (EVs) are lipid membranous vesicles released from almost all cell types. EVs carry complex molecular cargoes, such as proteins, RNAs (e.g., mRNA and

noncoding RNAs (microRNA, transfer RNA, circular RNA and long noncoding RNA)), and DNA fragments. The molecular contents of EVs largely reflect the cell of origin and thus show cell-type specificity. In particular, cancer-derived EVs contain and present on their surfaces cancer-specific molecules expressed by parental cancer cells (see, *e.g.*, Yáñez-Mó et al., 2015, J Extracell Vesicles. 4:27066; and Li *et al.*, 2015, Cell Res. 25:981-984)

[0110] In one embodiment, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure is used in a method of detecting a biomarker in a sample comprising EVs (*e.g.*, a liquid biopsy). In such embodiments, the biomarker is recognized by the anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure. The biomarker may be present on the surface of EVs. Exemplary methods of detecting the biomarker include, but are not limited to, immunoassays, such as immunoprecipitation; Western blot; ELISA; immunohistochemistry; immunocytochemistry; flow cytometry; and immuno-PCR. In some embodiments, an immunoassay can be a chemiluminescent immunoassay. In some embodiments, an immunoassay can be a high-throughput and/or automated immunoassay platform.

[0111] In some embodiments, the method of detecting a biomarker in a sample comprises contacting a sample with an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure. In some embodiments, such methods further comprise contacting the sample with one or more detection labels. In some embodiments, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure is labeled with one or more detection labels.

[0112] In some embodiments, a capture assay is performed to selectively capture EVs from a sample, such as a liquid biopsy sample. Exemplary examples of capture assays for EVs are described in US2021/0214806, which is hereby incorporated by reference in its entirety. In some embodiments, a capture assay is performed to selectively capture EVs of a certain size range, and/or certain characteristic(s), for example, EVs associated with cancer (*e.g.*, a tumor-associated glycoform of MUC4, for example a glycoform of MUC4 comprising the amino acid sequence CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) glycosylated with GalNAc on the serine and threonine residues shown in bold underlined text), glycosylated with GalNAc on the threonine residue shown in bold underlined text). In some such embodiments, prior to performing the capture assay, a sample may be pre-processed to remove non-EVs, including but not limited to, *e.g.*, soluble proteins and interfering entities such as, *e.g.*, cell debris. In some embodiments, EVs are purified from a sample using size exclusion chromatography.

[0113] In some embodiments, the method for detecting a biomarker comprises analyzing individual EVs (*e.g.*, a single EV assay). For example, such an assay may involve (i) a capture assay such as an antibody capture assay and (ii) one or more detection assays for at least one or more additional biomarkers, wherein the capture assay is performed prior to the detection assay. See, *e.g.*, US2021/0214806.

[0114] In some embodiments, a capture assay comprises a step of contacting a sample with at least one capture agent comprising an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure. The capture agent may be immobilized on a solid substrate. The solid substrate may be provided in a form that is suitable for capturing EVs and does not interfere with downstream handling, processing, and/or detection. For example, in some embodiments, a solid substrate may be or comprise a bead (*e.g.*, a magnetic bead). In some embodiments, a solid substrate may be or comprise a surface. For example, in some embodiments, such a surface may be a capture surface of an assay chamber (*e.g.*, a tube, a well, a microwell, a plate, a filter, a membrane, a matrix, etc.). In some embodiments, a capture agent is or comprises a magnetic bead comprising a capture moiety (*e.g.*, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure) conjugated thereto. See, *e.g.*, US2021/0214806.

[0115] In certain aspects, an anti-glyco-MUC4 antibody or antigen binding fragment of the disclosure competes with 2D5 or an antibody or antigen binding fragment comprising a heavy chain variable region of murine or humanized 2D5 (*e.g.,* SEQ ID NO:1 (murine) and SEQ ID NOS: 133-144 (exemplary humanized sequences)) and a light chain variable region of murine or humanized 2D5 (*e.g.*, SEQ ID NOS: 2 (murine) and SEQ ID NO:145-153 (exemplary humanized sequences)).

[0116] In other aspects, an anti-glyco-MUC4 antibody or antigen binding fragment of the disclosure competes with 5B8 or an antibody or antigen binding fragment comprising heavy and light chain variable regions of 5B8 (SEQ ID NOS: 23 and 24, respectively).

[0117] In other aspects, an anti-glyco-MUC4 antibody or antigen binding fragment of the disclosure competes with 15F3 or an antibody or antigen binding fragment comprising heavy and light chain variable regions of 15F3 (SEQ ID NOS: 45 and 46, respectively).

[0118] Competition can be assayed on cells that express the glyco-MUC4 epitope bound by 2D5, 5B8, or 15F3 or on a glycosylated MUC4 peptide containing the epitope bound by 2D5, 5B8, or 15F3, *e.g.*, the peptide CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) glycosylated with GalNAc on the serine and threonine residues shown in bold and underlined text. Cells that do not express the epitope or unglycosylated peptides can be used as controls.

[0119] Cells on which a competition assay can be carried out include, but are not limited to, the breast cancer cell line T47D and recombinant cells that are engineered to express the glyco-MUC4 epitope. In one non-limiting example, T47D cells, which express MUC4 but are inherently Tn-negative, are engineered to express the MUC4 Tn-antigen by knockout of the COSMC chaperone. Wildtype cells expressing the unglycosylated form of MUC4 can be used as a negative control.

[0120] Assays for competition include, but are not limited to, a radioactive material labeled immunoassay (RIA), an enzyme-linked immunosorbent assay (ELISA), a sandwich ELISA,

fluorescence activated cell sorting (FACS) assays, surface plasmon resonance (*e.g.*, Biacore) assays, and bio-layer interferometry (BLI) assays. In some embodiments, antibody competition assays can be carried out using BLI (*e.g.*, using an Octet-HTX system (Molecular Devices)). Antibody competition or epitope binning of monoclonal antibodies can be assessed in tandem against their specific antigen using BLI. In a BLI assay, the antigen can be immobilized onto a biosensor and presented to two competing antibodies in consecutive steps. The binding to non-overlapping epitopes occurs if saturation with the first antibody does not block the binding of the second antibody. In some embodiments, antibody competition assays can be carried out using surface plasmon resonance (e.g., using a Biacore system (Cytiva)). In a surface plasmon resonance assay, one or more antibodies can be immobilized onto a biosensor and presented with an analyte (*e.g.*, the glyco-MUC4 peptide of SEQ ID NO:154 or a negative control analyte such as an unglycosylated MUC4 peptide of SEQ ID NO:155). In some embodiments, the antibodies are contacted with a saturating concentration of the analyte, for example a concentration of at least about 0.5 μM. In some embodiments the saturating concentration is about 1 μM, about 1.5 μM, or about 2 μM. When comparing the binding affinities of two antibodies, the affinities of both antibodies are preferably measured using the same concentration of both antibodies, e.g., measured using a 1 μM concentration of each antibody.

[0121] In conducting an antibody competition assay between a reference antibody and a test antibody (irrespective of species or isotype), one may first label the reference with a detectable label, such as a fluorophore, biotin or an enzymatic (or even radioactive) label to enable subsequent identification. In this case, cells expressing glyco-MUC4 are incubated with unlabeled test antibody, labeled reference antibody is added, and the intensity of the bound label is measured. If the test antibody competes with the labeled reference antibody by binding to an overlapping epitope, the intensity will be decreased relative to a control reaction carried out without test antibody.

[0122] In a specific embodiment of this assay, the concentration of labeled reference antibody that yields 80% of maximal binding ("$conc_{80\%}$") under the assay conditions (*e.g.*, a specified density of cells) is first determined, and a competition assay may be then carried out with 10 x $conc_{80\%}$ of unlabeled test antibody and $conc_{80\%}$ of labeled reference antibody.

[0123] The inhibition can be expressed as an inhibition constant, or K_i, which is calculated according to the following formula:

$$K_i = IC_{50}/(1+[\text{reference Ab concentration}]/K_d),$$

where IC_{50} is the concentration of test antibody that yields a 50% reduction in binding of the reference antibody and K_d is the dissociation constant of the reference antibody, a measure of its affinity for glyco-MUC4. Antibodies that compete with anti-glyco-MUC4 antibodies disclosed herein can have a K_i from 10 pM to 10 nM under assay conditions described herein.

[0124] In various embodiments, a test antibody is considered to compete with a reference antibody if it decreases binding of the reference antibody by at least about 20% or more, for example, by at least about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or even more, or by a percentage ranging between any of the foregoing values, at a reference antibody concentration that is 80% of maximal binding under the specific assay conditions used, and a test antibody concentration that is 10-fold higher than the reference antibody concentration.

[0125] In one example of a competition assay, the glycosylated MUC4 peptide of SEQ ID NO:154 is adhered onto a solid surface, *e.g.*, a microwell plate, by contacting the plate with a solution of the peptide (*e.g.*, at a concentration of 1 µg/mL in PBS over night at 4°C). The plate is washed (*e.g.*, 0.1% Tween 20 in PBS) and blocked (*e.g.*, in Superblock, Thermo Scientific, Rockford, IL). A mixture of sub-saturating amount of biotinylated 2D5, 5B8, and 15F3 (*e.g.*, at a concentration of 80 ng/mL) and unlabeled 2D5, 5B8, and 15F3 (the “reference” antibody) or competing anti-glyco-MUC4 antibody (the "test" antibody) antibody in serial dilution (*e.g.*, at a concentration of 2.8 µg/mL, 8.3 µg/mL, or 25 µg/mL) in ELISA buffer (*e.g.*, 1% BSA and 0.1% Tween 20 in PBS) is added to wells and plates are incubated for 1 hour with gentle shaking. The plate is washed, 1 µg/mL HRP-conjugated Streptavidin diluted in ELISA buffer is added to each well and the plates incubated for 1 hour. Plates are washed and bound antibodies were detected by addition of substrate (*e.g.*, TMB, Biofx Laboratories Inc., Owings Mills, MD). The reaction is terminated by addition of stop buffer (*e.g.*, Bio FX Stop Reagents, Biofx Laboratories Inc., Owings Mills, MD) and the absorbance is measured at 650 nm using microplate reader (*e.g.*, VERSAmax, Molecular Devices, Sunnyvale, CA).

[0126] Variations on this competition assay can also be used to test competition between 2D5, 5B8, and 15F3 and another anti-glyco-MUC4 antibodies. For example, in certain aspects, the anti-glyco-MUC4 antibody is used as a reference antibody and 2D5, 5B8, or 15F3 is used as a test antibody. Additionally, instead of a glycosylated MUC4 peptide of SEQ ID NO:154, membrane-bound glyco-MUC4 expressed on cell surface (for example on the surface of one of the cell types mentioned above) in culture can be used. Generally, about 10^4 to 10^6 transfectants, *e.g.*, about 10^5 transfectants, are used. Other formats for competition assays are known in the art and can be employed.

[0127] In various embodiments, an anti-glyco-MUC4 antibody of the disclosure reduces the binding of labeled 2D5, 5B8, or 15F3 by at least 40%, by at least 50%, by at least 60%, by at least 70%, by at least 80%, by at least 90%, or by a percentage ranging between any of the foregoing values (*e.g.*, an anti-glyco-MUC4 antibody of the disclosure reduces the binding of labeled 2D5, 5B8, or 15F3 by 50% to 70%) when the anti-glyco-MUC4 antibody is used at a concentration of 0.08 µg/mL, 0.4 µg/mL, 2 µg/mL, 10 µg/mL, 50 µg/mL, 100 µg/mL or at a concentration ranging between any of the foregoing values (*e.g.*, at a concentration ranging from 2 µg/mL to 10 µg/mL).

[0128] In other embodiments, 2D5, 5B8, or 15F3 reduces the binding of a labeled anti-glyco-MUC4 antibody of the disclosure by at least 40%, by at least 50%, by at least 60%, by at least 70%, by at least 80%, by at least 90%, or by a percentage ranging between any of the foregoing values (*e.g.*, 2D5, 5B8, or 15F3 reduces the binding of a labeled an anti-glyco-MUC4 antibody of the disclosure by 50% to 70%) when 2D5, 5B8, or 15F3 is used at a concentration of 0.4 µg/mL, 2 µg/mL, 10 µg/mL, 50 µg/mL, 250 µg/mL or at a concentration ranging between any of the foregoing values (*e.g.*, at a concentration ranging from 2 µg/mL to 10 µg/mL).

[0129] In the foregoing assays, the 2D5, 5B8, or 15F3 antibody can be replaced by any antibody or antigen-binding fragment comprising the CDRs or the heavy and light chain variable regions of 2D5, 5B8, and 15F3, such as a humanized or chimeric counterpart of 2D5, 5B8, and 15F3. Exemplary humanize heavy and light chain variable regions of 2D5 are provided by SEQ ID NOS: 133-153.

[0130] In certain aspects, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure has an epitope which is the same or similar to the epitope of 2D5, 5B8, or 15F3. The epitope of an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure can be characterized by performing alanine scanning. A library of glycopeptides, each varying from the MUC4 peptide by an alanine point mutation at one of positions SEQ ID NO:154 (or, where the MUC4 peptide has an alanine, by a glycine point mutation). By measuring an antibody or antigen binding fragment's binding to each of the peptides by ELISA, the antibody or antigen binding fragment's epitope can be mapped.

[0131] In certain aspects, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure comprises heavy and/or light chain variable sequences (or encoded by the nucleotide sequences) set forth in Tables 1A-1C (murine) and 4A-4G (humanized). In other aspects, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure comprises heavy and/or light chain CDR sequences (or encoded by the nucleotide sequences) set forth in Tables 1-3. The framework sequences for such anti-glyco-MUC4 antibody and antigen-binding fragment can be the native murine framework sequences of the VH and VL sequences set forth in Tables 1A-1C or can be non-native (*e.g.*, humanized or human) framework sequences. Humanized framework sequences of the VH and VL sequences of 2D5 are set forth in Tables 4A-4G.

[0132] In yet other aspects, the disclosure provides an anti-MUC4 antibody or antigen binding fragment having heavy and light chain variable regions having at least 85%, 90%, 95%, 98%, 99%, or 99.5% sequence identity of SEQ ID NOS: 1 and 2, respectively.

[0133] In yet other aspects, the disclosure provides an anti-MUC4 antibody or antigen binding fragment having heavy and light chain variable regions having at least 85%, 90%, 95%, 98%, 99%, or 99.5% sequence identity of SEQ ID NOS: 23 and 24, respectively.

[0134] In yet other aspects, the disclosure provides an anti-MUC4 antibody or antigen binding fragment having heavy and light chain variable regions having at least 85%, 90%, 95%, 98%, 99%, or 99.5% sequence identity of SEQ ID NOS: 45 and 46, respectively.

[0135] In yet other aspects, the disclosure provides an anti-MUC4 antibody or antigen binding fragment having a heavy chain variable region having at least 95%, 98%, 99%, or 99.5% sequence identity of one of SEQ ID NOS: 133-144 and light chain variable regions having at least 95%, 98%, 99%, or 99.5% sequence identity of one of SEQ ID NOS: 145 and 153.

[0136] In yet other aspects, an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure is a single-chain variable fragment (scFv). An exemplary scFv comprises the heavy chain variable fragment N-terminal to the light chain variable fragment. Another exemplary scFv comprises the light chain variable fragment N-terminal to the heavy chain variable fragment. In some embodiments, the scFv heavy chain variable fragment and light chain variable fragment are covalently bound to a linker sequence of 4-15 amino acids. The scFv can be in the form of a bi-specific T-cell engager or within a chimeric antigen receptor (CAR).

5.1.1. Antibody Specificity

[0137] In some embodiments, the anti-glyco-MUC4 antibodies of the disclosure specifically bind to the MUC4 glycoprotein CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154), glycosylated with GalNAc on the serine and threonine residues shown in bold underlined text.

[0138] In certain embodiments, the anti-glyco-MUC4 antibodies of the disclosure specifically binds to a MUC4 glycoprotein described above, and does not specifically bind to one or more of: the unglycosylated MUC4 peptide CTIPSTAMHTRSTAAPIPILP (SEQ ID NO:155) (the "unglycosylated MUC4 peptide"); the MUC1 tandem repeat $(VTSAPDTRPAPGSTAPPAHG)_3$ (SEQ ID NO:201) that has been glycosylated *in vitro* using purified recombinant human glycosyltransferases GalNAc-T1, GalNAc-T2, and GalNAc-T4 ("the first MUC1 glycopeptide"); the MUC1 peptide TAPPAHGV**TS**APD**T**RPAPG**ST**APPAHGVT (SEQ ID NO:202) that has been glycosylated *in vitro* with GalNAc on the serine and threonine residues shown with bold and underlined text (the "second MUC1 glycopeptide"); the CD44v6 peptide GYRQ**T**PKEDSH**S**TTGTAAA (SEQ ID NO:218) that has been glycosylated *in vitro* with GalNAc on the threonine and serine residues shown with bold and underlined text (the "CD44v6 glycopeptide"); the LAMP1 peptide CEQDRP**S**P**TT**APPAPPSPSP (SEQ ID NO:219) that has been glycosylated *in vitro* with GalNAc on the serine and threonine residues shown with bold and underlined text (the "LAMP1 glycopeptide"); and the cMET peptide PTKSFISGG**ST**ITGVGKNLN (SEQ ID NO:220) that has been glycosylated *in vitro* with GalNAc on the serine and threonine residues shown with bold and underlined text (the "cMET glycopeptide").

[0139] In some embodiments, an anti-glyco-MUC4 antibody of the disclosure has a binding affinity to the MUC4 glycopeptide which is at least 3 times, at least 5 times, at least 10 times, at least 20 times, at least 50 times, at least 100 times, or at least 1000 times the binding affinity of the anti-glyco-MUC4 antibody to the unglycosylated MUC4 peptide.

[0140] In some embodiments, an anti-glyco-MUC4 antibody of the disclosure has a binding affinity to the MUC4 glycopeptide which is at least 3 times, at least 5 times, at least 10 times, at least 20 times, at least 50 times, at least 100 times, or at least 1000 times the binding affinity of the anti-glyco-MUC4 antibody to the first MUC1 glycopeptide.

[0141] In some embodiments, an anti-glyco-MUC4 antibody of the disclosure has a binding affinity to the MUC4 glycopeptide which is at least 3 times, at least 5 times, at least 10 times, at least 20 times, at least 50 times, at least 100 times, or at least 1000 times the binding affinity of the anti-glyco-MUC4 antibody to the second MUC1 glycopeptide.

[0142] In some embodiments, an anti-glyco-MUC4 antibody of the disclosure has a binding affinity to the MUC4 glycopeptide which is at least 3 times, at least 5 times, at least 10 times, at least 20 times, at least 50 times, at least 100 times, or at least 1000 times the binding affinity of the anti-glyco-MUC4 antibody to the CD44v6 glycopeptide.

[0143] In some embodiments, an anti-glyco-MUC4 antibody of the disclosure has a binding affinity to the MUC4 glycopeptide which is at least 3 times, at least 5 times, at least 10 times, at least 20 times, at least 50 times, at least 100 times, or at least 1000 times the binding affinity of the anti-glyco-MUC4 antibody to the LAMP1 glycopeptide.

[0144] In some embodiments, an anti-glyco-MUC4 antibody of the disclosure has a binding affinity to the MUC4 glycopeptide which is at least 3 times, at least 5 times, at least 10 times, at least 20 times, at least 50 times, at least 100 times, or at least 1000 times the binding affinity of the anti-glyco-MUC4 antibody to the cMET glycopeptide.

[0145] Assays for determining affinity, including relative affinity, include but are not limited to a radioactive material labeled immunoassay (RIA), an enzyme-linked immunosorbent assay (ELISA), a sandwich ELISA, fluorescence activated cell sorting (FACS) assays, surface plasmon resonance (*e.g.*, Biacore) assays, and bio-layer interferometry (BLI) assays. In some embodiments, affinity is measured by surface plasmon resonance (*e.g.*, Biacore). In other embodiments, affinity

[0146] Exemplary anti-glyco-MUC4 antibodies and fragments thereof are described in numbered embodiments 1 to 414.

5.2 Antibody-Drug Conjugates

[0147] Another aspect of the disclosure concerns antibody drug conjugates (ADCs) including the anti-glyco-MUC4 antibodies and antigen-binding fragments of the disclosure. The ADCs

generally comprise an anti-glyco-MUC4 antibody and/or binding fragment as described herein having one or more cytotoxic and/or cytostatic agents linked thereto by way of one or more linkers. In specific embodiments, the ADCs are compounds according to structural formula (I):

$$[D\text{-}L\text{-}XY]_n\text{-}Ab$$

or salts thereof, where each "D" represents, independently of the others, a cytotoxic and/or cytostatic agent ("drug"); each "L" represents, independently of the others, a linker; "Ab" represents an anti-glyco-MUC4 antigen binding domain, such as an anti-glyco-MUC4 antibody or binding fragment described herein; each "XY" represents a linkage formed between a functional group R^x on the linker and a "complementary" functional group R^y on the antibody, and n represents the number of drugs linked to, or drug-to-antibody ratio (DAR), of the ADC.

[0148] Specific embodiments of the various antibodies (Ab) that can comprise the ADCs include the various embodiments of anti-glyco-MUC4 antibodies and/or binding fragments described above.

[0149] In some specific embodiments of the ADCs and/or salts of structural formula (I), each D is the same and/or each L is the same.

[0150] In some embodiments, the ADC comprises an amanitin toxin. Amanitins are bicyclic peptides of eight amino acids that are naturally occurring poisons found in several species of the *Amanita* genus of mushrooms. Amanitin toxins inhibit RNA polymerase II, which results in apoptosis of a cell. Exemplary amantin toxins that can be conjugated and an anti-glyco-MUC4 antibody of the disclosure and methods of their conjugation are described in US 2019/0328899 and US 2021/0077571, which are incorporated by reference herein in their entireties.

[0151] In some embodiments, a glycan of an anti-glyco-MUC4 antibodies and antigen-binding fragments of the disclosure (*e.g.*, at or around Asn-297 of an IgG Fc (Kabat numbering)) can be modified and a cytotoxic and/or cytostatic agent attached to the glycan. Van Geel *et al.*, 2015, Bioconjugate Chem. 26(11):2233-2242. A chemoenzymatic protocol provides for the highly controlled attachment of a drug to an N-glycan at or around Asn-297 via two stages: i) enzymatic remodeling via trimming and tagging with azide; and ii) ligation of a drug via copper-free click chemistry. Such methods are applicable to any IgG isotype, irrespective of glycosylation profile. Exemplary compositions and methods for conjugating a drug to a glycan of an anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure are described, for example, in WO 2015/057063; WO 2015/057064; WO 2015/057065; WO 2015/057066; WO 2015/112013; WO 2016/022027; WO 2016/053107; WO 2016/170186; WO 2017/137423; WO 2017/137456; and WO 2017/137457, each of which is hereby incorporated by reference in its entirety.

[0152] Specific embodiments of cytotoxic and/or cytostatic agents (D) and linkers (L) that can comprise the anti-glyco-MUC4 ADCs of the disclosure, as well as the number of cytotoxic and/or cytostatic agents linked to the ADCs, are described in more detail below.

5.2.1. Cytotoxic and/or Cytostatic Agents

[0153] The cytotoxic and/or cytostatic agents may be any agents known to inhibit the growth and/or replication of and/or kill cells, and in particular cancer and/or tumor cells. Numerous agents having cytotoxic and/or cytostatic properties are known in the literature. Non-limiting examples of classes of cytotoxic and/or cytostatic agents include, by way of example and not limitation, radionuclides, alkylating agents, topoisomerase I inhibitors, topoisomerase II inhibitors, DNA intercalating agents (*e.g.*, groove binding agents such as minor groove binders), RNA/DNA antimetabolites, cell cycle modulators, kinase inhibitors, protein synthesis inhibitors, histone deacetylase inhibitors, mitochondria inhibitors, and antimitotic agents.

[0154] Specific non-limiting examples of agents within certain of these various classes are provided below.

[0155] Alkylating Agents: asaley ((L-Leucine, N-[N-acetyl-4-[bis-(2-chloroethyl)amino]-DL-phenylalanyl]-, ethylester; NSC 167780; CAS Registry No. 3577897)); AZQ ((1,4-cyclohexadiene-1,4-dicarbamic acid, 2,5-bis(1-aziridinyl)-3,6-dioxo-, diethyl ester; NSC 182986; CAS Registry No. 57998682)); BCNU ((N,N'-Bis(2-chloroethyl)-N-nitrosourea; NSC 409962; CAS Registry No. 154938)); busulfan (1,4-butanediol dimethanesulfonate; NSC 750; CAS Registry No. 55981); (carboxyphthalato)platinum (NSC 27164; CAS Registry No. 65296813); CBDCA ((cis-(1,1-cyclobutanedicarboxylato)diammineplatinum(II)); NSC 241240; CAS Registry No. 41575944)); CCNU ((N-(2-chloroethyl)-N'-cyclohexyl-N-nitrosourea; NSC 79037; CAS Registry No. 13010474)); CHIP (iproplatin; NSC 256927); chlorambucil (NSC 3088; CAS Registry No. 305033); chlorozotocin ((2-[[[(2-chloroethyl) nitrosoamino]carbonyl]amino]-2-deoxy-D-glucopyranose; NSC 178248; CAS Registry No. 54749905)); cis-platinum (cisplatin; NSC 119875; CAS Registry No. 15663271); clomesone (NSC 338947; CAS Registry No. 88343720); cyanomorpholinodoxorubicin (NCS 357704; CAS Registry No. 88254073); cyclodisone (NSC 348948; CAS Registry No. 99591738); dianhydrogalactitol (5,6-diepoxydulcitol; NSC 132313; CAS Registry No. 23261203); fluorodopan ((5-[(2-chloroethyl)-(2-fluoroethyl)amino]-6-methyl-uracil; NSC 73754; CAS Registry No. 834913); hepsulfam (NSC 329680; CAS Registry No. 96892578); hycanthone (NSC 142982; CAS Registry No. 23255938); melphalan (NSC 8806; CAS Registry No. 3223072); methyl CCNU ((1-(2-chloroethyl)-3-(trans-4-methylcyclohexane)-1-nitrosourea; NSC 95441; 13909096); mitomycin C (NSC 26980; CAS Registry No. 50077); mitozolamide (NSC 353451; CAS Registry No. 85622953); nitrogen mustard ((bis(2-chloroethyl)methylamine hydrochloride; NSC 762; CAS Registry No. 55867); PCNU ((1-(2-chloroethyl)-3-(2,6-dioxo-3-piperidyl)-1-nitrosourea; NSC 95466; CAS Registry No. 13909029)); piperazine alkylator ((1-(2-chloroethyl)-4-(3-chloropropyl)-piperazine dihydrochloride; NSC 344007)); piperazinedione (NSC 135758; CAS Registry No. 41109802); pipobroman ((N,N-bis(3-bromopropionyl) piperazine; NSC 25154; CAS Registry No. 54911)); porfiromycin (N-methylmitomycin C; NSC 56410; CAS Registry No. 801525); spirohydantoin mustard (NSC 172112; CAS Registry No. 56605164); teroxirone

(triglycidylisocyanurate; NSC 296934; CAS Registry No. 2451629); tetraplatin (NSC 363812; CAS Registry No. 62816982); thio-tepa (N,N',N''-tri-1,2-ethanediylthio phosphoramide; NSC 6396; CAS Registry No. 52244); triethylenemelamine (NSC 9706; CAS Registry No. 51183); uracil nitrogen mustard (desmethyldopan; NSC 34462; CAS Registry No. 66751); Yoshi-864 ((bis(3-mesyloxy propyl)amine hydrochloride; NSC 102627; CAS Registry No. 3458228).

[0156] Topoisomerase I Inhibitors: camptothecin (NSC 94600; CAS Registry No. 7689-03-4); various camptothecin derivatives and analogs (for example, NSC 100880, NSC 603071, NSC 107124, NSC 643833, NSC 629971, NSC 295500, NSC 249910, NSC 606985, NSC 74028, NSC 176323, NSC 295501, NSC 606172, NSC 606173, NSC 610458, NSC 618939, NSC 610457, NSC 610459, NSC 606499, NSC 610456, NSC 364830, and NSC 606497); morpholinisoxorubicin (NSC 354646; CAS Registry No. 89196043); SN-38 (NSC 673596; CAS Registry No. 86639-52-3).

[0157] Topoisomerase II Inhibitors: doxorubicin (NSC 123127; CAS Registry No. 25316409); amonafide (benzisoquinolinedione; NSC 308847; CAS Registry No. 69408817); m-AMSA ((4'-(9-acridinylamino)-3'-methoxymethanesulfonanilide; NSC 249992; CAS Registry No. 51264143)); anthrapyrazole derivative ((NSC 355644); etoposide (VP-16; NSC 141540; CAS Registry No. 33419420); pyrazoloacridine ((pyrazolo[3,4,5-kl]acridine-2(6H)-propanamine, 9-methoxy-N, N-dimethyl-5-nitro-, monomethanesulfonate; NSC 366140; CAS Registry No. 99009219); bisantrene hydrochloride (NSC 337766; CAS Registry No. 71439684); daunorubicin (NSC 821151; CAS Registry No. 23541506); deoxydoxorubicin (NSC 267469; CAS Registry No. 63950061); mitoxantrone (NSC 301739; CAS Registry No. 70476823); menogaril (NSC 269148; CAS Registry No. 71628961); N,N-dibenzyl daunomycin (NSC 268242; CAS Registry No. 70878512); oxanthrazole (NSC 349174; CAS Registry No. 105118125); rubidazone (NSC 164011; CAS Registry No. 36508711); teniposide (VM-26; NSC 122819; CAS Registry No. 29767202).

[0158] DNA Intercalating Agents: anthramycin (CAS Registry No. 4803274); chicamycin A (CAS Registry No. 89675376); tomaymycin (CAS Registry No. 35050556); DC-81 (CAS Registry No. 81307246); sibiromycin (CAS Registry No. 12684332); pyrrolobenzodiazepine derivative (CAS Registry No. 945490095); SGD-1882 ((S)-2-(4-aminophenyl)-7-methoxy-8-(3-4(S)-7-methoxy-2-(4-methoxyphenyl)-- 5-oxo-5,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propox- y)-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5(11aH)-one); SG2000 (SJG-136; (11aS,11a'S)-8,8'-(propane-1,3-diylbis(oxy))bis(7-methoxy-2-methylene-2,3- -dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5(11aH)-one); NSC 694501; CAS Registry No. 232931576).

[0159] RNA/DNA Antimetabolites: L-alanosine (NSC 153353; CAS Registry No. 59163416); 5-azacytidine (NSC 102816; CAS Registry No. 320672); 5-fluorouracil (NSC 19893; CAS Registry No. 51218); acivicin (NSC 163501; CAS Registry No. 42228922); aminopterin

derivative N-[2-chloro-5-[[(2,4-diamino-5-methyl-6-quinazolinyl)methyl]amino]benzoyl-]L-aspartic acid (NSC 132483); aminopterin derivative N-[4-[[(2,4-diamino-5-ethyl-6-quinazolinyl)methyl]amino]benzoyl]L-asparti- c acid (NSC 184692); aminopterin derivative N-[2-chloro-4-[[(2,4-diamino-6-pteridinyl)methyl]amino]benzoyl]L-aspartic acid monohydrate (NSC 134033); an antifo ((N$^{\alpha}$ -(4-amino-4-deoxypteroyl)-N^{7}-hemiphthaloyl-L-ornithin- e; NSC 623017)); Baker's soluble antifol (NSC 139105; CAS Registry No. 41191042); dichlorallyl lawsone ((2-(3,3-dichloroallyl)-3-hydroxy-1,4-naphthoquinone; NSC 126771; CAS Registry No. 36417160); brequinar (NSC 368390; CAS Registry No. 96201886); ftorafur ((pro-drug; 5-fluoro-1-(tetrahydro-2-furyl)-uracil; NSC 148958; CAS Registry No. 37076689); 5,6-dihydro-5-azacytidine (NSC 264880; CAS Registry No. 62402317); methotrexate (NSC 740; CAS Registry No. 59052); methotrexate derivative (N-[[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]-1-naphthalenyl]car- bonyl]L-glutamic acid; NSC 174121); PALA ((N-(phosphonoacetyl)-L-aspartate; NSC 224131; CAS Registry No. 603425565); pyrazofurin (NSC 143095; CAS Registry No. 30868305); trimetrexate (NSC 352122; CAS Registry No. 82952645).

[0160] DNA Antimetabolites: 3-HP (NSC 95678; CAS Registry No. 3814797); 2'-deoxy-5-fluorouridine (NSC 27640; CAS Registry No. 50919); 5-HP (NSC 107392; CAS Registry No. 19494894); α-TGDR (α-2'-deoxy-6-thioguanosine; NSC 71851 CAS Registry No. 2133815); aphidicolin glycinate (NSC 303812; CAS Registry No. 92802822); ara C (cytosine arabinoside; NSC 63878; CAS Registry No. 69749); 5-aza-2'-deoxycytidine (NSC 127716; CAS Registry No. 2353335); β-TGDR (β-2'-deoxy-6-thioguanosine; NSC 71261; CAS Registry No. 789617); cyclocytidine (NSC 145668; CAS Registry No. 10212256); guanazole (NSC 1895; CAS Registry No. 1455772); hydroxyurea (NSC 32065; CAS Registry No. 127071); inosine glycodialdehyde (NSC 118994; CAS Registry No. 23590990); macbecin II (NSC 330500; CAS Registry No. 73341738); pyrazoloimidazole (NSC 51143; CAS Registry No. 6714290); thioguanine (NSC 752; CAS Registry No. 154427); thiopurine (NSC 755; CAS Registry No. 50442).

[0161] Cell Cycle Modulators: silibinin (CAS Registry No. 22888-70-6); epigallocatechin gallate (EGCG; CAS Registry No. 989515); procyanidin derivatives (*e.g.*, procyanidin A1 [CAS Registry No. 103883030], procyanidin B1 [CAS Registry No. 20315257], procyanidin B4 [CAS Registry No. 29106512], arecatannin B1 [CAS Registry No. 79763283]); isoflavones (*e.g.*, genistein [4%5,7-trihydroxyisoflavone; CAS Registry No. 446720], daidzein [4',7-dihydroxyisoflavone, CAS Registry No. 486668]; indole-3-carbinol (CAS Registry No. 700061); quercetin (NSC 9219; CAS Registry No. 117395); estramustine (NSC 89201; CAS Registry No. 2998574); nocodazole (CAS Registry No. 31430189); podophyllotoxin (CAS Registry No. 518285); vinorelbine tartrate (NSC 608210; CAS Registry No. 125317397); cryptophycin (NSC 667642; CAS Registry No. 124689652).

[0162] Kinase Inhibitors: afatinib (CAS Registry No. 850140726); axitinib (CAS Registry No. 319460850); ARRY-438162 (binimetinib) (CAS Registry No. 606143899); bosutinib (CAS Registry No. 380843754); cabozantinib (CAS Registry No. 1140909483); ceritinib (CAS Registry No. 1032900256); crizotinib (CAS Registry No. 877399525); dabrafenib (CAS Registry No. 1195765457); dasatinib (NSC 732517; CAS Registry No. 302962498); erlotinib (NSC 718781; CAS Registry No. 183319699); everolimus (NSC 733504; CAS Registry No. 159351696); fostamatinib (NSC 745942; CAS Registry No. 901119355); gefitinib (NSC 715055; CAS Registry No. 184475352); ibrutinib (CAS Registry No. 936563961); imatinib (NSC 716051; CAS Registry No. 220127571); lapatinib (CAS Registry No. 388082788); lenvatinib (CAS Registry No. 857890392); mubritinib (CAS 366017096); nilotinib (CAS Registry No. 923288953); nintedanib (CAS Registry No. 656247175); palbociclib (CAS Registry No. 571190302); pazopanib (NSC 737754; CAS Registry No. 635702646); pegaptanib (CAS Registry No. 222716861); ponatinib (CAS Registry No. 1114544318); rapamycin (NSC 226080; CAS Registry No. 53123889); regorafenib (CAS Registry No. 755037037); AP 23573 (ridaforolimus) (CAS Registry No. 572924540); INCB018424 (ruxolitinib) (CAS Registry No. 1092939177); ARRY-142886 (selumetinib) (NSC 741078; CAS Registry No. 606143-52-6); sirolimus (NSC 226080; CAS Registry No. 53123889); sorafenib (NSC 724772; CAS Registry No. 475207591); sunitinib (NSC 736511; CAS Registry No. 341031547); tofacitinib (CAS Registry No. 477600752); temsirolimus (NSC 683864; CAS Registry No. 163635043); trametinib (CAS Registry No. 871700173); vandetanib (CAS Registry No. 443913733); vemurafenib (CAS Registry No. 918504651); SU6656 (CAS Registry No. 330161870); CEP-701 (lesaurtinib) (CAS Registry No. 111358884); XL019 (CAS Registry No. 945755566); PD-325901 (CAS Registry No. 391210109); PD-98059 (CAS Registry No. 167869218); ATP-competitive TORC1/TORC2 inhibitors including PI-103 (CAS Registry No. 371935749), PP242 (CAS Registry No. 1092351671), PP30 (CAS Registry No. 1092788094), Torin 1 (CAS Registry No. 1222998368), LY294002 (CAS Registry No. 154447366), XL-147 (CAS Registry No. 934526893), CAL-120 (CAS Registry No. 870281348), ETP-45658 (CAS Registry No. 1198357797), PX 866 (CAS Registry No. 502632668), GDC-0941 (CAS Registry No. 957054307), BGT226 (CAS Registry No. 1245537681), BEZ235 (CAS Registry No. 915019657), XL-765 (CAS Registry No. 934493762).

[0163] Protein Synthesis Inhibitors: acriflavine (CAS Registry No. 65589700); amikacin (NSC 177001; CAS Registry No. 39831555); arbekacin (CAS Registry No. 51025855); astromicin (CAS Registry No. 55779061); azithromycin (NSC 643732; CAS Registry No. 83905015); bekanamycin (CAS Registry No. 4696768); chlortetracycline (NSC 13252; CAS Registry No. 64722); clarithromycin (NSC 643733; CAS Registry No. 81103119); clindamycin (CAS Registry No. 18323449); clomocycline (CAS Registry No. 1181540); cycloheximide (CAS Registry No. 66819); dactinomycin (NSC 3053; CAS Registry No. 50760); dalfopristin (CAS Registry No. 112362502); demeclocycline (CAS Registry No. 127333); dibekacin (CAS Registry No.

34493986); dihydrostreptomycin (CAS Registry No. 128461); dirithromycin (CAS Registry No. 62013041); doxycycline (CAS Registry No. 17086281); emetine (NSC 33669; CAS Registry No. 483181); erythromycin (NSC 55929; CAS Registry No. 114078); flurithromycin (CAS Registry No. 83664208); framycetin (neomycin B; CAS Registry No. 119040); gentamycin (NSC 82261; CAS Registry No. 1403663); glycylcyclines, such as tigecycline (CAS Registry No. 220620097); hygromycin B (CAS Registry No. 31282049); isepamicin (CAS Registry No. 67814760); josamycin (NSC 122223; CAS Registry No. 16846245); kanamycin (CAS Registry No. 8063078); ketolides such as telithromycin (CAS Registry No. 191114484), cethromycin (CAS Registry No. 205110481), and solithromycin (CAS Registry No. 760981837); lincomycin (CAS Registry No. 154212); lymecycline (CAS Registry No. 992212); meclocycline (NSC 78502; CAS Registry No. 2013583); metacycline (rondomycin; NSC 356463; CAS Registry No. 914001); midecamycin (CAS Registry No. 35457808); minocycline (NSC 141993; CAS Registry No. 10118908); miocamycin (CAS Registry No. 55881077); neomycin (CAS Registry No. 119040); netilmicin (CAS Registry No. 56391561); oleandomycin (CAS Registry No. 3922905); oxazolidinones, such as eperezolid (CAS Registry No. 165800044), linezolid (CAS Registry No. 165800033), posizolid (CAS Registry No. 252260029), radezolid (CAS Registry No. 869884786), ranbezolid (CAS Registry No. 392659380), sutezolid (CAS Registry No. 168828588), tedizolid (CAS Registry No. 856867555); oxytetracycline (NSC 9169; CAS Registry No. 2058460); paromomycin (CAS Registry No. 7542372); penimepicycline (CAS Registry No. 4599604); peptidyl transferase inhibitors, *e.g.*, chloramphenicol (NSC 3069; CAS Registry No. 56757) and derivatives such as azidamfenicol (CAS Registry No. 13838089), florfenicol (CAS Registry No. 73231342), and thiamphenicol (CAS Registry No. 15318453), and pleuromutilins such as retapamulin (CAS Registry No. 224452668), tiamulin (CAS Registry No. 55297955), valnemulin (CAS Registry No. 101312929); pirlimycin (CAS Registry No. 79548735); puromycin (NSC 3055; CAS Registry No. 53792); quinupristin (CAS Registry No. 120138503); ribostamycin (CAS Registry No. 53797356); rokitamycin (CAS Registry No. 74014510); rolitetracycline (CAS Registry No. 751973); roxithromycin (CAS Registry No. 80214831); sisomicin (CAS Registry No. 32385118); spectinomycin (CAS Registry No. 1695778); spiramycin (CAS Registry No. 8025818); streptogramins such as pristinamycin (CAS Registry No. 270076603), quinupristin/dalfopristin (CAS Registry No. 126602899), and virginiamycin (CAS Registry No. 11006761); streptomycin (CAS Registry No. 57921); tetracycline (NSC 108579; CAS Registry No. 60548); tobramycin (CAS Registry No. 32986564); troleandomycin (CAS Registry No. 2751099); tylosin (CAS Registry No. 1401690); verdamicin (CAS Registry No. 49863481).

[0164] Histone Deacetylase Inhibitors: abexinostat (CAS Registry No. 783355602); belinostat (NSC 726630; CAS Registry No. 414864009); chidamide (CAS Registry No. 743420022); entinostat (CAS Registry No. 209783802); givinostat (CAS Registry No. 732302997); mocetinostat (CAS Registry No. 726169739); panobinostat (CAS Registry No. 404950807);

quisinostat (CAS Registry No. 875320299); resminostat (CAS Registry No. 864814880); romidepsin (CAS Registry No. 128517077); sulforaphane (CAS Registry No. 4478937); thioureidobutyronitrile (Kevetrin™; CAS Registry No. 6659890); valproic acid (NSC 93819; CAS Registry No. 99661); vorinostat (NSC 701852; CAS Registry No. 149647789); ACY-1215 (rocilinostat; CAS Registry No. 1316214524); CUDC-101 (CAS Registry No. 1012054599); CHR-2845 (tefinostat; CAS Registry No. 914382608); CHR-3996 (CAS Registry No. 1235859138); 4SC-202 (CAS Registry No. 910462430); CG200745 (CAS Registry No. 936221339); SB939 (pracinostat; CAS Registry No. 929016966).

[0165] Mitochondria Inhibitors: pancratistatin (NSC 349156; CAS Registry No. 96281311); rhodamine-123 (CAS Registry No. 63669709); edelfosine (NSC 324368; CAS Registry No. 70641519); d-alpha-tocopherol succinate (NSC 173849; CAS Registry No. 4345033); compound 11β (CAS Registry No. 865070377); aspirin (NSC 406186; CAS Registry No. 50782); ellipticine (CAS Registry No. 519233); berberine (CAS Registry No. 633658); cerulenin (CAS Registry No. 17397896); GX015-070 (Obatoclax®; 1H-Indole, 2-(2-((3,5-dimethyl-1H-pyrrol-2-yl)methylene)-3-methoxy-2H-pyrrol-5-yl)-; NSC 729280; CAS Registry No. 803712676); celastrol (tripterine; CAS Registry No. 34157830); metformin (NSC 91485; CAS Registry No. 1115704); Brilliant green (NSC 5011; CAS Registry No. 633034); ME-344 (CAS Registry No. 1374524556).

[0166] Antimitotic Agents: allocolchicine (NSC 406042); auristatins, such as MMAE (monomethyl auristatin E; CAS Registry No. 474645-27-7) and MMAF (monomethyl auristatin F; CAS Registry No. 745017-94-1; halichondrin B (NSC 609395); colchicine (NSC 757; CAS Registry No. 64868); cholchicine derivative (N-benzoyl-deacetyl benzamide; NSC 33410; CAS Registry No. 63989753); dolastatin 10 (NSC 376128; CAS Registry No 110417-88-4); maytansine (NSC 153858; CAS Registry No. 35846-53-8); rhozoxin (NSC 332598; CAS Registry No. 90996546); taxol (NSC 125973; CAS Registry No. 33069624); taxol derivative ((2'-N-[3-(dimethylamino)propyl]glutaramate taxol; NSC 608832); thiocolchicine (3-demethylthiocolchicine; NSC 361792); trityl cysteine (NSC 49842; CAS Registry No. 2799077); vinblastine sulfate (NSC 49842; CAS Registry No. 143679); vincristine sulfate (NSC 67574; CAS Registry No. 2068782).

[0167] Any of these agents that include or that may be modified to include a site of attachment to an antibody may be included in the ADCs disclosed herein.

[0168] In a specific embodiment, the cytotoxic and/or cytostatic agent is an antimitotic agent.

[0169] In another specific embodiment, the cytotoxic and/or cytostatic agent is an auristatin, for example, monomethyl auristatin E ("MMAE") or monomethyl auristatin F ("MMAF").

5.2.2. Linkers

[0170] In the anti-glyco-MUC4 ADCs of the disclosure, the cytotoxic and/or cytostatic agents are linked to the antibody by way of linkers. The linker linking a cytotoxic and/or cytostatic agent to the antibody of an ADC may be short, long, hydrophobic, hydrophilic, flexible or rigid, or may be composed of segments that each independently have one or more of the above-mentioned properties such that the linker may include segments having different properties. The linkers may be polyvalent such that they covalently link more than one agent to a single site on the antibody, or monovalent such that covalently they link a single agent to a single site on the antibody.

[0171] As will be appreciated by skilled artisans, the linkers link cytotoxic and/or cytostatic agents to the antibody by forming a covalent linkage to the cytotoxic and/or cytostatic agent at one location and a covalent linkage to antibody at another. The covalent linkages are formed by reaction between functional groups on the linker and functional groups on the agents and antibody. As used herein, the expression “linker” is intended to include (i) unconjugated forms of the linker that include a functional group capable of covalently linking the linker to a cytotoxic and/or cytostatic agent and a functional group capable of covalently linking the linker to an antibody; (ii) partially conjugated forms of the linker that includes a functional group capable of covalently linking the linker to an antibody and that is covalently linked to a cytotoxic and/or cytostatic agent, or vice versa; and (iii) fully conjugated forms of the linker that is covalently linked to both a cytotoxic and/or cytostatic agent and an antibody. In some specific embodiments of linkers and anti-glyco-MUC4 ADCs of the disclosure, as well as synthons used to conjugate linker-agents to antibodies, moieties comprising the functional groups on the linker and covalent linkages formed between the linker and antibody are specifically illustrated as R_x and XY, respectively.

[0172] The linkers are preferably, but need not be, chemically stable to conditions outside the cell, and may be designed to cleave, immolate and/or otherwise specifically degrade inside the cell. Alternatively, linkers that are not designed to specifically cleave or degrade inside the cell may be used. Choice of stable versus unstable linker may depend upon the toxicity of the cytotoxic and/or cytostatic agent. For agents that are toxic to normal cells, stable linkers are preferred. Agents that are selective or targeted and have lower toxicity to normal cells may utilize, chemical stability of the linker to the extracellular milieu is less important. A wide variety of linkers useful for linking drugs to antibodies in the context of ADCs are known in the art. Any of these linkers, as well as other linkers, may be used to link the cytotoxic and/or cytostatic agents to the antibody of the anti-glyco-MUC4 ADCs of the disclosure.

[0173] Exemplary polyvalent linkers that may be used to link many cytotoxic and/or cytostatic agents to a single antibody molecule are described, for example, in WO 2009/073445; WO 2010/068795; WO 2010/138719; WO 2011/120053; WO 2011/171020; WO 2013/096901; WO

2014/008375; WO 2014/093379; WO 2014/093394; WO 2014/093640, the content of which are incorporated herein by reference in their entireties. For example, the Fleximer linker technology developed by Mersana *et al.* has the potential to enable high-DAR ADCs with good physicochemical properties. As shown below, the Mersana technology is based on incorporating drug molecules into a solubilizing poly-acetal backbone via a sequence of ester bonds. The methodology renders highly-loaded ADCs (DAR up to 20) while maintaining good physicochemical properties.

[0174] Additional examples of dendritic type linkers can be found in US 2006/116422; US 2005/271615; de Groot *et al.* (2003) Angew. Chem. Int. Ed. 42:4490-4494; Amir *et al.* (2003) Angew. Chem. Int. Ed. 42:4494-4499; Shamis *et al.* (2004) J. Am. Chem. Soc. 126:1726-1731; Sun *et al.* (2002) Bioorganic & Medicinal Chemistry Letters 12:2213-2215; Sun *et al.* (2003) Bioorganic & Medicinal Chemistry 11:1761-1768; King *et al.*(2002) Tetrahedron Letters 43:1987-1990, each of which is incorporated herein by reference.

[0175] Exemplary monovalent linkers that may be used are described, for example, in Nolting, 2013, Antibody-Drug Conjugates, Methods in Molecular Biology 1045:71-100; Kitson *et al.*, 2013, CROs/CMOs--Chemica Oggi--Chemistry Today 31(4):30-38; Ducry *et al.*, 2010, Bioconjugate Chem. 21:5-13; Zhao *et al.*, 2011, J. Med. Chem. 54:3606-3623; U.S. Pat. No. 7,223,837; U.S. Pat. No. 8,568,728; U.S. Pat. No. 8,535,678; and WO2004010957, each of which is incorporated herein by reference.

[0176] Additional exemplary linkers and associated methods and chemistries are provided that are stable in blood, provide for site-specific and stable conjugation, and provides for cancer-specific activation via specific enzymes found in cancer cells. Site specific conjugation allows for production of homogenous ADCs, while plasma-stable linkers enable cancer-specific toxin release. In some embodiments, a functionalized prenyl substrate can be covalently joined to Cys of CaaX amino acid sequence introduced at the C-terminus of a light chain by prenyl transferase (*e.g.*, farnesyl transferase). Drug conjugation may then occur via click chemistry or oxime ligation between isoprenoid and linker functionalities. Exemplary linkers, associate methods, and associate chemistries that may be used are described in, for example, WO 2012/153193, WO 2015/182984; WO 2017/089890; WO 2017/089894; WO 2017/089895; WO 2017/051249; WO 2017/051254; WO 2018/182341; WO 2020/222573; WO 2021/137646; and WO 2020/141923, each of which is hereby incorporated by reference in its entirety.

[0177] By way of example and not limitation, some cleavable and noncleavable linkers that may be included in the anti-glyco-MUC4 ADCs of the disclosure are described below.

5.2.3. Cleavable Linkers

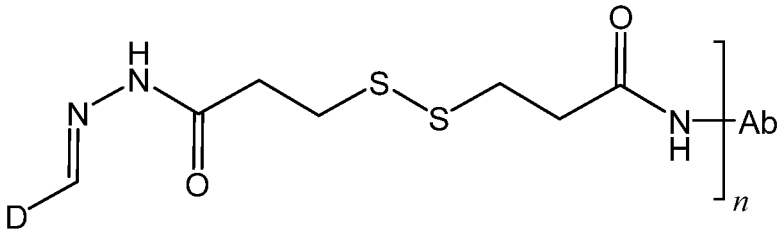
[0178] In certain embodiments, the linker selected is cleavable *in vivo*. Cleavable linkers may include chemically or enzymatically unstable or degradable linkages. Cleavable linkers

generally rely on processes inside the cell to liberate the drug, such as reduction in the cytoplasm, exposure to acidic conditions in the lysosome, or cleavage by specific proteases or other enzymes within the cell. Cleavable linkers generally incorporate one or more chemical bonds that are either chemically or enzymatically cleavable while the remainder of the linker is noncleavable. In certain embodiments, a linker comprises a chemically labile group such as hydrazone and/or disulfide groups. Linkers comprising chemically labile groups exploit differential properties between the plasma and some cytoplasmic compartments. The intracellular conditions to facilitate drug release for hydrazone containing linkers are the acidic environment of endosomes and lysosomes, while the disulfide containing linkers are reduced in the cytosol, which contains high thiol concentrations, *e.g.*, glutathione. In certain embodiments, the plasma stability of a linker comprising a chemically labile group may be increased by introducing steric hindrance using substituents near the chemically labile group.

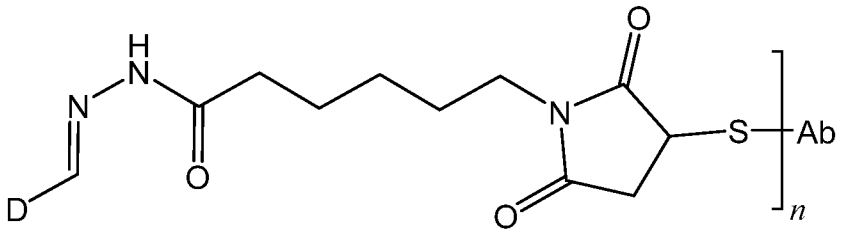
[0179] Acid-labile groups, such as hydrazone, remain intact during systemic circulation in the blood's neutral pH environment (pH 7.3-7.5) and undergo hydrolysis and release the drug once the ADC is internalized into mildly acidic endosomal (pH 5.0-6.5) and lysosomal (pH 4.5-5.0) compartments of the cell. This pH dependent release mechanism has been associated with nonspecific release of the drug. To increase the stability of the hydrazone group of the linker, the linker may be varied by chemical modification, *e.g.*, substitution, allowing tuning to achieve more efficient release in the lysosome with a minimized loss in circulation.

[0180] Hydrazone-containing linkers may contain additional cleavage sites, such as additional acid-labile cleavage sites and/or enzymatically labile cleavage sites. ADCs including exemplary hydrazone-containing linkers include the following structures:

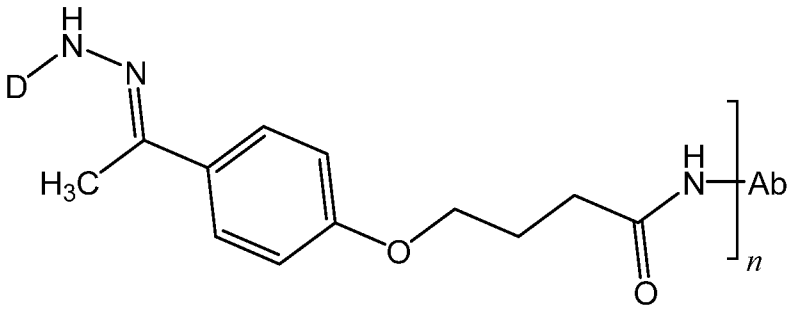
(Ig)



(Ih)



(Ii)



wherein D and Ab represent the cytotoxic and/or cytostatic agent (drug) and Ab, respectively, and n represents the number of drug-linkers linked to the antibody. In certain linkers such as linker (Ig), the linker comprises two cleavable groups--a disulfide and a hydrazone moiety. For such linkers, effective release of the unmodified free drug requires acidic pH or disulfide reduction and acidic pH. Linkers such as (Ih) and (Ii) have been shown to be effective with a single hydrazone cleavage site.

[0181] Additional linkers which remain intact during systemic circulation and undergo hydrolysis and release the drug when the ADC is internalized into acidic cellular compartments include carbonates. Such linkers can be useful in cases where the cytotoxic and/or cytostatic agent can be covalently attached through an oxygen.

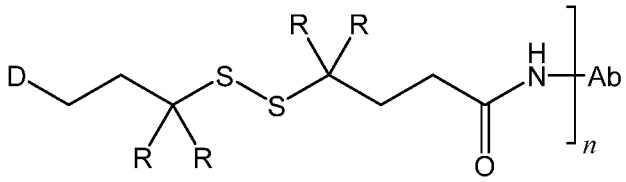
[0182] Other acid-labile groups that may be included in linkers include cis-aconityl-containing linkers. cis-Aconityl chemistry uses a carboxylic acid juxtaposed to an amide bond to accelerate amide hydrolysis under acidic conditions.

[0183] Cleavable linkers may also include a disulfide group. Disulfides are thermodynamically stable at physiological pH and are designed to release the drug upon internalization inside cells, wherein the cytosol provides a significantly more reducing environment compared to the extracellular environment. Scission of disulfide bonds generally requires the presence of a cytoplasmic thiol cofactor, such as (reduced) glutathione (GSH), such that disulfide-containing

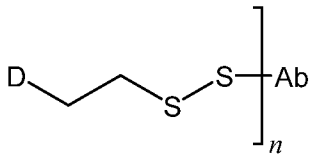
linkers are reasonably stable in circulation, selectively releasing the drug in the cytosol. The intracellular enzyme protein disulfide isomerase, or similar enzymes capable of cleaving disulfide bonds, may also contribute to the preferential cleavage of disulfide bonds inside cells. GSH is reported to be present in cells in the concentration range of 0.5-10 mM compared with a significantly lower concentration of GSH or cysteine, the most abundant low-molecular weight thiol, in circulation at approximately 5 Tumor cells, where irregular blood flow leads to a hypoxic state, result in enhanced activity of reductive enzymes and therefore even higher glutathione concentrations. In certain embodiments, the *in vivo* stability of a disulfide-containing linker may be enhanced by chemical modification of the linker, *e.g.*, use of steric hindrance adjacent to the disulfide bond.

[0184] ADCs including exemplary disulfide-containing linkers include the following structures:

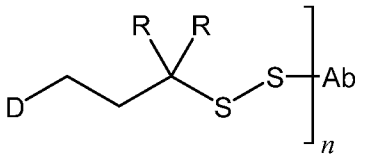
(Ij)



(Ik)



(Il)



wherein D and Ab represent the drug and antibody, respectively, n represents the number of drug-linkers linked to the antibody and R is independently selected at each occurrence from hydrogen or alkyl, for example. In certain embodiments, increasing steric hindrance adjacent to the disulfide bond increases the stability of the linker. Structures such as (Ij) and (Il) show increased *in vivo* stability when one or more R groups is selected from a lower alkyl, such as methyl.

[0185] Another type of cleavable linker that may be used is a linker that is specifically cleaved by an enzyme. Such linkers are typically peptide-based or include peptidic regions that act as substrates for enzymes. Peptide based linkers tend to be more stable in plasma and extracellular milieu than chemically labile linkers. Peptide bonds generally have good serum stability, as lysosomal proteolytic enzymes have very low activity in blood due to endogenous inhibitors and the unfavorably high pH value of blood compared to lysosomes. Release of a

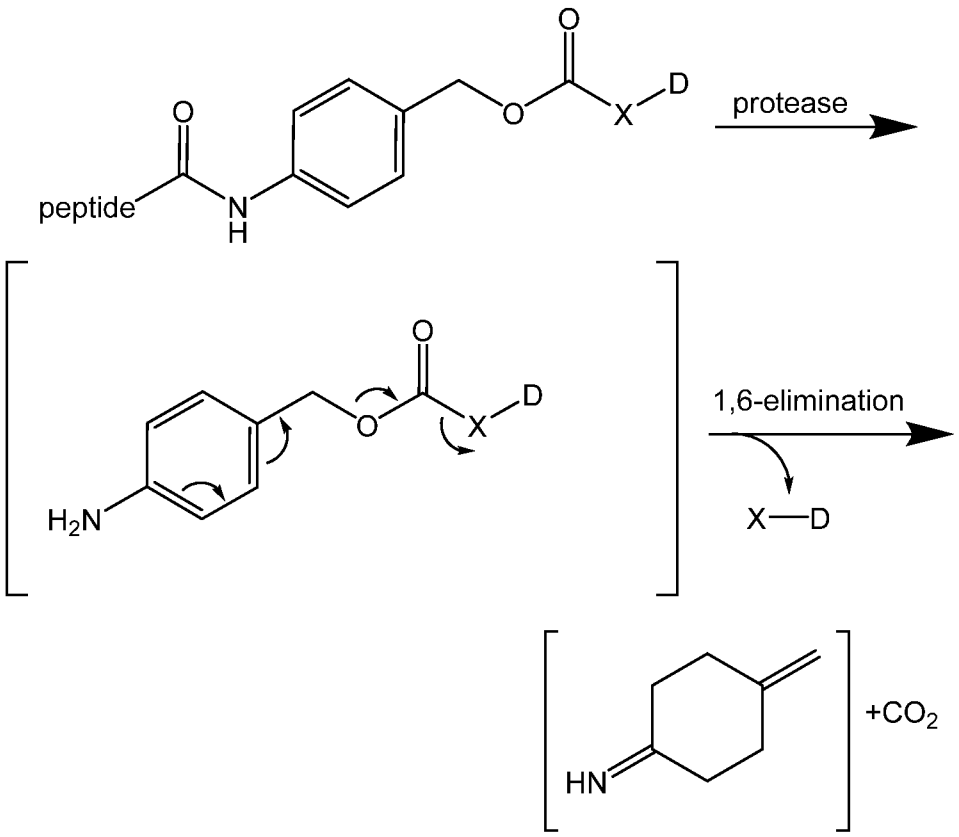
drug from an antibody occurs specifically due to the action of lysosomal proteases, *e.g.*, cathepsin and plasmin. These proteases may be present at elevated levels in certain tumor cells.

[0186] In exemplary embodiments, the cleavable peptide is selected from tetrapeptides such as Gly-Phe-Leu-Gly (SEQ ID NO:157), Ala-Leu-Ala-Leu (SEQ ID NO:158) or dipeptides such as Val-Cit, Val-Ala, Met-(D)Lys, Asn-(D)Lys, Val-(D)Asp, Phe-Lys, Ile-Val, Asp-Val, His-Val, NorVal-(D)Asp, Ala-(D)Asp 5, Met-Lys, Asn-Lys, Ile-Pro, Me3Lys-Pro, PhenylGly-(D)Lys, Met-(D)Lys, Asn-(D)Lys, Pro-(D)Lys, Met-(D)Lys, Asn-(D)Lys, AM Met-(D)Lys, Asn-(D)Lys, AW Met-(D)Lys, and Asn-(D)Lys. In certain embodiments, dipeptides are preferred over longer polypeptides due to hydrophobicity of the longer peptides.

[0187] A variety of dipeptide-based cleavable linkers useful for linking drugs such as doxorubicin, mitomycin, camptothecin, pyrrolobenzodiazepine, tallysomycin and auristatin/auristatin family members to antibodies have been described (see, Dubowchik *et al.*, 1998, J. Org. Chem. 67:1866-1872; Dubowchik *et al.*, 1998, Bioorg. Med. Chem. Lett. 8(21):3341-3346; Walker *et al.*, 2002, Bioorg. Med. Chem. Lett. 12:217-219; Walker *et al.*, 2004, Bioorg. Med. Chem. Lett. 14:4323-4327; Sutherland *et al.*, 2013, Blood 122: 1455-1463; and Francisco *et al.*, 2003, Blood 102:1458-1465, of each of which is incorporated herein by reference). All of these dipeptide linkers, or modified versions of these dipeptide linkers, may be used in the anti-glyco-MUC4 ADCs of the disclosure. Other dipeptide linkers that may be used include those found in ADCs such as Seattle Genetics' Brentuximab Vendotin SGN-35 (Adcetris™), Seattle Genetics SGN-75 (anti-CD-70, Val-Cit-monomethyl auristatin F(MMAF), Seattle Genetics SGN-CD33A (anti-CD-33, Val-Ala-(SGD-1882)), Celldex Therapeutics glembatumumab (CDX-011) (anti-NMB, Val-Cit-monomethyl auristatin E (MMAE), and Cytogen PSMA-ADC (PSMA-ADC-1301) (anti-PSMA, Val-Cit-MMAE).

[0188] Enzymatically cleavable linkers may include a self-immolative spacer to spatially separate the drug from the site of enzymatic cleavage. The direct attachment of a drug to a peptide linker can result in proteolytic release of an amino acid adduct of the drug, thereby impairing its activity. The use of a self-immolative spacer allows for the elimination of the fully active, chemically unmodified drug upon amide bond hydrolysis.

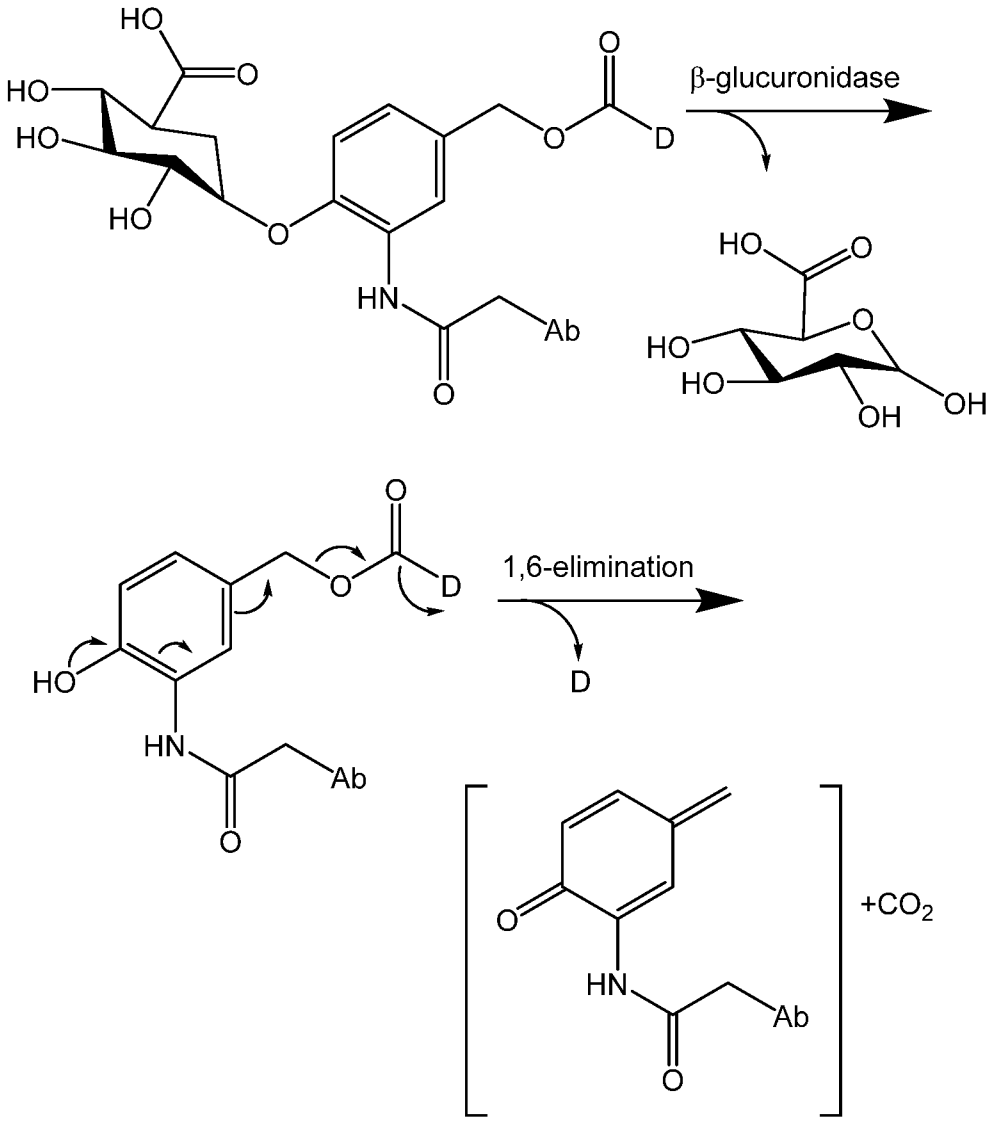
[0189] One self-immolative spacer is the bifunctional para-aminobenzyl alcohol group, which is linked to the peptide through the amino group, forming an amide bond, while amine containing drugs may be attached through carbamate functionalities to the benzylic hydroxyl group of the linker (PABC). The resulting prodrugs are activated upon protease-mediated cleavage, leading to a 1,6-elimination reaction releasing the unmodified drug, carbon dioxide, and remnants of the linker group. The following scheme depicts the fragmentation of p-amidobenzyl ether and release of the drug:



wherein X-D represents the unmodified drug.

[0190] Heterocyclic variants of this self-immolative group have also been described. See for example, U.S. Pat. No. 7,989,434, incorporated herein by reference.

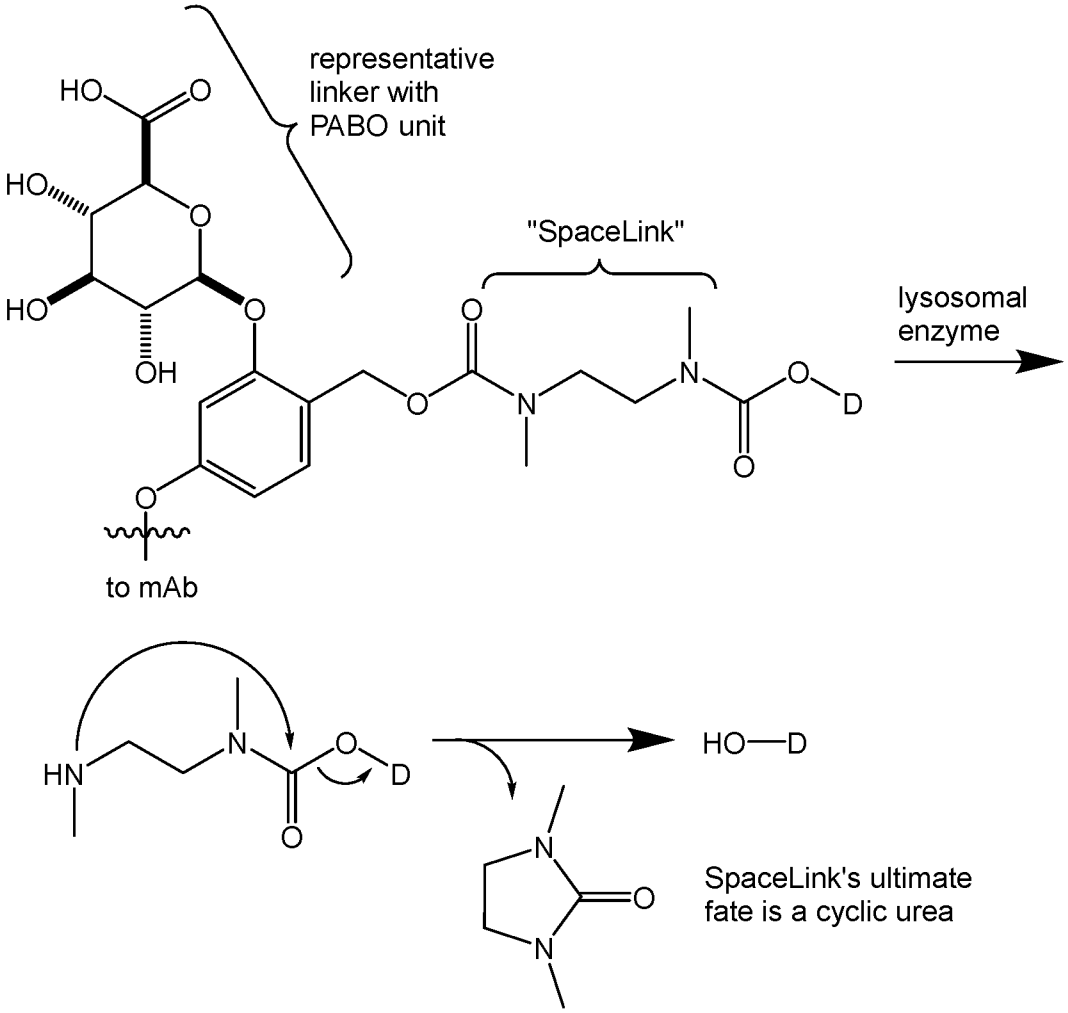
[0191] In some embodiments, the enzymatically cleavable linker is a β-glucuronic acid-based linker. Facile release of the drug may be realized through cleavage of the β-glucuronide glycosidic bond by the lysosomal enzyme β-glucuronidase. This enzyme is present abundantly within lysosomes and is overexpressed in some tumor types, while the enzyme activity outside cells is low. β-Glucuronic acid-based linkers may be used to circumvent the tendency of an ADC to undergo aggregation due to the hydrophilic nature of β-glucuronides. In some embodiments, β-glucuronic acid-based linkers are preferred as linkers for ADCs linked to hydrophobic drugs. The following scheme depicts the release of the drug from and ADC containing a β-glucuronic acid-based linker:



[0192] A variety of cleavable β-glucuronic acid-based linkers useful for linking drugs such as auristatins, camptothecin and doxorubicin analogues, CBI minor-groove binders, and psymberin to antibodies have been described (see, see Nolting, Chapter 5 "Linker Technology in Antibody-Drug Conjugates," In: Antibody-Drug Conjugates: Methods in Molecular Biology, vol. 1045, pp. 71-100, Laurent Ducry (Ed.), Springer Science & Business Medica, LLC, 2013; Jeffrey *et al.*, 2006, Bioconjug. Chem. 17:831-840; Jeffrey *et al.*, 2007, Bioorg. Med. Chem. Lett. 17:2278-2280; and Jiang *et al.*, 2005, J. Am. Chem. Soc. 127:11254-11255, each of which is incorporated herein by reference). All of these β-glucuronic acid-based linkers may be used in the anti-glyco-MUC4 ADCs of the disclosure.

[0193] Additionally, cytotoxic and/or cytostatic agents containing a phenol group can be covalently bonded to a linker through the phenolic oxygen. One such linker, described in WO 2007/089149, relies on a methodology in which a diamino-ethane "SpaceLink" is used in conjunction with traditional "PABO"-based self-immolative groups to deliver phenols. The

cleavage of the linker is depicted schematically below, where D represents a cytotoxic and/or cytostatic agent having a phenolic hydroxyl group.

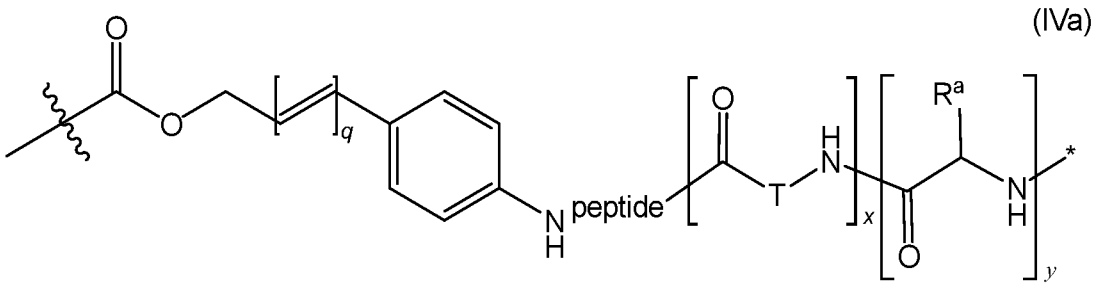


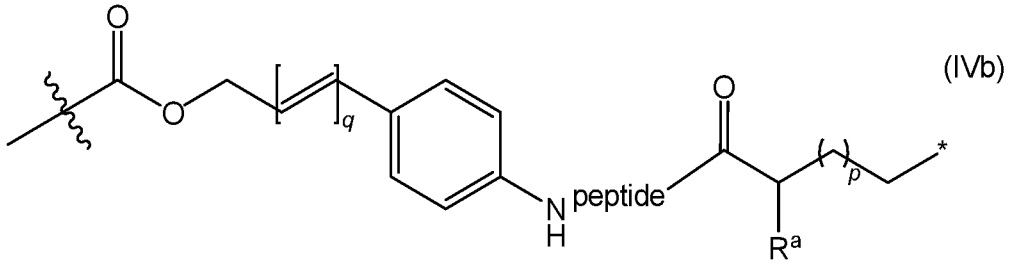
[0194] Cleavable linkers may include noncleavable portions or segments, and/or cleavable segments or portions may be included in an otherwise non-cleavable linker to render it cleavable. By way of example only, polyethylene glycol (PEG) and related polymers may include cleavable groups in the polymer backbone. For example, a polyethylene glycol or polymer linker may include one or more cleavable groups such as a disulfide, a hydrazone or a dipeptide.

[0195] Other degradable linkages that may be included in linkers include ester linkages formed by the reaction of PEG carboxylic acids or activated PEG carboxylic acids with alcohol groups on a biologically active agent, wherein such ester groups generally hydrolyze under physiological conditions to release the biologically active agent. Hydrolytically degradable linkages include, but are not limited to, carbonate linkages; imine linkages resulting from reaction of an amine and an aldehyde; phosphate ester linkages formed by reacting an alcohol with a phosphate group; acetal linkages that are the reaction product of an aldehyde and an

alcohol; orthoester linkages that are the reaction product of a formate and an alcohol; and oligonucleotide linkages formed by a phosphoramidite group, including but not limited to, at the end of a polymer, and a 5' hydroxyl group of an oligonucleotide.

[0196] In certain embodiments, the linker comprises an enzymatically cleavable peptide moiety, for example, a linker comprising structural formula (IVa) or (IVb):



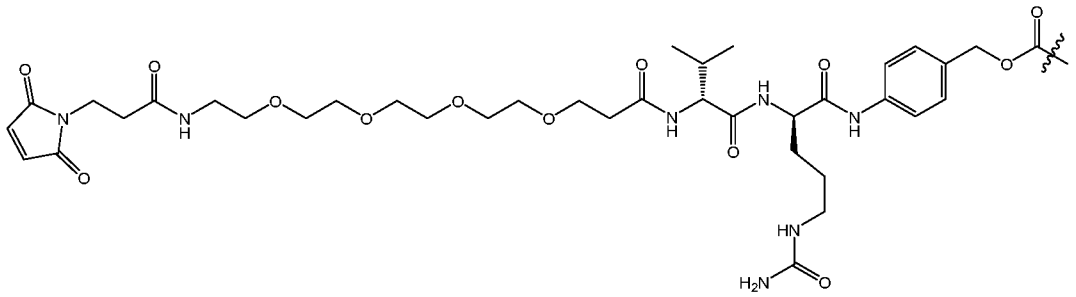


or a salt thereof, wherein: peptide represents a peptide (illustrated C→N and not showing the carboxy and amino "termini") cleavable by a lysosomal enzyme; T represents a polymer comprising one or more ethylene glycol units or an alkylene chain, or combinations thereof; R^a is selected from hydrogen, alkyl, sulfonate and methyl sulfonate; p is an integer ranging from 0 to 5; q is 0 or 1; x is 0 or 1; y is 0 or 1; ⁄ represents the point of attachment of the linker to a cytotoxic and/or cytostatic agent; and * represents the point of attachment to the remainder of the linker.

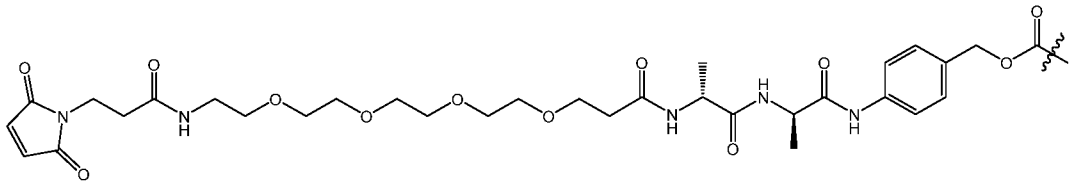
[0197] In certain embodiments, the peptide is selected from a tripeptide or a dipeptide. In particular embodiments, the dipeptide is selected from: Val-Cit; Cit-Val; Ala-Ala; Ala-Cit; Cit-Ala; Asn-Cit; Cit-Asn; Cit-Cit; Val-Glu; Glu-Val; Ser-Cit; Cit-Ser; Lys-Cit; Cit-Lys; Asp-Cit; Cit-Asp; Ala-Val; Val-Ala; Phe-Lys; Val-Lys; Ala-Lys; Phe-Cit; Leu-Cit; Ile-Cit; Phe-Arg; and Trp-Cit. In certain embodiments, the dipeptide is selected from: Cit-Val; and Ala-Val.

[0198] Specific exemplary embodiments of linkers according to structural formula (IVa) that may be included in the anti-glyco-MUC4 ADCs of the disclosure include the linkers illustrated below (as illustrated, the linkers include a group suitable for covalently linking the linker to an antibody):

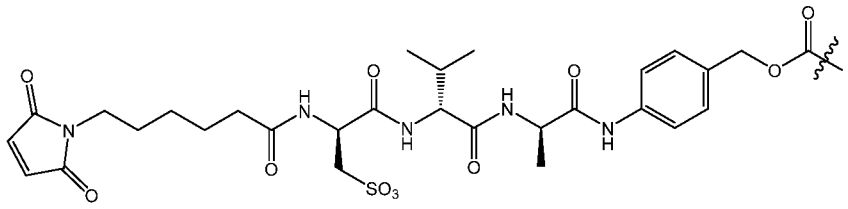
(IVa.1)



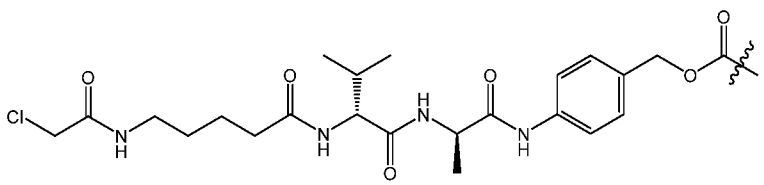
(IVa.2)



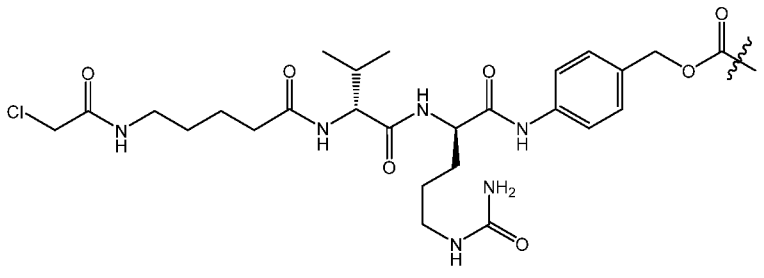
(IVa.3)



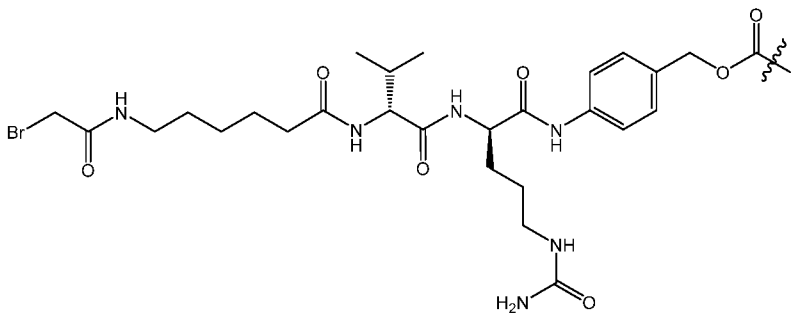
(IVa.4)



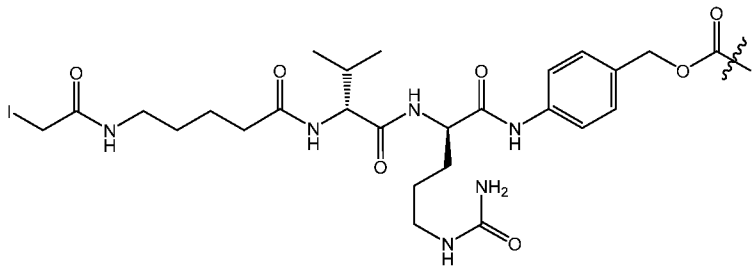
(IVa.5)



(IVa.6)

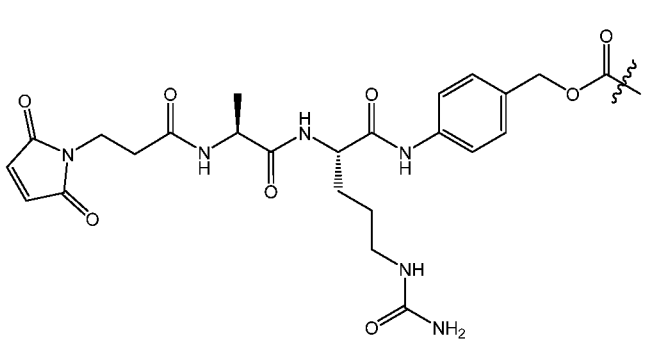


(IVa.7)

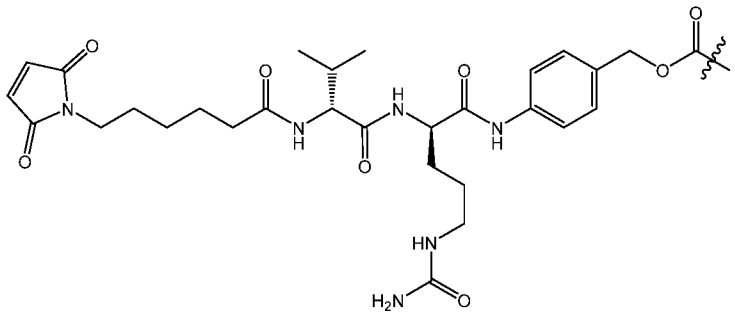


[0199] Specific exemplary embodiments of linkers according to structural formula (IVb) that may be included in the anti-glyco-MUC4 ADCs of the disclosure include the linkers illustrated below (as illustrated, the linkers include a group suitable for covalently linking the linker to an antibody):

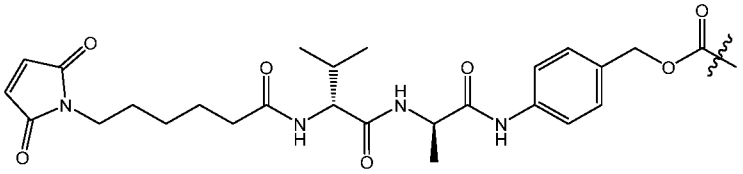
(IVb.1)



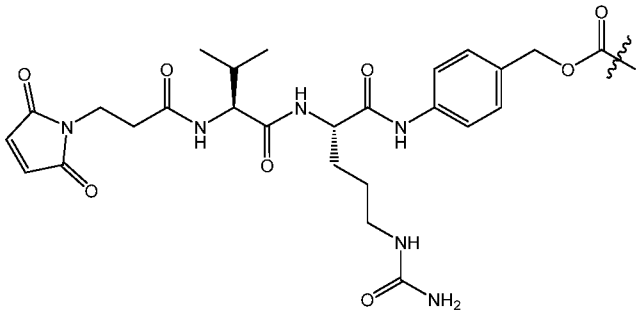
(IVb.2)



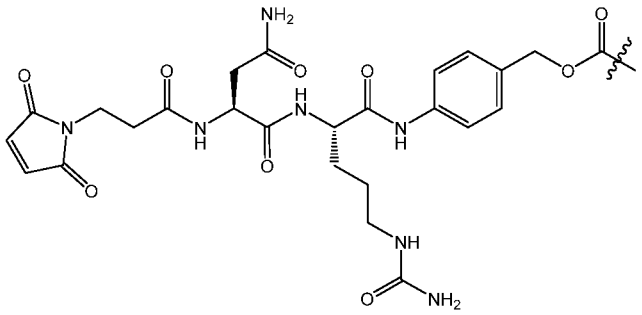
(IVb.3)



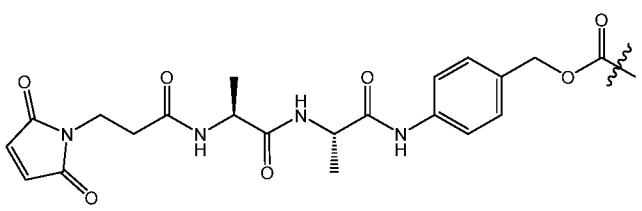
(IVb.4)



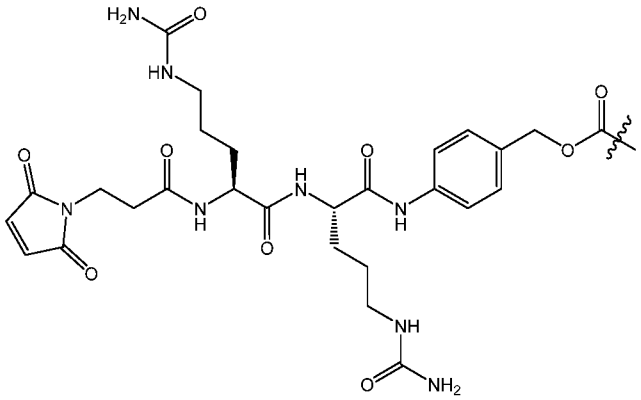
(IVb.5)



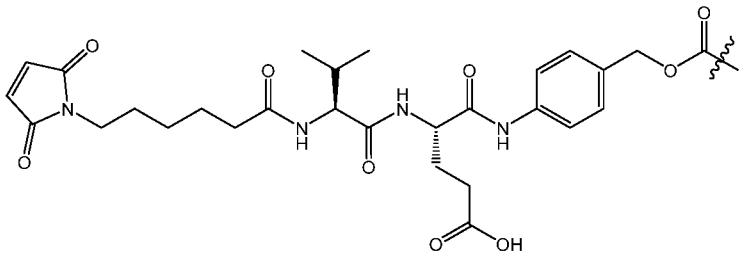
(IVb.6)



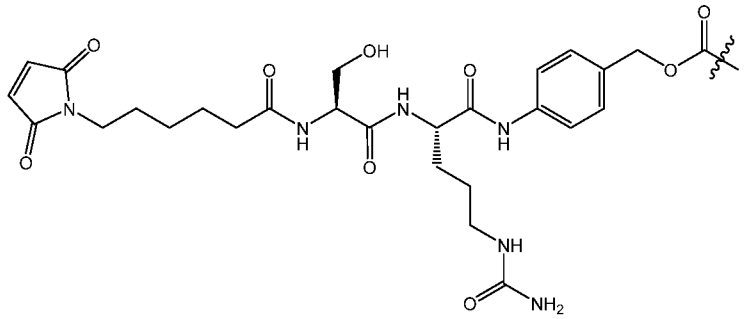
(IVb.7)



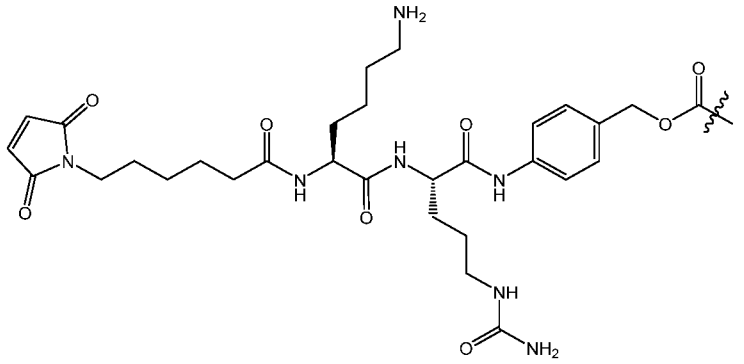
(IVb.8)



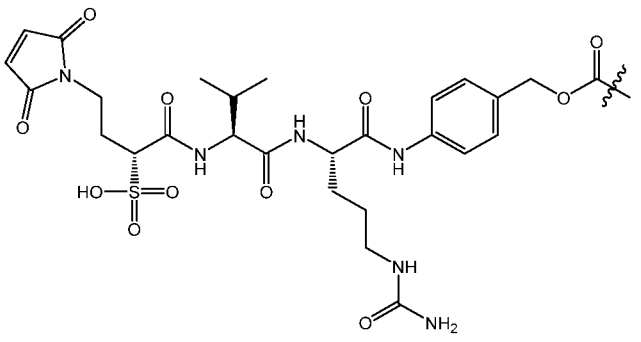
(IVb.9)



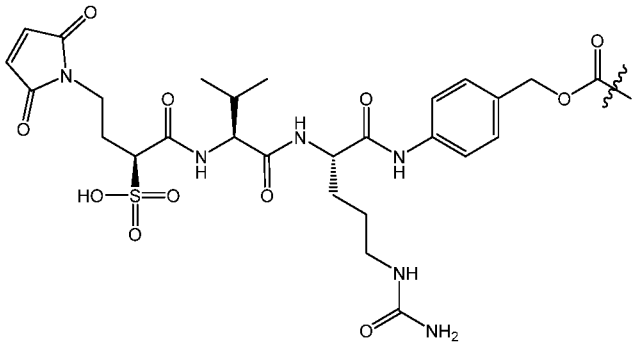
(IVb.10)



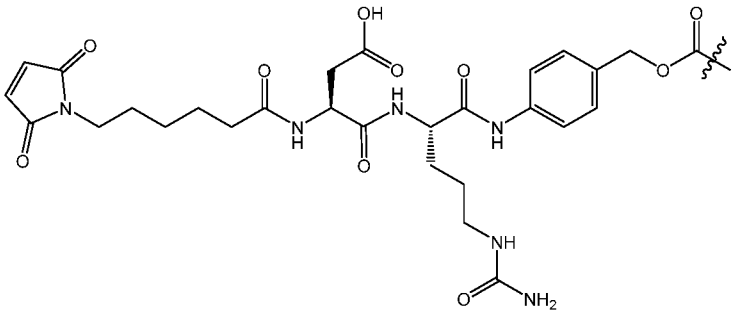
(IVb.11)



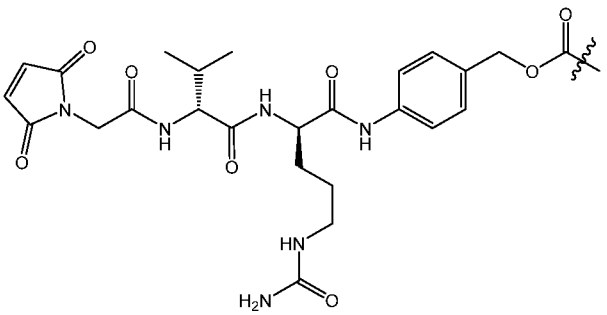
(IVb.12)



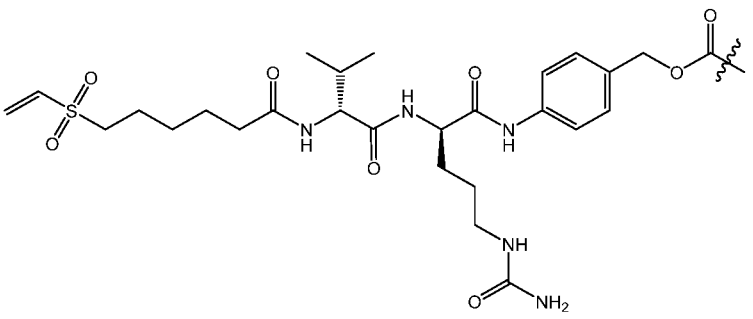
(IVb.13)



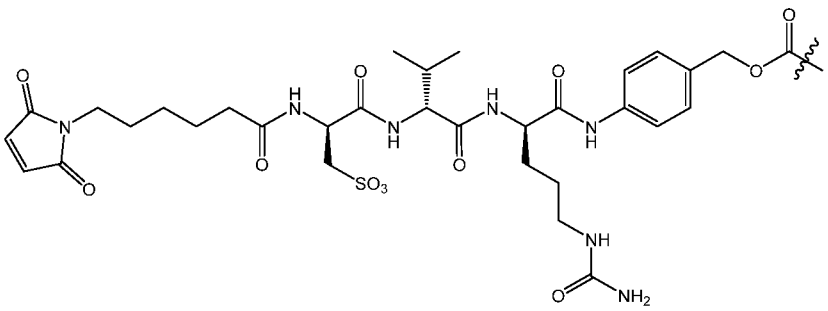
(IVb.14)



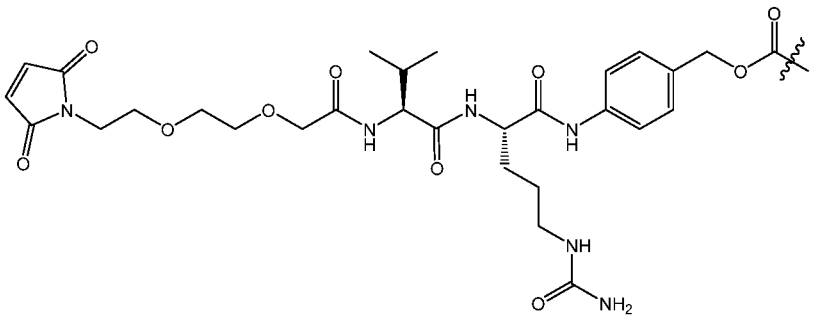
(IVb.15)



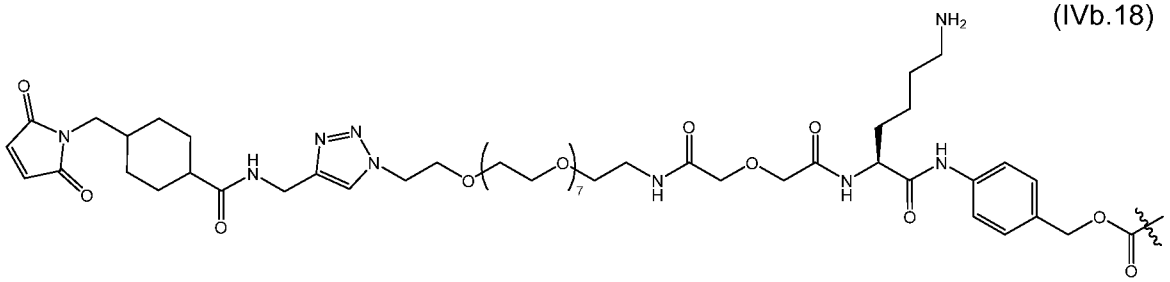
(IVb.16)



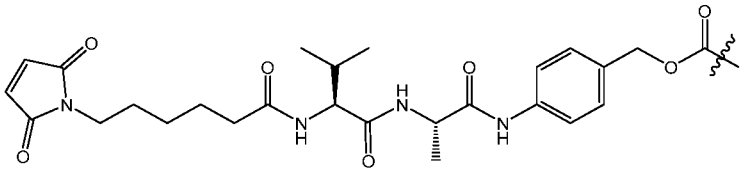
(IVb.17)



(IVb.18)

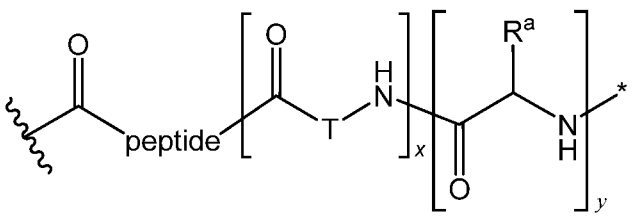


(IVb.19)

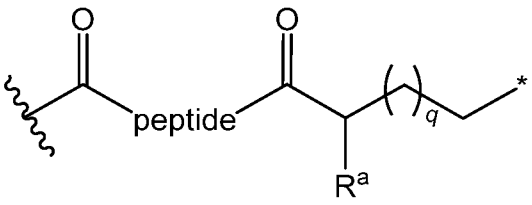


[0200] In certain embodiments, the linker comprises an enzymatically cleavable peptide moiety, for example, a linker comprising structural formula (IVc) or (IVd):

(IVc)



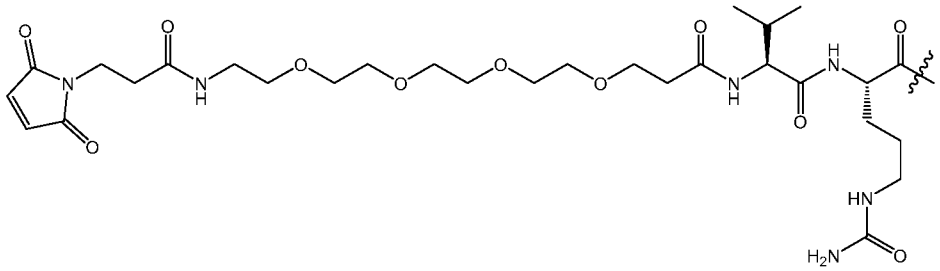
(IVd)



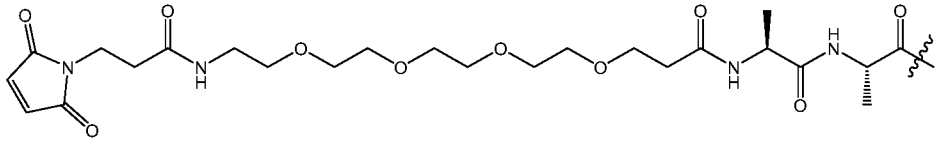
or a salt thereof, wherein: peptide represents a peptide (illustrated C→N and not showing the carboxy and amino "termini") cleavable by a lysosomal enzyme; T represents a polymer comprising one or more ethylene glycol units or an alkylene chain, or combinations thereof; R^a is selected from hydrogen, alkyl, sulfonate and methyl sulfonate; p is an integer ranging from 0 to 5; q is 0 or 1; x is 0 or 1; y is 0 or 1; x represents the point of attachment of the linker to a cytotoxic and/or cytostatic agent; and * represents the point of attachment to the remainder of the linker.

[0201] Specific exemplary embodiments of linkers according to structural formula (IVc) that may be included in the anti-glyco-MUC4 ADCs of the disclosure include the linkers illustrated below (as illustrated, the linkers include a group suitable for covalently linking the linker to an antibody):

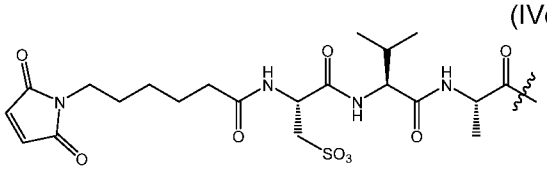
(IVc.1)



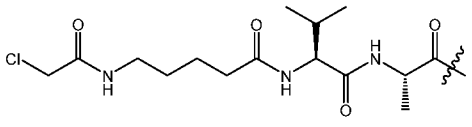
(IVc.2)



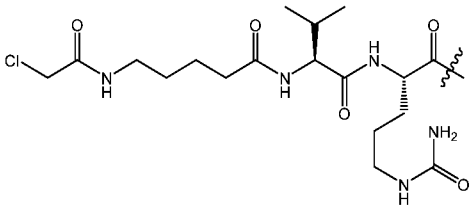
(IVc.3)



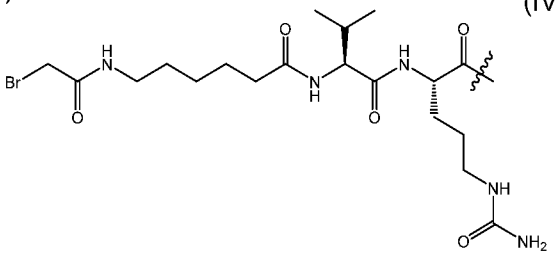
(IVc.4)



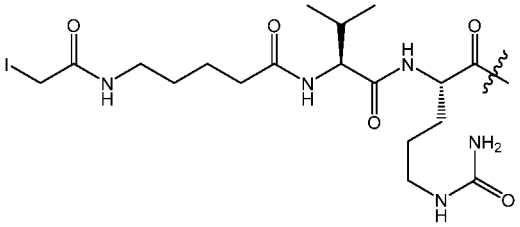
(IVc.5)



(IVc.6)

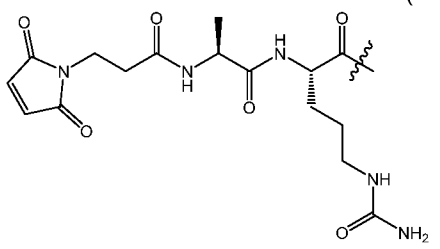


(IVc.7)

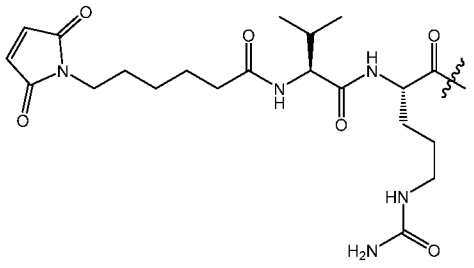


[0202] Specific exemplary embodiments of linkers according to structural formula (IVd) that may be included in the anti-glyco-MUC4 ADCs of the disclosure include the linkers illustrated below (as illustrated, the linkers include a group suitable for covalently linking the linker to an antibody):

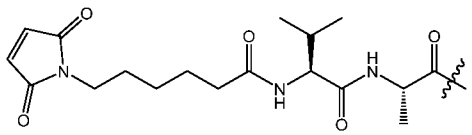
(IVd.1)



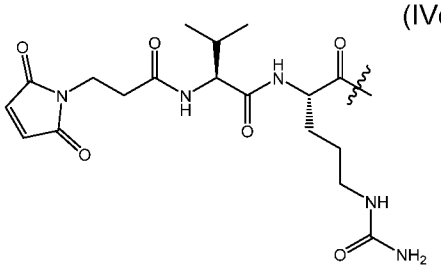
(IVd.2)



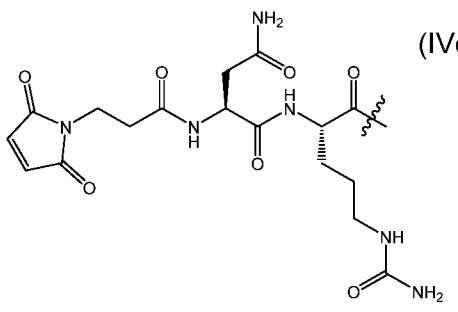
(IVd.3)



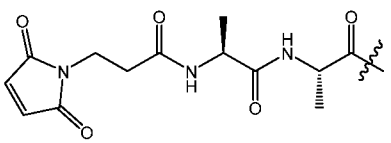
(IVd.4)



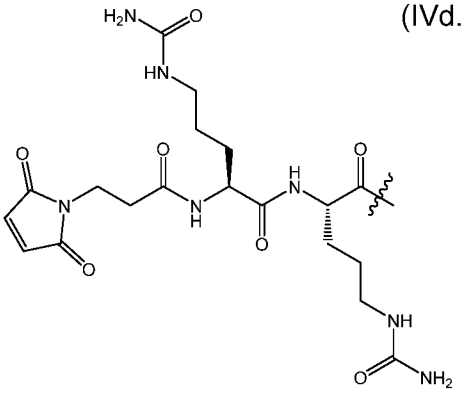
(IVd.5)



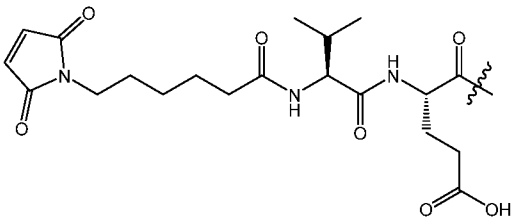
(IVd.6)



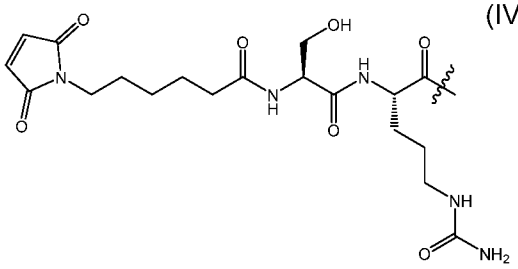
(IVd.7)



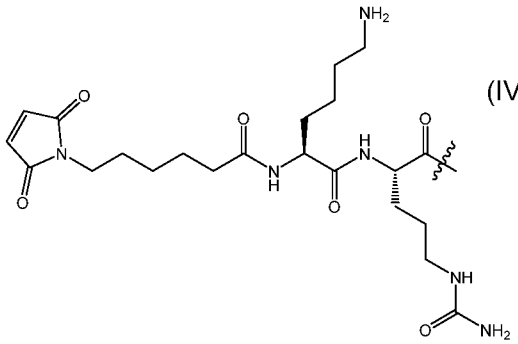
(IVd.8)



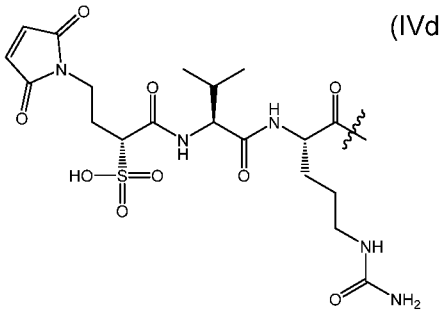
(IVd.9)



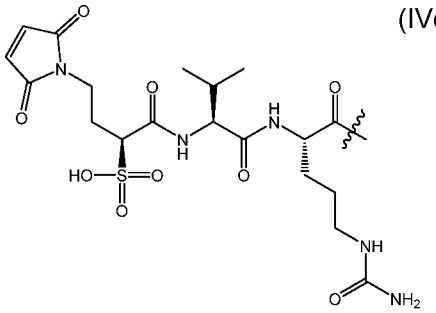
(IVd.10)



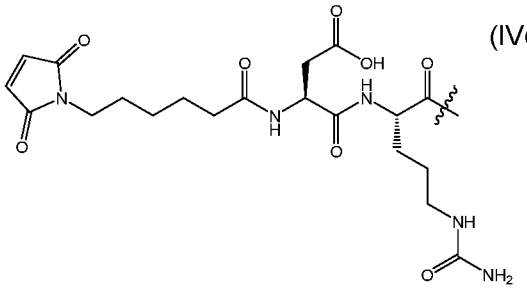
(IVd.11)



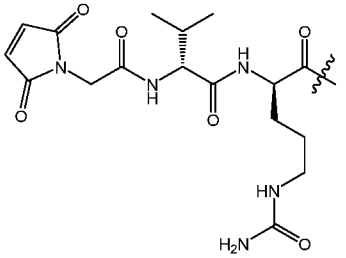
(IVd.12)



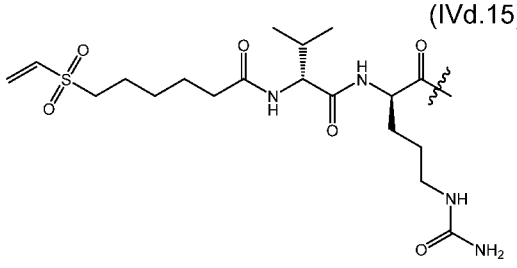
(IVd.13)



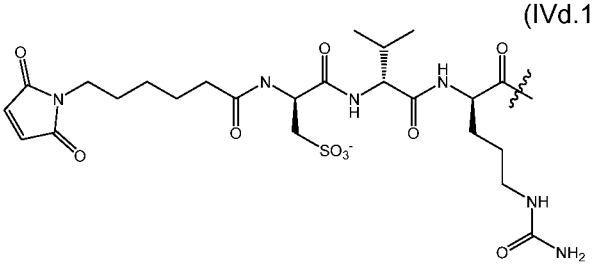
(IVd.14)

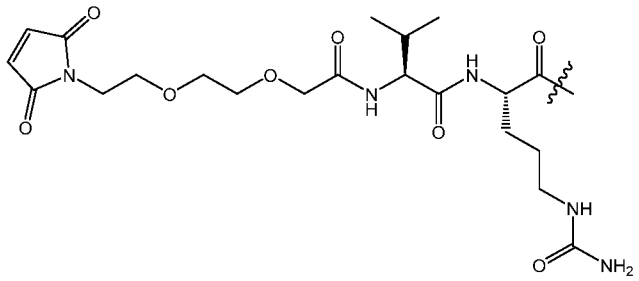


(IVd.15)



(IVd.16)





(IVd.17)

[0203] In certain embodiments, the linker comprising structural formula (IVa), (IVb), (IVc), or (IVd) further comprises a carbonate moiety cleavable by exposure to an acidic medium. In particular embodiments, the linker is attached through an oxygen to a cytotoxic and/or cytostatic agent.

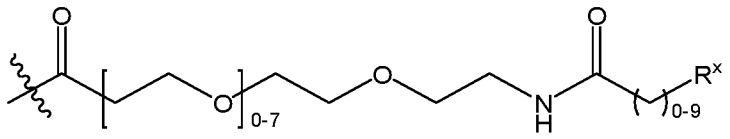
5.2.4. Non-Cleavable Linkers

[0204] Although cleavable linkers may provide certain advantages, the linkers comprising the anti-glyco-MUC4 ADC of the disclosure need not be cleavable. For noncleavable linkers, the release of drug does not depend on the differential properties between the plasma and some cytoplasmic compartments. The release of the drug is postulated to occur after internalization of the ADC via antigen-mediated endocytosis and delivery to lysosomal compartment, where the antibody is degraded to the level of amino acids through intracellular proteolytic degradation. This process releases a drug derivative, which is formed by the drug, the linker, and the amino acid residue to which the linker was covalently attached. The amino acid drug metabolites from conjugates with noncleavable linkers are more hydrophilic and generally less membrane permeable, which leads to less bystander effects and less nonspecific toxicities compared to conjugates with a cleavable linker. In general, ADCs with noncleavable linkers have greater stability in circulation than ADCs with cleavable linkers. Non-cleavable linkers may be alkylene chains, or maybe polymeric in natures, such as, for example, based upon polyalkylene glycol polymers, amide polymers, or may include segments of alkylene chains, polyalkylene glocols and/or amide polymers.

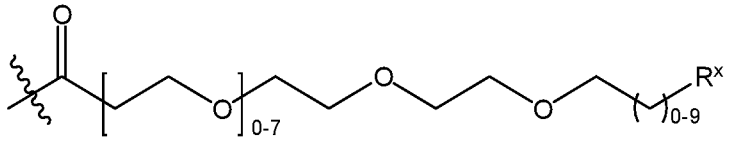
[0205] A variety of non-cleavable linkers used to link drugs to antibodies have been described. See, Jeffrey *et al.*, 2006, Bioconjug. Chem. 17; 831-840; Jeffrey *et al.*, 2007, Bioorg. Med. Chem. Lett. 17:2278-2280; and Jiang *et al.*, 2005, J. Am. Chem. Soc. 127:11254-11255, each of which is incorporated herein by reference. All of these linkers may be included in the anti-glyco-MUC4 ADCs of the disclosure.

[0206] In certain embodiments, the linker is non-cleavable *in vivo*, for example a linker according to structural formula (VIa), (VIb), (VIc) or (VId) (as illustrated, the linkers include a group suitable for covalently linking the linker to an antibody:

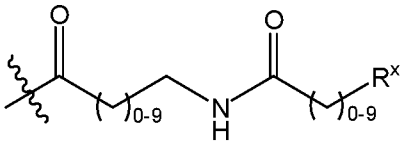
(VIa)



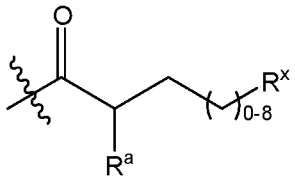
(VIb)



(VIc)



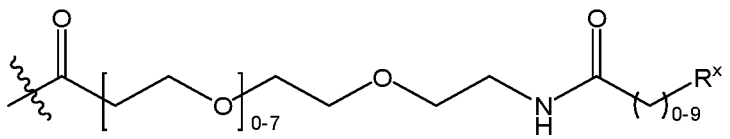
(VId)



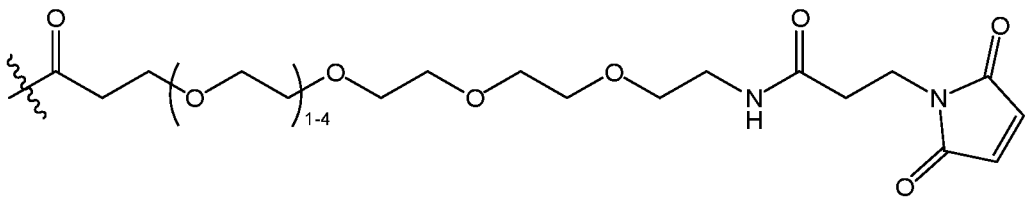
or salts thereof, wherein: R^a is selected from hydrogen, alkyl, sulfonate and methyl sulfonate; R^x is a moiety including a functional group capable of covalently linking the linker to an antibody; and ⁄ represents the point of attachment of the linker to a cytotoxic and/or cytostatic agent.

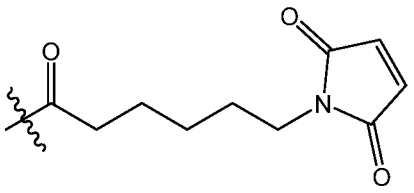
[0207] Specific exemplary embodiments of linkers according to structural formula (VIa)-(VId) that may be included in the anti-glyco-MUC4 ADCs of the disclosure include the linkers illustrated below (as illustrated, the linkers include a group suitable for covalently linking the linker to an antibody, and ⁄ represents the point of attachment to a cytotoxic and/or cytostatic agent):

(VIa)

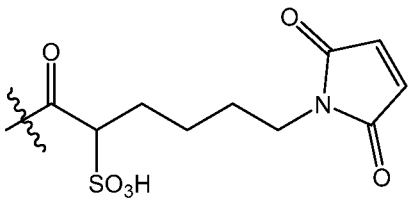


(VIa.1)



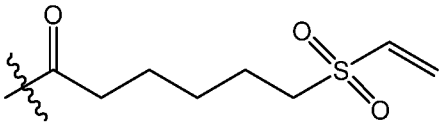


(VId.1)



(VId.2)

(VId.3)

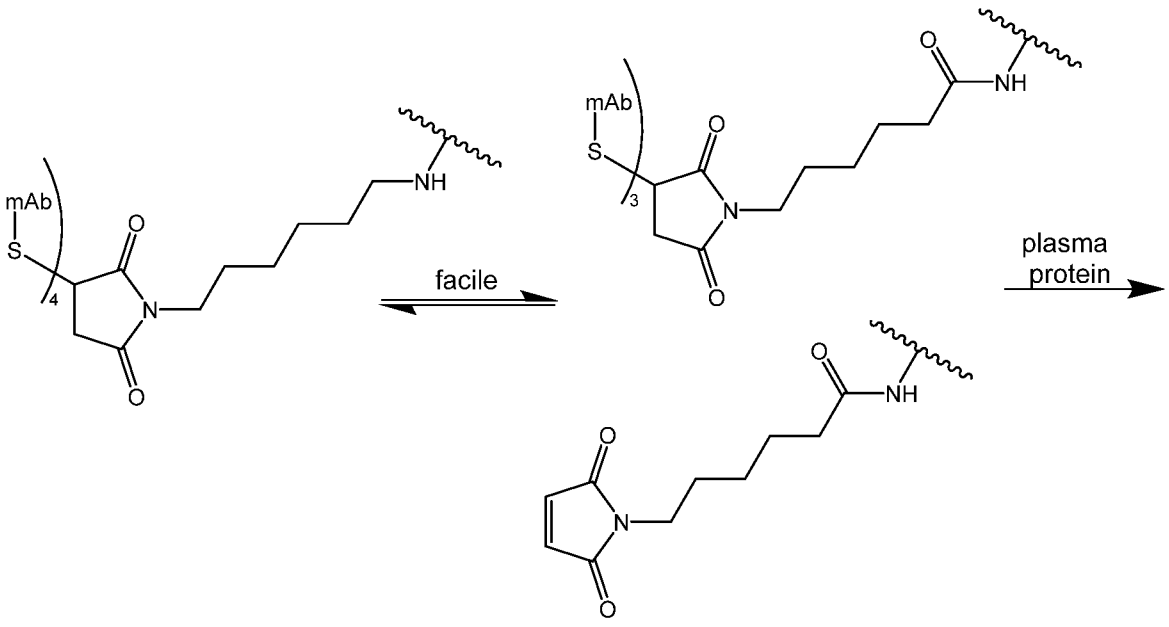


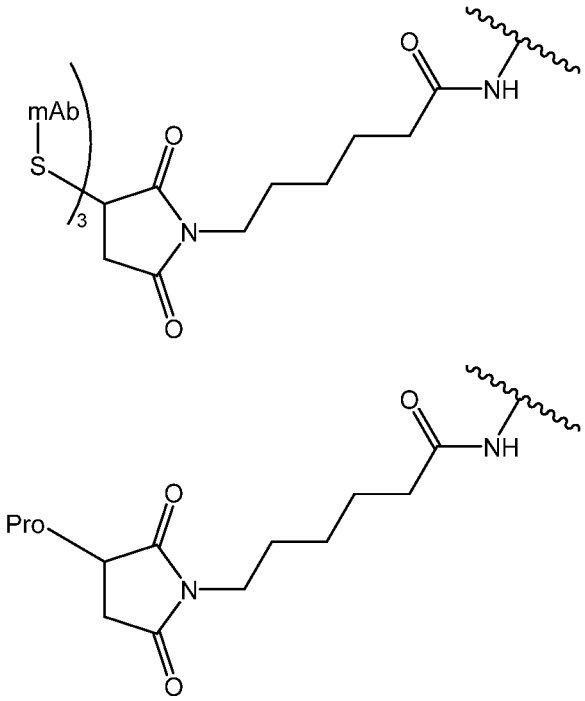
5.2.5. Groups Used to Attach Linkers to Antibodies

[0208] A variety of groups may be used to attach linker-drug synthons to antibodies to yield ADCs. Attachment groups can be electrophilic in nature and include: maleimide groups, activated disulfides, active esters such as NHS esters and HOBt esters, haloformates, acid halides, alkyl and benzyl halides such as haloacetamides. As discussed below, there are also emerging technologies related to "self-stabilizing" maleimides and "bridging disulfides" that can be used in accordance with the disclosure. The specific group used will depend, in part, on the site of attachment to the antibody.

[0209] One example of a "self-stabilizing" maleimide group that hydrolyzes spontaneously under antibody conjugation conditions to give an ADC species with improved stability is depicted in the schematic below. See US20130309256 A1; also Lyon *et al.*, Nature Biotech published online, doi:10.1038/nbt.2968.

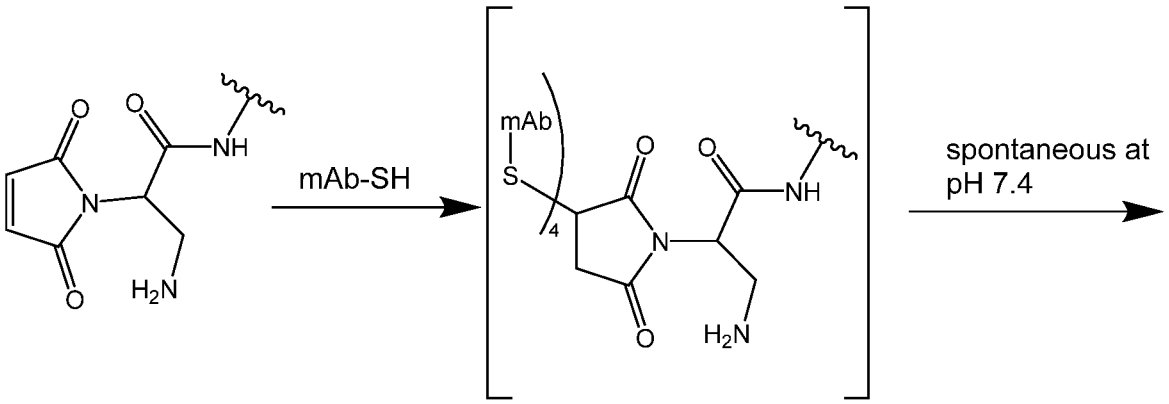
Normal system:



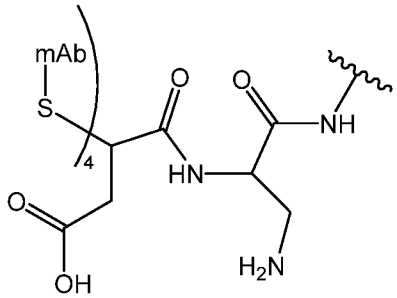


Leads to "DAR loss" over time

SGN MalDPR (maleimido dipropylamino) system:

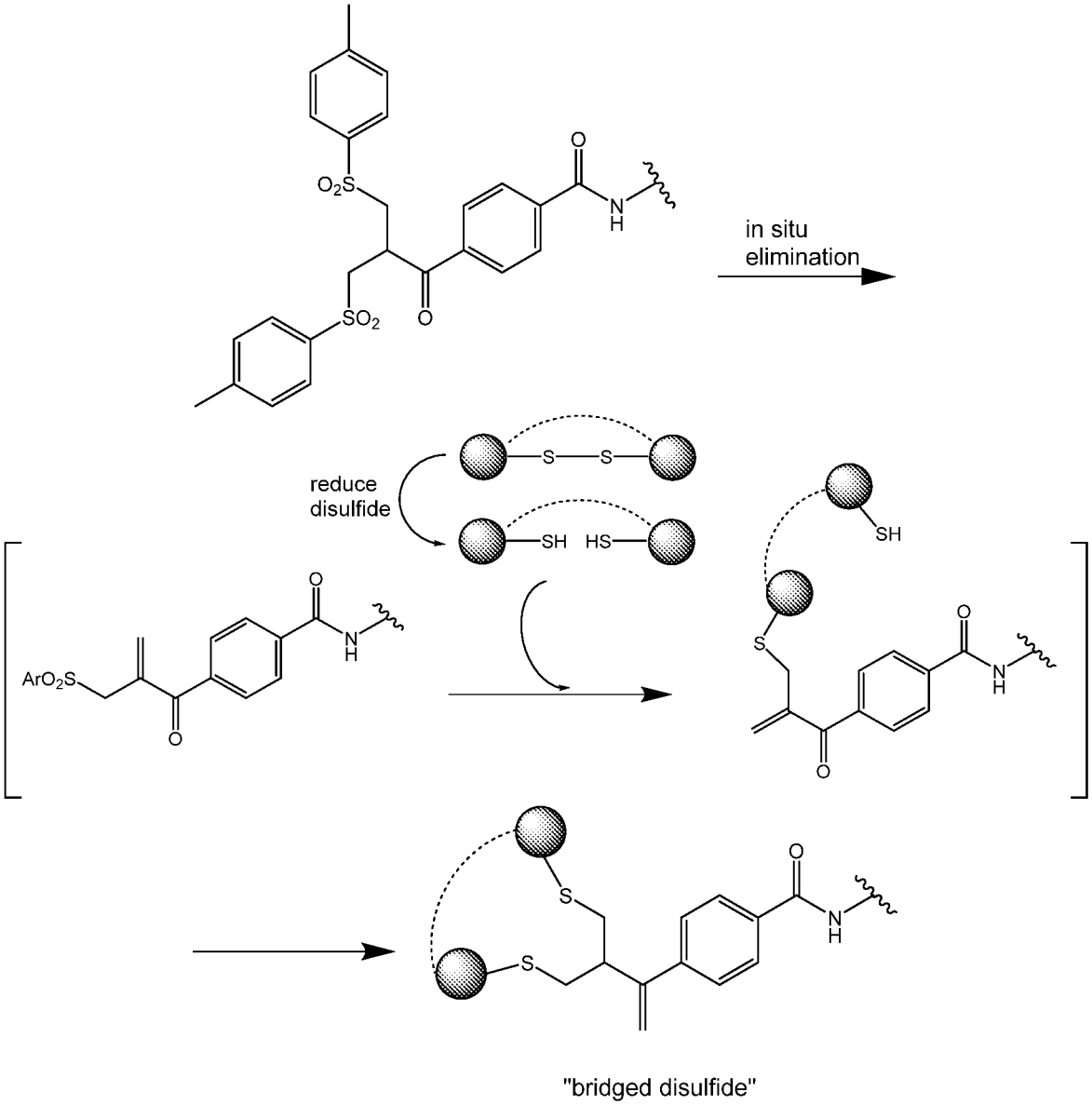


US20130309256A1

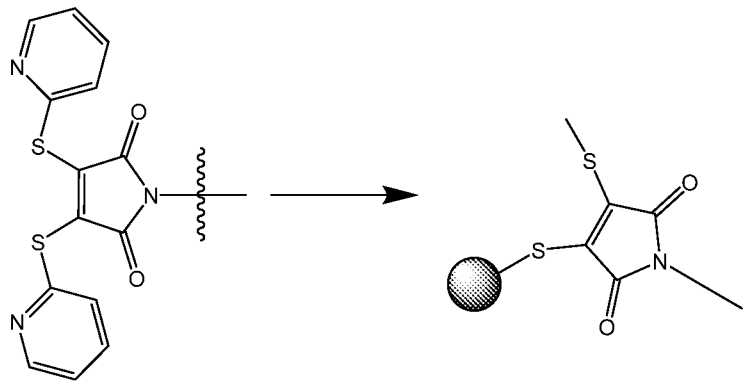


stable in plasma
(retro hetero-Michael
reaction shown above slow)

[0210] Polytherics has disclosed a method for bridging a pair of sulfhydryl groups derived from reduction of a native hinge disulfide bond. See, Badescu *et al.*, 2014, Bioconjugate Chem. 25:1124-1136. The reaction is depicted in the schematic below. An advantage of this methodology is the ability to synthesize enriched DAR4 ADCs by full reduction of IgGs (to give 4 pairs of sulfhydryls) followed by reaction with 4 equivalents of the alkylating agent. ADCs containing "bridged disulfides" are also claimed to have increased stability.



[0211] Similarly, as depicted below, a maleimide derivative (1, below) that is capable of bridging a pair of sulfhydryl groups has been developed. See WO2013/085925.



5.2.6. Linker Selection Considerations

[0212] As is known by skilled artisans, the linker selected for a particular ADC may be influenced by a variety of factors, including but not limited to, the site of attachment to the antibody (*e.g.*, lys, cys or other amino acid residues), structural constraints of the drug pharmacophore and the lipophilicity of the drug. The specific linker selected for an ADC should seek to balance these different factors for the specific antibody/drug combination. For a review of the factors that are influenced by choice of linkers in ADCs, see Nolting, Chapter 5 "Linker Technology in Antibody-Drug Conjugates," In: Antibody-Drug Conjugates: Methods in Molecular Biology, vol. 1045, pp. 71-100, Laurent Ducry (Ed.), Springer Science & Business Medica, LLC, 2013.

[0213] For example, ADCs have been observed to effect killing of bystander antigen-negative cells present in the vicinity of the antigen-positive tumor cells. The mechanism of bystander cell killing by ADCs has indicated that metabolic products formed during intracellular processing of the ADCs may play a role. Neutral cytotoxic metabolites generated by metabolism of the ADCs in antigen-positive cells appear to play a role in bystander cell killing while charged metabolites may be prevented from diffusing across the membrane into the medium and therefore cannot affect bystander killing. In certain embodiments, the linker is selected to attenuate the bystander killing effect caused by cellular metabolites of the ADC. In certain embodiments, the linker is selected to increase the bystander killing effect.

[0214] The properties of the linker may also impact aggregation of the ADC under conditions of use and/or storage. Typically, ADCs reported in the literature contain no more than 3-4 drug molecules per antibody molecule (see, *e.g.*, Chari, 2008, Acc Chem Res 41:98-107). Attempts to obtain higher drug-to-antibody ratios ("DAR") often failed, particularly if both the drug and the linker were hydrophobic, due to aggregation of the ADC (King *et al.*, 2002, J Med Chem 45:4336-4343; Hollander *et al.*, 2008, Bioconjugate Chem 19:358-361; Burke *et al.*, 2009 Bioconjugate Chem 20:1242-1250). In many instances, DARs higher than 3-4 could be beneficial as a means of increasing potency. In instances where the cytotoxic and/or cytostatic agent is hydrophobic in nature, it may be desirable to select linkers that are relatively

hydrophilic as a means of reducing ADC aggregation, especially in instances where DARS greater than 3-4 are desired. Thus, in certain embodiments, the linker incorporates chemical moieties that reduce aggregation of the ADCs during storage and/or use. A linker may incorporate polar or hydrophilic groups such as charged groups or groups that become charged under physiological pH to reduce the aggregation of the ADCs. For example, a linker may incorporate charged groups such as salts or groups that deprotonate, *e.g.*, carboxylates, or protonate, *e.g.*, amines, at physiological pH.

[0215] Exemplary polyvalent linkers that have been reported to yield DARs as high as 20 that may be used to link numerous cytotoxic and/or cytostatic agents to an antibody are described in WO 2009/073445; WO 2010/068795; WO 2010/138719; WO 2011/120053; WO 2011/171020; WO 2013/096901; WO 2014/008375; WO 2014/093379; WO 2014/093394; WO 2014/093640, the content of which are incorporated herein by reference in their entireties.

[0216] In particular embodiments, the aggregation of the ADCs during storage or use is less than about 10% as determined by size-exclusion chromatography (SEC). In particular embodiments, the aggregation of the ADCs during storage or use is less than 10%, such as less than about 5%, less than about 4%, less than about 3%, less than about 2%, less than about 1%, less than about 0.5%, less than about 0.1%, or even lower, as determined by size-exclusion chromatography (SEC).

5.2.7. Methods of Making Anti-Glyco-MUC4 ADCs

[0217] The anti-glyco-MUC4 ADCs of the disclosure may be synthesized using chemistries that are well-known. The chemistries selected will depend upon, among other things, the identity of the cytotoxic and/or cytostatic agent(s), the linker and the groups used to attach linker to the antibody. Generally, ADCs according to formula (I) may be prepared according to the following scheme:

$$D\text{-}L\text{-}R^x + Ab\text{-}R^y \rightarrow [D\text{-}L\text{-}XY]_n\text{-}Ab \quad (I)$$

where D, L, Ab, XY and n are as previously defined, and R^x and R^y represent complementary groups capable of forming a covalent linkages with one another, as discussed above.

[0218] The identities of groups R^x and R^y will depend upon the chemistry used to link synthon D-L- R^x to the antibody. Generally, the chemistry used should not alter the integrity of the antibody, for example its ability to bind its target. Preferably, the binding properties of the conjugated antibody will closely resemble those of the unconjugated antibody. A variety of chemistries and techniques for conjugating molecules to biological molecules such as antibodies are known in the art and in particular to antibodies, are well-known. See, *e.g.*, Amon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy," in: Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* Eds., Alan R. Liss, Inc., 1985; Hellstrom *et al.*, "Antibodies For Drug Delivery," in: Controlled Drug Delivery, Robinson *et al.*

Eds., Marcel Dekker, Inc., 2nd Ed. 1987; Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review," in: Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera *et al.*, Eds., 1985; "Analysis, Results, and Future Prospective of the Therapeutic Use of Radiolabeled Antibody In Cancer Therapy," in: Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin *et al.*, Eds., Academic Press, 1985; Thorpe *et al.*, 1982, Immunol. Rev. 62:119-58; PCT publication WO 89/12624. Any of these chemistries may be used to link the synthons to an antibody.

[0219] A number of functional groups R^x and chemistries useful for linking synthons to accessible lysine residues are known and include, by way of example and not limitation, NHS-esters and isothiocyanates.

[0220] A number of functional groups R^x and chemistries useful for linking synthons to accessible free sulfhydryl groups of cysteine residues are known and include, by way of example and not limitation, haloacetyls and maleimides.

[0221] However, conjugation chemistries are not limited to available side chain groups. Side chains such as amines may be converted to other useful groups, such as hydroxyls, by linking an appropriate small molecule to the amine. This strategy can be used to increase the number of available linking sites on the antibody by conjugating multifunctional small molecules to side chains of accessible amino acid residues of the antibody. Functional groups R^x suitable for covalently linking the synthons to these "converted" functional groups are then included in the synthons.

[0222] The antibody may also be engineered to include amino acid residues for conjugation. An approach for engineering antibodies to include non-genetically encoded amino acid residues useful for conjugating drugs in the context of ADCs is described by Axup *et al.*, 2012, Proc Natl Acad Sci USA. 109(40):16101-16106, as are chemistries and functional group useful for linking synthons to the non-encoded amino acids.

[0223] Typically, the synthons are linked to the side chains of amino acid residues of the antibody, including, for example, the primary amino group of accessible lysine residues or the sulfhydryl group of accessible cysteine residues. Free sulfhydryl groups may be obtained by reducing interchain disulfide bonds.

[0224] For linkages where R^y is a sulfhydryl group (for example, when R^x is a maleimide), the antibody is generally first fully or partially reduced to disrupt interchain disulfide bridges between cysteine residues.

[0225] Cysteine residues that do not participate in disulfide bridges may engineered into an antibody by mutation of one or more codons. Reducing these unpaired cysteines yields a sulfhydryl group suitable for conjugation. Preferred positions for incorporating engineered cysteines include, by way of example and not limitation, positions S112C, S113C, A114C,

S115C, A176C, 5180C, S252C, V286C, V292C, S357C, A359C, S398C, S428C (Kabat numbering) on the human IgG_1 heavy chain and positions V110C, S114C, S121C, S127C, S168C, V205C (Kabat numbering) on the human Ig kappa light chain (see, *e.g.*, U.S. Pat. No. 7,521,541, U.S. Pat. No. 7,855,275 and U.S. Pat. No. 8,455,622).

[0226] As will appreciated by skilled artisans, the number of cytotoxic and/or cytostatic agents linked to an antibody molecule may vary, such that a collection of ADCs may be heterogeneous in nature, where some antibodies contain one linked agent, some two, some three, *etc.* (and some none). The degree of heterogeneity will depend upon, among other things, the chemistries used for linking the cytotoxic and/or cytostatic agents. For example, where the antibodies are reduced to yield sulfhydryl groups for attachment, heterogeneous mixtures of antibodies having zero, 2, 4, 6 or 8 linked agents per molecule are often produced. Furthermore, by limiting the molar ratio of attachment compound, antibodies having zero, 1, 2, 3, 4, 5, 6, 7 or 8 linked agents per molecule are often produced. Thus, it will be understood that depending upon context, stated DARs may be averages for a collection of antibodies. For example, “DAR4” can refer to an ADC preparation that has not been subjected to purification to isolate specific DAR peaks and can comprise a heterogeneous mixture of ADC molecules having different numbers of cytostatic and/or cytotoxic agents attached per antibody (*e.g.*, 0, 2, 4, 6, 8 agents per antibody), but has an average drug-to-antibody ratio of 4. Similarly, in some embodiments, “DAR2” refers to a heterogeneous ADC preparation in which the average drug-to-antibody ratio is 2.

[0227] When enriched preparations are desired, antibodies having defined numbers of linked cytotoxic and/or cytostatic agents may be obtained via purification of heterogeneous mixtures, for example, via column chromatography, *e.g.*, hydrophobic interaction chromatography.

[0228] Purity may be assessed by a variety of methods, as is known in the art. As a specific example, an ADC preparation may be analyzed via HPLC or other chromatography and the purity assessed by analyzing areas under the curves of the resultant peaks.

5.3 Chimeric Antigen Receptors

[0229] The present disclosure provides chimeric antigen receptors (CARs) comprising the anti-glyco-MUC4 antibodies or antigen-binding fragments described herein. In some embodiments, the CAR comprises one or more scFvs (*e.g.*, one or two) as described herein. For example, a CAR can comprise two scFvs covalently connected by a linker sequence (*e.g.*, of 4-15 amino acids). Exemplary linkers include GGGGS (SEQ ID NO:159) and $(GGGGS)_3$ (SEQ ID NO:160).

[0230] The CARs of the disclosure typically comprise an extracellular domain operably linked to a transmembrane domain which is in turn operably linked to an intracellular domain for signaling. The CARs can further comprise a signal peptide at the N-terminus of the extracellular domain (*e.g.*, a human CD8 signal peptide). In some embodiments, a CAR of the disclosure

comprises a human CD8 signal peptide comprising the amino acid sequence MALPVTALLLPLALLLHAARP (SEQ ID NO:161).

[0231] The extracellular domains of the CARs of the disclosure comprise the sequence of an anti-glyco-MUC4 antibody or antigen-binding fragment (*e.g.*, as described in Section 5.1 or numbered embodiments 1 to 414).

[0232] Exemplary transmembrane domain sequence and intracellular domain sequences are described in Sections 5.3.1 and 5.3.2, respectively.

[0233] Several fusion proteins described herein (*e.g.*, numbered embodiments 421 to 445) are CARs (*e.g.*, numbered embodiments 446 to 479), and the CAR-related disclosures apply to such fusion proteins. Other fusion proteins described herein are chimeric T cell receptors (TCRs) (*e.g.*, numbered embodiments 490 to 584), and the chimeric TCR-related disclosures apply to such fusion proteins.

5.3.1. Transmembrane Domain

[0234] With respect to the transmembrane domain, the CAR can be designed to comprise a transmembrane domain that is operably linked (*e.g.*, fused) to the extracellular domain of the CAR.

[0235] The transmembrane domain may be derived either from a natural or from a synthetic source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein. Transmembrane regions of particular use in this disclosure may be derived from (*i.e.*, comprise at least the transmembrane region(s) of) the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154. In some instances, a variety of human hinges can be employed as well including the human Ig (immunoglobulin) hinge.

[0236] In one embodiment, the transmembrane domain is synthetic (*i.e.*, non-naturally occurring). Examples of synthetic transmembrane domains are peptides comprising predominantly hydrophobic residues such as leucine and valine. Preferably a triplet of phenylalanine, tryptophan and valine will be found at each end of a synthetic transmembrane domain. Optionally, a short oligo- or polypeptide linker, preferably between 2 and 10 amino acids in length may form the linkage between the transmembrane domain and the cytoplasmic signaling domain of the CAR. A glycine-serine doublet provides a particularly suitable linker.

[0237] In one embodiment, the transmembrane domain in the CAR of the disclosure is the CD8 transmembrane domain. In one embodiment, the CD8 transmembrane domain comprises the amino acid sequence YLHLGALGRDLWGPSPVTGYHPLL (SEQ ID NO:162).

[0238] In one embodiment, the transmembrane domain in the CAR of the disclosure is the CD28 transmembrane domain. In one embodiment, the CD28 transmembrane domain comprises the amino acid sequence FWVLVVVGGVLACYSLLVTVAFIIFWV (SEQ ID NO:163).

[0239] In some instances, the transmembrane domain of the CAR of the disclosure is linked to the extracellular domain by a CD8a hinge domain. In one embodiment, the CD8a hinge domain comprises the amino acid sequence TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC (SEQ ID NO:221). In another embodiment, the CD8a hinge domain comprises the amino acid sequence TTTPAPRPPTPAPTIASPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO:165). In another embodiment, the CD8a hinge domain comprises the amino acid sequence TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO:223).

[0240] In some instances, the transmembrane domain of the CAR of the disclosure is linked to the extracellular domain by a human IgG4-short hinge. In one embodiment, the human IgG4-short hinge comprises the amino acid sequence ESKYGPPCPSCP (SEQ ID NO:166).

[0241] In some instances, the transmembrane domain of the CAR of the disclosure is linked to the extracellular domain by a human IgG4-long hinge. In one embodiment, the human IgG4-long hinge comprises the amino acid sequence ESKYGPPCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDG VEVHNAKTKPREEQFQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPR EPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGKM (SEQ ID NO:167).

5.3.2. Intracellular Domain

[0242] The intracellular signaling domain of the CAR of the disclosure is responsible for activation of at least one of the normal effector functions of the immune cell in which the CAR is expressed. The term “effector function” refers to a specialized function of a cell. Effector function of a T cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines. Thus, the term “intracellular signaling domain” refers to the portion of a protein which transduces the effector function signal and directs the cell to perform a specialized function. While usually the entire intracellular signaling domain can be employed, in many cases it is not necessary to use the entire chain. To the extent that a truncated portion of the intracellular signaling domain is used, such truncated portion may be used in place of the intact chain as long as it transduces the effector function signal. The term intracellular signaling domain is thus meant to include any truncated portion of the intracellular signaling domain sufficient to transduce the effector function signal.

[0243] Preferred examples of intracellular signaling domains for use in the CAR of the disclosure include cytoplasmic sequences of the T cell receptor (TCR) and co-receptors that act

in concert to initiate signal transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any synthetic sequence that has the same functional capability.

[0244] Signals generated through the TCR alone may be insufficient for full activation of the T cell and a secondary or co-stimulatory signal is also required. Thus, T cell activation can be said to be mediated by two distinct classes of cytoplasmic signaling sequence: those that initiate antigen-dependent primary activation through the TCR (primary cytoplasmic signaling sequences) and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal (secondary cytoplasmic signaling sequences).

[0245] Primary cytoplasmic signaling sequences regulate primary activation of the TCR complex either in a stimulatory way, or in an inhibitory way. Primary cytoplasmic signaling sequences that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs.

[0246] Examples of ITAM containing primary cytoplasmic signaling sequences that are of particular use in the CARs of the disclosure include those derived from TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d. It is particularly preferred that cytoplasmic signaling molecule in the CAR of the disclosure comprises a cytoplasmic signaling sequence from CD3-zeta.

[0247] In a preferred embodiment, the cytoplasmic domain of the CAR is designed to include an ITAM containing primary cytoplasmic signaling sequences domain (*e.g.*, that of CD3-zeta) by itself or combined with any other desired cytoplasmic domain(s) useful in the context of the CAR of the disclosure. For example, the cytoplasmic domain of the CAR can include a CD3 zeta chain portion and a costimulatory signaling region.

[0248] The costimulatory signaling region refers to a portion of the CAR comprising the intracellular domain of a costimulatory molecule. A costimulatory molecule is a cell surface molecule other than an antigen receptor or its ligands that is required for an efficient response of lymphocytes to an antigen. Examples of such molecules include CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, and a ligand that specifically binds with CD83, DAP10, GITR, and the like.

[0249] The cytoplasmic signaling sequences within the cytoplasmic signaling portion of the CAR of the disclosure may be linked to each other in a random or specified order. Optionally, a short oligo- or polypeptide linker, preferably between 2 and 10 amino acids in length may form the linkage. A glycine-serine doublet provides a particularly suitable linker.

[0250] In one embodiment, the cytoplasmic domain comprises the signaling domain of CD3-zeta and the signaling domain of CD28. In some embodiments, the signaling domain of CD3-

zeta comprises the amino acid sequence RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYN ELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO:168). In some embodiments, the signaling domain of CD28 comprises the amino acid sequence RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS (SEQ ID NO:169).

[0251] In another embodiment, the cytoplasmic domain comprises the signaling domain of CD3-zeta and the signaling domain of 4-1BB.

[0252] In another embodiment, the cytoplasmic domain comprises the signaling domain of CD3-zeta and the signaling domain of CD2. In some embodiments, the signaling domain of CD2 comprises the amino acid sequence TKRKKQRSRRNDEELETRAHRVATEERGRKPHQIPASTPQNPATSQHPPPPPGHRSQAPSHR PPPPGHRVQHQPQKRPPAPSGTQVHQQKGPPLPRPRVQPKPPHGAAENSLSPSSN (SEQ ID NO:217).

[0253] In another embodiment, the cytoplasmic domain comprises the signaling domain of CD3-zeta, the signaling domain of CD28, and the signaling domain of CD2.

[0254] In another embodiment, the cytoplasmic domain comprises the signaling domain of CD3-zeta, the signaling domain of 4-1BB, and the signaling domain of CD2.

[0255] Inclusion of the CD2 signaling domain in the cytoplasmic domain allows for the tuning of CAR T cell cytokine production (*see* US Pat. No. 9,783,591, the contents of which are incorporated herein by reference in their entireties). As disclosed in US Pat. No. 9,783,591, inclusion of the CD2 signaling domain in the CAR cytoplasmic domain significantly alters CAR T cell cytokine production in both positive and negative directions, with the effect being dependent on the presence and identity of other costimulatory molecules in the costimulatory signaling region of the cytoplasmic domain. For example, in some embodiments, inclusion of the CD2 signaling domain and the CD28 signaling domain in the costimulatory signaling region of the cytoplasmic domain results in the release of significantly less IL2 relative to T cells expressing a CAR with CD28 but not CD2. A CAR T cell releasing less IL2 can result in reduced proliferation of immunosuppressive Treg cells. In some embodiments, inclusion of the CD2 signaling domain in the costimulatory signaling region of the cytoplasmic domain significantly reduces calcium influx in the CAR T cell. This has been shown to reduce activation-induced CAR T cell death.

5.4 Chimeric T Cell Receptors

[0256] The present disclosure provides chimeric T cell receptors (TCRs) comprising the anti-glyco-MUC4 antibodies or antigen-binding fragments described herein. The chimeric TCRs provide an anti-glyco-MUC4 specific antibody and TCR chimera that specifically binds to anti-glyco-MUC4, and are capable of recruiting at least one TCR-associated signaling molecule

(*e.g.*, CD3γε, CD3δε, and ζζ). In some embodiments, the chimeric TCR comprises one or more antigen-binding fragments capable of binding glyco-MUC4. Examples of antigen-binding fragments include by way of example and not limitation, Fab, Fab', F $(ab')_2$, Fv fragments, single chain Fv fragments (scFv) and single domain fragments. In some embodiments, an antigen-binding fragment of a chimeric T cell receptor comprises at least one anti-glyco-MUC4 variable heavy chain and at least one anti-glyco-MUC4 variable light chain as described herein.

[0257] TCRs occur as either an αβ heterodimer or as a γδ heterodimer, with T cells expressing either the αβ form or the γδ form TCR on the cell surface. The four chains (α, β, γ, δ) each have a characteristic extracellular structure consisting of a highly polymorphic "immunoglobulin variable region"-like N-terminal domain and an "immunoglobulin constant region"-like second domain. Each of these domains has a characteristic intra-domain disulfide bridge. The constant region is proximal to the cell membrane, followed by a connecting peptide, a transmembrane region and a short cytoplasmic tail. The covalent linkage between the 2 chains of the heterodimeric TCR is formed by the cysteine residue located within the short connecting peptide sequence bridging the extracellular constant domain and the transmembrane region which forms a disulfide bond with the paired TCR chain cysteine residue at the corresponding position (Lefranc and Lefranc, "The T Cell Receptor FactsBook," Academic Press, 2001).

[0258] Several examples of chimeric TCRs are known in the art. See, *e.g.*, Kuwana *et al.*, Biochem Biophys Res Commun. 149(3):960-968; Gross *et al.*, 1989, Proc Natl Acad Sci USA. 86:10024-10028; Gross & Eshhar, 1992, FASEB J. 6(15):3370-3378; Liu *et al.*, 2021, Sci Transl Med, 13:eabb5191, WO 2016/187349, WO 2017/070608, WO 2020/029774, and US Patent No. 7,741,465, the contents of each of which are incorporated herein by reference in their entireties.

[0259] A chimeric TCR generally comprises a first polypeptide chain comprising a first TCR domain, a second polypeptide chain comprising a second TCR domain, and an anti-glyco-MUC4 antigen binding fragment described herein. In some embodiments, the chimeric TCR comprises a single anti-glyco-MUC4 antigen binding fragment. In other embodiments, the chimeric TCR comprises a two or more anti-glyco-MUC4 antigen binding fragments. In certain embodiments, the chimeric TCR comprises two anti-glyco-MUC4 antigen binding fragments.

[0260] In some embodiments, the anti-glyco-MUC4 antigen binding fragment is an scFv described herein. In embodiments in which the chimeric TCR includes a single anti-glyco-MUC4 antigen binding fragment, a single anti-glyco-MUC4 scFv can be included in either the first polypeptide chain or the second polypeptide chain of the chimeric TCR. In embodiments in which the chimeric TCR includes, *e.g.*, two anti-glyco-MUC4 antigen binding fragments, two anti-glyco-MUC4 scFvs can be included in either the first polypeptide chain or the second polypeptide chain of the chimeric TCR, or a first scFv can be included in the first polypeptide chain and a second scFv can be included in the second polypeptide chain. In embodiments in

which two scFvs are included in one of either the first polypeptide chain or the second polypeptide chain of the chimeric TCR, the two scFvs can be linked via a peptide linker. In some embodiments, the chimeric TCR comprises two or more anti-glyco-MUC4 scFvs having the same amino acid sequence. In other embodiments, the chimeric TCR comprises two or more anti-glyco-MUC4 scFvs having different amino acid sequences.

[0261] In other embodiments, the anti-glyco-MUC4 antigen binding fragment is an Fv fragment. In some embodiments, an anti-glyco-MUC4 variable heavy chain (VH) described herein is included in one of the two polypeptide chains that associate to form the chimeric TCR. An anti-glyco-MUC4 variable light chain (VL) described herein can be included in the polypeptide chain that does not include the anti-glyco-MUC4 VH. When the first and second polypeptide chains dimerize, the anti-glyco-MUC4 VH and VL are brought together to form an anti-glyco-MUC4 Fv fragment. In some embodiments, the VH is included in the first polypeptide chain and the VL is included in the second polypeptide chain. In other embodiments, the VH is included in the second polypeptide chain and the VL is included in the first polypeptide chain.

[0262] In other embodiments, the anti-glyco-MUC4 antigen fragment is a Fab- domain, comprising VH, VL, CH1, and CL domains. In some embodiments, an anti-glyco-MUC4 variable heavy chain (VH) described herein and a CH1 domain is included in the first or second polypeptide chain. In some embodiments, an anti-glyco-MUC4 variable light chain (VL) described herein and a CL domain are included in the first or second polypeptide chain that does not include the anti-glyco-MUC4 VH and CH1. In other embodiments, an anti-glyco-MUC4 variable heavy chain (VH) and a CL domain is included in the first or second polypeptide chain. In some embodiments, an anti-glyco-MUC4 variable light chain (VL) and a CH1 domain are included in the polypeptide chain that does not include the anti-glyco-MUC4 VH and CL. When the first and second polypeptide chains dimerize, the anti-glyco-MUC4 VH and VL, and the CH1 and CL, are brought together to form an anti-glyco-MUC4 Fab domain. In some embodiments, the VH and the CH1 or CL is included in the first polypeptide chain, and the VL and the CL or CH1 is included in the second polypeptide chain. In other embodiments, the VH and the CH1 or CL is included in the second polypeptide chain, and the VL and the CH1 or CL is included in the first polypeptide chain.

[0263] In other embodiments, the anti-glyco-MUC4 VH and CH1 or CL are included in the first polypeptide chain of the second polypeptide chain, and the chimeric TCR further comprises a third polypeptide comprising the VL and either a CL domain or a CH1 domain. The third polypeptide is capable of associating with the VH and CH1 or CL of the first or second polypeptide chain, thus forming a Fab domain. In some embodiments, both the first and second polypeptide chains include a VH and a CH1 domain or a CL domain. Where both the first and second polypeptide chains include a VH and a CH1 or CL, a third polypeptide comprising a VL and a CL or CH1 associates with the first polypeptide chain to form a first Fab domain, and a

fourth polypeptide comprising a VL and a CL or CH1 associates with the second polypeptide chain to form a second Fab domain.

[0264] First and second TCR domains are included in the first and second polypeptide chains, respectively, with the first TCR domain comprising a first TCR transmembrane domain from a first TCR subunit and the second TCR domain comprising a second TCR transmembrane domain from a second TCR subunit. In some embodiments, the first TCR subunit is a TCR α chain and the second TCR subunit is a TCR β chain. In other embodiments, the first TCR subunit is a TCR β chain and the second TCR subunit is a TCR α chain. In In some embodiments, the first TCR subunit is a TCR γ chain and the second TCR subunit is a TCR δ chain. In other embodiments, the first TCR subunit is a TCR δ chain and the second TCR subunit is a TCR γ chain. A TCR transmembrane domain from a TCR subunit can be a native TCR transmembrane domain, a natural or engineered variant thereof, or a fragment of the native or variant TCR transmembrane domain. In some embodiments, the first and/or second TCR transmembrane domains comprise, individually, an amino acid sequence of a TCR transmembrane domain contained in one of SEQ ID NOS:77-80 of WO 2017/070608, which is incorporated by reference in its entirety. In other embodiments, the first and/or second TCR transmembrane domains comprise, individually, an amino acid sequence of SEQ ID NOS:1-4 of WO 2017/070608.

[0265] In some embodiments, in addition to the first and second TCR transmembrane domains, the first and second TCR domains also include first and second connecting peptides, respectively. The first and second connecting peptides are positioned at the N-terminus of the first and second TCR transmembrane domains, respectively. In some embodiments, the first connecting peptide comprises all or a portion of the connecting peptide of the first TCR subunit and/or the second connecting peptide comprises all or a portion of the connecting peptide of the second TCR subunit. In some embodiments, the first transmembrane domain and the first connecting peptide are derived from different TCR subunits and/or the second transmembrane domain and the second connecting peptide are derived from different TCR subunits. A connecting peptide from a TCR subunit can be a native TCR connecting peptide, a natural or engineered variant thereof, or a fragment of the native or variant TCR connecting peptide. In some embodiments, the first and/or second connecting peptides comprise, individually, an amino acid sequence of a connecting peptide contained in one of SEQ ID NOS:77-80 of WO 2017/070608. In other embodiments, the first and/or second connecting peptides comprise, individually, an amino acid sequence of SEQ ID NOS:5-12 of WO 2017/070608.

[0266] In some embodiments, the first and second TCR domains comprise a first and second TCR constant domain, respectively. The first and second TCR constant domains are positioned at the C-terminus of the first and second TCR transmembrane domains, respectively. If the first and/or second TCR domains include a TCR connecting peptide, the TCR constant domain can

be positioned at the C-terminus of the TCR connecting peptide. In some embodiments, the first TCR constant domain comprises all or a portion of the constant domain of the first TCR subunit and/or the second TCR constant domain comprises all or a portion of the constant domain of the second TCR subunit. For example, in some embodiments, the first and/or second TCR constant domains are derived from TCR a and β subunit constant domains, or TCR γ and δ subunit constant domains. A TCR constant domain from a TCR subunit can be a native TCR intra constant cellular domain, a natural or engineered variant thereof, or a fragment of the native or variant TCR constant domain. In some embodiments, the first and/or second TCR constant domain comprise, individually an amino acid sequence of SEQ ID NOS:172, 174, 176, 178, 180, or 182, or the wildtype equivalent thereof.

[0267] In some embodiments, the first and second TCR domains comprise first and second TCR intracellular domains, respectively. The first and second TCR intracellular domains are positioned at the C-terminus of the first and second TCR transmembrane domains, respectively. In some embodiments, the first TCR intracellular domain comprises all or a portion of the intracellular domain of the first TCR subunit and/or the second TCR intracellular domain comprises all or a portion of the intracellular domain of the second TCR subunit. A TCR intracellular domain from a TCR subunit can be a native TCR intracellular domain, a natural or engineered variant thereof, or a fragment of the native or variant TCR intracellular domain. In some embodiments, the first and/or second TCR intracellular domains comprise, individually, an amino acid sequence of a TCR intracellular domain contained in one of SEQ ID NOS:77-80 of WO 2017/070608. In other embodiments, the first and/or second TCR intracellular domain comprise, individually, an amino acid sequence of SEQ ID NOS:13-14 of WO 2017/070608.

[0268] In some embodiments, the first polypeptide chain of the chimeric TCR further comprises a first accessory intracellular domain C-terminal to the first TCR transmembrane domain and/or the second polypeptide chain of the chimeric TCR further comprises a second accessory intracellular domain C-terminal to the second transmembrane domain. In some embodiments, the first and/or second accessory intracellular domains comprise a TCR costimulatory domain. In some embodiments, the TCR costimulatory domain comprises all or a portion of the amino acid sequence of SEQ ID NO: 70 or 71 of WO 2017/070608.

[0269] In some embodiments the first TCR domain is a fragment of the first TCR subunit and/or the second TCR subunit is a fragment of the second TCR subunit.

[0270] The first and second polypeptide chains that form the chimeric TCR are linked. In some embodiments, the first and second polypeptide chains that form the chimeric TCR are linked by a disulfide bond. In some embodiments, first and second polypeptide chains that form the chimeric TCR are linked by a disulfide bond between a residue in the first connecting peptide and a residue in the second connecting peptide.

[0271] In some embodiments, the first and second polypeptide chains are linked or otherwise associate. In some embodiments, the associated first and second polypeptide chains are capable of recruiting at least one TCR-associated signaling modules, such as, *e.g.*, CD3δε, CD3γε, and ζζ. In certain embodiments, the associated first and second polypeptide chains are capable of recruiting each of CD3δε, CD3γε, and ζζ, forming a TCR-CD3 complex.

[0272] In some embodiments, the first polypeptide chain comprises a first linker between the first TCR domain and an anti-glyco-MUC4 VH or VL of the scFv, Fv, or Fab fragment included in the first polypeptide chain. In some embodiments, the second polypeptide chain comprises a second linker between the second TCR domain and an anti-glyco-MUC4 VH or VL of the scFv, Fv, or Fab fragment included in the second polypeptide chain. In some embodiments, the first peptide linker and/or the second peptide linker comprises between about 5 to about 70 amino acids. In some embodiment, the first and/or second linker comprises a constant domain or fragment thereof from an immunoglobulin or T cell receptor subunit. In some embodiments, the first and/or second linker comprises an immunoglobulin constant domain or fragment thereof. For example, in those embodiments described above comprising a CH1 or CL domain, the CH1 or CL domain functions as a linker between the TCR domain and the anti-glyco-MUC4 binding fragment, or a subpart (*e.g.*, VH or VL) thereof. The immunoglobulin constant domain can also be, in addition to CH1 or CL, a CH2, CH3, or CH4 domain or fragment thereof. The immunoglobulin constant domains can be derived from an IgG (*e.g.*, IgG1, IgG2, IgG3, or IgG4), IgA (*e.g.*, IgA1 or IgA2), IgD, IgM, or IgE heavy chain. In some embodiments the constant domains can be derived from a human (*e.g.*, IgG1, IgG2, IgG3, or IgG4), IgA (*e.g.*, IgA1 or IgA2), IgD, IgM, or IgE heavy chain. In other embodiments, a TCR constant domain or fragment thereof described above functions as a linker between the TCR domain and the anti-glyco-MUC4 binding fragment, or a subpart (*e.g.*, VH or VL) thereof. In some embodiments, the first and second linkers are capable of binding to one another.

[0273] In some embodiments, the first and second polypeptide chains are connected, at least temporarily, by a cleavable peptide linker. In some embodiments, the cleavable peptide linker is a furin-p2A cleavable peptide. The cleavable peptide linker can facilitate expression of the two polypeptide chains. The cleavable peptide linker can be configured to temporarily associate the first polypeptide chain with the second polypeptide chain during and/or shortly after protein translation.

[0274] In some embodiments, the chimeric TCR is a synthetic T cell receptor and antigen receptor (STAR), as described in Liu et al., 2021, Sci Transl Med, and WO 2020/029774, the contents of each of which are incorporated herein by reference in their entireties.

[0275] In some aspects, the STAR comprises, from N- to C-terminus, a first polypeptide chain comprising an anti-glyco-MUC4 variable heavy chain and a TCRα chain constant region

domain; a cleavable peptide linker; and a second polypeptide chain comprising an anti-glyco-MUC4 variable light chain and a TCRβ constant region domain (configuration STAR 1).

[0276] In other aspects, the STAR comprises, from N- to C-terminus, a first polypeptide chain comprising an anti-glyco-MUC4 variable heavy chain and a TCRβ chain constant region domain; a cleavable peptide linker; and a second polypeptide chain comprising an anti-glyco-MUC4 variable light chain and a TCRα constant region domain (configuration STAR 2).

[0277] In other aspects, the STAR comprises, from N- to C-terminus, a first polypeptide chain comprising an anti-glyco-MUC4 variable light chain and a TCRα chain constant region domain; a cleavable peptide linker; and a second polypeptide chain comprising an anti-glyco-MUC4 variable heavy chain and a TCRβ constant region domain (configuration STAR 3).

[0278] In other aspects, the STAR comprises, from N- to C-terminus, a first polypeptide chain comprising an anti-glyco-MUC4 variable light chain and a TCRβ chain constant region domain; a cleavable peptide linker; and a second polypeptide chain comprising an anti-glyco-MUC4 variable heavy chain and a TCRα constant region domain (configuration STAR 4).

[0279] In certain embodiments, the TCRα chain constant region domain and the TCRβ chain constant region domain of any one of configurations STAR 1 through STAR 4 can be replaced by TCRγ and TCRδ constant region domains, respectively.

[0280] The chimeric TCRs of the present disclosure can form complexes with TCR-associated signaling molecules (*e.g.*, CD3γε, CD3δε, and ζζ) endogenously expressed in T cells. These complexes provide for TCR signaling controlled by binding of the anti-glyco- MUC4 heavy and light variable chains by its target.

[0281] Chimeric TCRs of the disclosure are further described in numbered embodiments 490 to 584.

5.4.1. TCR Constant Domains

[0282] With respect to the TCR constant domains, the chimeric TCR can be designed to comprise constant regions that are derived from, *e.g.*, human peripheral blood T cells. Nucleotide and corresponding amino acid sequences for TCR constant regions for use in chimeric TCRs according to the disclosure are provided in Table 5.

Table 5
Nucleotide and Amino Acid Sequences for TCR Constant Regions

Description	Sequence	SEQ ID NO:
TCRα Constant Region – Nucleic Acid (human)	Gatatccagaaccctgaccctgctgtctatcaactccgggactctaaatcca gtgacaagtctgtctgcctattcaccgattttgattctcaaacaaatgtgtcac aaagtaaggattctgatgtgtatatcacagacaaatgtgtgctagacatgag gtctatggacttcaagagcaacagtgctgtggcctggagcaacaaatctga ctttgcatgtgcaaacgccttcaacaacagcattattccagaagacaccttct tccccagcccagaaagttcctgtgatgtcaagctggtcgagaaaagctttg aaacagatacgaacctaaactttcaaaacctgtcagtgattgggttccgaat cctcctcctgaaagtggccgggtttaatctgctcatgacgctgcggctgtggt ccagc	170

Table 5 Nucleotide and Amino Acid Sequences for TCR Constant Regions		
Description	**Sequence**	**SEQ ID NO:**
TCRα Constant Region – Amino Acid (human)	XIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQS KDSDVYITDKCVLDMRSMDFKSNSAVAWSNKSDFAC ANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLN FQNLSVIGFRILLLKVAGFNLLMTLRLWSS X=Asp, Asn, His, Tyr	171
TCRα Constant Region – Amino Acid (murine); Cysteine mutant	Aatatccagaacccagaacctgctgtgtaccagttaaaagatcctcggtct caggacagcaccctctgcctgttcaccgactttgactcccaaatcaatgtgc cgaaaaccatggaatctggaacgttcatcactgacaaaactgtgctggac atgaaagctatggattccaagagcaatggggccattgcctggagcaacca gacaagcttcacctgccaagatatcttcaaagagaccaacgccacctacc ccagttcagacgttccctgtgatgccacgttgactgagaaaagctttgaaac agatatgaacctaaactttcaaaacctgtcagttatgggactccgaatcctcc tgctgaaagtagccggatttaacctgctcatgacgctgaggctgtggtccag ttga	172
TCRα Constant Region – Amino Acid (murine); Cysteine mutant	XIQNPEPAVYQLKDPRSQDSTLCLFTDFDSQINVPKT MESGTFITDKTVLDMKAMDSKSNGAIAWSNQTSFTC QDIFKETNATYPSSDVPCDATLTEKSFETDMNLNFQN LSVMGLRILLLKVAGFNLLMTLRLWSS X at 1, x=Asp, Asn, His, Tyr	173
TCRβ Constant Region – Nucleic Acid (human)	gaggacctgaaaaacgtgttcccacccgaagtggccgtcttcgaaccatc agaagcagagatctcccacacccaaaaggccacactggtgtgcctggcc acaggcttcttccccgaccacgtggagctgagctggtgggtgaatgggaa ggaggtgcacagtggggtctgcacagacccgcagcccctcaaggagca gcccgccctcaatgactccagatactgcctgagcagccgcctgagggtctc ggccaccttctggcagaacccccgcaaccacttccgctgtcaagtccagtt ctacgggctctcggagaatgacgagtggacccaggatagggccaaaccc gtcacccagatcgtcagcgccgaggcctggggtagagcagactgtggcttt acctcggtgtcctaccagcaaggggtcctgtctgccaccatcctctatgaga tcctgctagggaaggccaccctgtatgctgtgctggtcagcgcccttgtgttg atggccatggtcaagagaaaggatttc	174
TCRβ Constant Region – Amino Acid (human)	EDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFFP DHVELSWWVNGKEVHSGVCTDPQPLKEQPALNDSR YCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDE WTQDRAKPVTQIVSAEAWGRADCGFTSVSYQQGVL SATILYEILLGKATLYAVLVSALVLMAMVKRKD	175
TCRβ Constant Region – Amino Acid (murine); Cysteine mutant	gaggatctgagaaatgtgactccacccaaggtctccttgtttgagccatcaa aagcagagattgcaaacaaacaaaaggctaccctcgtgtgcttggccag gggcttcttccctgaccacgtggagctgagctggtgggtgaatggcaagga ggtccacagtggggtcagcacggaccctcaggcctacaaggagagcaat tatagctactgcctgagcagccgcctgagggtctctgctaccttctggcaca atcctcgcaaccacttccgctgccaagtgcagttccatgggctttcagagga ggacaagtggccagagggctcacccaaacctgtcacacagaacatcagt gcagaggcctggggccgagcagactgtgggattacctcagcatcctatca acaaggggtcttgtctgccaccatcctctatgagatcctgctagggaaagcc accctgtatgctgtgcttgtcagtacactggtggtgatggctatggtcaaaag aaagaattca	176
TCRβ Constant Region – Amino Acid (murine); Cysteine mutant	EDLRNVTPPKVSLFEPSKAEIANKQKATLVCLARGFFP DHVELSWWVNGKEVHSGVSTDPQAYKESNYSYCLS SRLRVSATFWHNPRNHFRCQVQFHGLSEEDKWPEG SPKPVTQNISAEAWGRADCGITSASYQQGVLSATILY EILLGKATLYAVLVSTLVVMAMVKRKNS	177
TCRγ Constant Region – Amino Acid (human)	DKQLDADVSPKPTIFLPSIAETKLQKAGTYLCLLEKFFP DVIKIHWQEKKSNTILGSQEGNTMKTNDTYMKFSWLT VPEKSLDKEHRCIVRHENNKNGVDQEIIFPPIKTDVITM DPKDNCSKDANDTLLLQLTNTSAYYMYLLLLLKSVVY FAIITCCLLRRTAFCCNGEKS	178

Table 5 Nucleotide and Amino Acid Sequences for TCR Constant Regions		
Description	**Sequence**	**SEQ ID NO:**
TCRγ Constant Region – Amino Acid (murine)	XKRLDADISPKPTIFLPSVAETNLHKTGTYLCLLEKFFP DVIRVYWKEKDGNTILDSQEGDTLKTNDTYMKFSWLT VPERAMGKEHRCIVKHENNKGGADQEIFFPSIKKVAV STKPTTCWQDKNDVLQLQFTITSAYYTYLLLLLKSVIYL AIISFSLLRRTSVCGNEKKS X = any naturally occurring amino acid	179
TCRδ Constant Region – Amino Acid (human)	SQPHTKPSVFVMKNGTNVACLVKEFYPKDIRINLVSS KKITEFDPAIVISPSGKYNAVKLGKYEDSNSVTCSVQH DNKTVHSTDFEVKTDSTDHVKPKETENTKQPSKSCH KPKAIVHTEKVNMMSLTVLGLRMLFAKTVAVNFLLTAK LFFL	180
TCRδ Constant Region – Amino Acid (murine)	XSQPPAKPSVFIMKNGTNVACLVKDFYPKEVTISLRSS KKIVEFDPAIVISPSGKYSAVKLGQYGDSNSVTCSVQH NSETVHSTDFEPYANSFNNEKLPEPENDTQISEPCYG PRVTVHTEKVNMMSLTVLGLRLLFAKTIAINFLLTVKLF F X = any naturally occurring amino acid	181

[0283] In certain embodiments the TCR constant regions of the chimeric TCR can be modified to provide for additional bonds between two TCR constant domains of the chimeric TCR. In some embodiments, the residue corresponding to position 48 of the wildtype human TCRα constant domain is mutated to cysteine and the residue corresponding to position 57 of the wildtype human TCRβ constant domain is mutated to cysteine. This results in the formation of a disulfide linkage between TCRα and TCRβ constant domains, resulting in a disulfide bond between the first and second polypeptide chains of the chimeric TCR. In some embodiments, the residue corresponding to position 85 of the wildtype human TCRα constant domain is mutated to alanine and the residue corresponding to position 88 of the wildtype human TCRβ constant domain is mutated to glycine. Again, this results in the formation of a disulfide linkage between TCRα and TCRβ constant domains.

5.4.2. Cleavable Linkers

[0284] The two polypeptide chains of the chimeric TCRs of the disclosure can be linked via a peptide linker. In some embodiments, the two polypeptide chains of the chimeric TCR are linked via a furin-P2A peptide linker, which provides a protease cleavage site between the two polypeptide chains. The two polypeptide chains can thus be transcribed and translated into a fusion protein, which is subsequently cleaved by a protease into two distinct protein subunits. In some embodiments, the two resulting protein subunits are covalently bound through disulfide bonds, and subsequently form a complex with the endogenous CD3 subunits (ε, δ, λ, and ζ) of T cells.

[0285] In some embodiments, the furin-P2A peptide linker comprises the sequence RAKRSGSGATNFSLLKQAGDVEENPGP (SEQ ID NO:199).

[0286] In some embodiments, the furin-P2A peptide linker comprises the sequence ATNFSLLKQAGDVEENPGP (SEQ ID NO:200).

5.5 MicAbodies

[0287] The present disclosure provides MicAbodies comprising the anti-glyco-MUC4 antibodies and antigen-binding fragments of the disclosure. MicAbodies are fusion proteins comprising an antibody or antigen-binding fragment and an engineered MHC-class I-chain-related (MIC) protein domain. MIC proteins are the natural ligands of human NKG2D receptors expressed on the surface of NK cells, and the α1-α2 domain of MIC proteins provides the binding site for the NKG2D receptor. By fusing an engineered MIC protein domain (*e.g.*, an engineered α1-α2 domain) to a cancer-targeting antibody or antigen-binding fragment, T-cells expressing an engineered NKG2D receptor capable of binding the engineered MIC protein domain can be targeted to cancer cells. Engineered MIC protein domains that can be included in MicAbodies of the disclosure, and NKG2D receptors capable of binding the engineered MIC protein domains, CARs and CAR T cells comprising the NKG2D receptors are described in U.S. publication nos. US 2011/0183893, US2011/0311561, US 2015/0165065, and US 2016/0304578 and PCT publication nos. WO 2016/090278, WO 2017/024131, WO 2017/222556, and WO 2019/191243, the contents of which are incorporated herein by reference in their entireties.

[0288] In some embodiments, the MicAbodies of the disclosure comprise α1-α2 domains which are at least 80% identical or homologous to the α1-α2 domain of an NKG2D ligand (*e.g.*, MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, or OMCP). Exemplary amino acid sequences of MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, and OMCP are set forth as SEQ ID NOs: 1-9 of WO 2019/191243, respectively, the sequences of which are incorporated herein by reference. In other embodiments, the α1-α2 domain is 85% identical to a native or natural α1-α2 domain of an NKG2D ligand. In yet other embodiments, the α1-α2 domain is 90% identical to a native or natural α1-α2 domain of a natural NKG2D ligand protein and binds non-natural NKG2D.

[0289] In some embodiments, the MicAbodies of the disclosure comprise α1-α2 domains which are at least 80% identical or homologous to a native or natural α1-α2 domain of a human MICA or MICB protein and bind NKG2D. In some embodiments, the α1-α2 domain is 85% identical to a native or natural α1-α2 domain of a human MICA or MICB protein and binds NKG2D. In other embodiments, the α1-α2 domain is 90%, 95%, 96%, 97%, 98%, or 99% identical to a native or natural α1-α2 platform domain of a human MICA or MICB protein and binds NKG2D.

[0290] In some embodiments, specific mutations in α1-α2 domains of NKG2D ligands can be made to create non-natural α1-α2 domains that bind non-natural NKG2D receptors, themselves engineered so as to have reduced affinity for natural NKG2D ligands. This can be done, for example, through genetic engineering. A non-natural NKG2D receptor so modified can be used

to create on the surface of NK- or T-cells of the immune system an NKG2D-based CAR that can preferentially bind to and be activated by molecules comprised of the non-natural α1-α2 domains. These pairs of non-natural NKG2D receptors and their cognate non-natural NKG2D ligands can provide important safety, efficacy, and manufacturing advantages for treating cancer and viral infections as compared to traditional CAR-T cells and CAR-NK cells. Activation of CAR-T cells and CAR-NK cells having a NKG2D-based CAR can be controlled by administration of a MicAbody. In the event that an adverse event develops, the dosing regimen of the MicAbody can be modified rather than having to deploy an induced suicide mechanism to destroy the infused CAR cells.

[0291] MicAbodies can be generated by attaching an antibody or antigen-binding fragment to an engineered α1-α2 domain via a linker, *e.g.*, APTSSSGGGGS (SEQ ID NO:182) or GGGS (SEQ ID NO:183). For example, an α1-α2 domain can be fused to the C-terminus of an IgG heavy chain or light chain, for example, as described in WO 2019/191243.

[0292] In some embodiments, the MicAbodies of the disclosure comprise an engineered α1-α2 domain comprising the amino acid sequence EPHSLRYNLTVLSWDGSVQSGFLTEVHLDGQPFLRCDRQKCRAKPQGQWAEDVLGNKTWD RETRDLTGWGTTLLMTLAHIKDQKEGLHSLQEIRVCEIHEDNSTRSSQHFYYDGELFLSQNLET LEWTMPQSSRAQTLAMNVRNFLKEDAMETDIGYRLMRADCLSELRRYLKSGVVLRRTV (SEQ ID NO:184) (MICA25.17).

[0293] In other embodiments, the MicAbodies of the disclosure comprise an engineered α1-α2 domain comprising the amino acid sequence EPHSLRYNLTVLSWDGSVQSGFLTEVHLDGQPFLRCDRQKCRAKPQGQWAEDVLGNKTWD RETRDLTGWGTFLRMTLAHIKDQKEGLHSLQEIRVCEIHEDNSTRSSQHFYYDGELFLSQNLET LEWTMPQSSRAQTLAMNVRNFLKEDAMETDRSGLLMRADCLSELRRYLKSGVVLRRTV (SEQ ID NO:185) (MICA25.18).

[0294] In other embodiments, the MicAbodies of the disclosure comprise an engineered α1-α2 domain comprising the amino acid sequence AAEPHSLSYDITVIPKFRPGPRWCAVQGQVDEKTFLHYDCGNKTVTPVSPLGKKLNVTTAWKA QNPVLREVVDILTEQLWDIQLENYTPKEPLTLQARMSCEQKAEGHSSGSWQFSFDGQIFLLFD SEKRMWTTVHPGARKMKEKWENDKVVATTLYTWSMGDCIGWLEDFLMGMDSTLEPSAGAP (SEQ ID NO:186) (ULBP2.S1).

[0295] In other embodiments, the MicAbodies of the disclosure comprise an engineered α1-α2 domain comprising the amino acid sequence AAEPHSLSYDITVIPKFRPGPRWCAVQGQVDEKTFLHYDCGNKTVTPVSPLGKKLNVTTAWKA QNPVLREVVDILTEQLWDIQLENYTPKEPLTLQARMSCEQKAEGHSSGSWQFSFDGQIFLLFD SEKRMWTTVHPGARKMKEKWENDKVVATLMRIWSMGDCIGWLEDFLMGMDSTLEPSAGAP (SEQ ID NO:187) (ULBP2.S2).

[0296] In other embodiments, the MicAbodies of the disclosure comprise an engineered α1-α2 domain comprising the amino acid sequence AAEPHSLSYDITVIPKFRPGPRWCAVQGQVDEKTFLHYDCGNKTVTPVSPLGKKLNVTTAWKA QNPVLREVVDILTEQLWDIQLENYTPKEPLTLQARMSCEQKAEGHSSGSWQFSFDGQIFLLFD SEKRMWTTVHPGARKMKEKWENDKVVATKLYLWSMGDCIGWLEDFLMGMDSTLEPSAGAP (SEQ ID NO:188) (ULBP2.S3).

[0297] In other embodiments, the MicAbodies of the disclosure comprise an engineered α1-α2 domain comprising the amino acid sequence AAEPHSLWYNFTIIHLPRHGQQWCEVQSQVDQKNFLSYDCGSDKVLSMGHLEEQLYATDAW GKQLEMLREVGQRLRLELADTELEDFTPSGPLTLQVRMSCESEADGYIRGSWQFSFDGRKFL LFDSNNRKWTVVHAGARRMKEKWEKDSGLTTDLIRRSMGDCKSWLRDFLMHRKKRLEPTAP (SEQ ID NO:189) (ULBP3.S1).

[0298] In other embodiments, the MicAbodies of the disclosure comprise an engineered α1-α2 domain comprising the amino acid sequence AAEPHSLWYNFTIIHLPRHGQQWCEVQSQVDQKNFLSYDCGSDKVLSMGHLEEQLYATDAW GKQLEMLREVGQRLRLELADTELEDFTPSGPLTLQVRMSCESEADGYIRGSWQFSFDGRKFL LFDSNNRKWTVVHAGARRMKEKWEKDSGLTTYFYLRSMGDCKSWLRDFLMHRKKRLEPTAP (SEQ ID NO:190) (ULBP3.S2).

[0299] In other embodiments, the MicAbodies of the disclosure comprise an engineered α1-α2 domain comprising the amino acid sequence EPHSLSYDITVIPKFRPGPRWCAVQGQVDEKTFLHYDCGNKTVTPVSPLGKKLNVTTAWKAQN PVLREVVDILTEQLWDIQLENYTPKEPLTLQARMSCEQKAEGHSSGSWQFSFDGQIFLLFDSE KRMWTTVHPGARKMKEKWENDKVVATILWQTSMGDCIGWLEDFLMGMDSTLEPS (SEQ ID NO:191) (ULBP2.C).

[0300] In other embodiments, the MicAbodies of the disclosure comprise an engineered α1-α2 domain comprising the amino acid sequence EPHSLSYDITVIPKFRPGPRWCAVQGQVDEKTFLHYDCGNKTVTPVSPLGKKLNVTTAWKAQN PVLREVVDILTEQLWDIQLENYTPKEPLTLQARMSCEQKAEGHSSGSWQFSFDGQIFLLFDSE KRMWTTVHPGARKMKEKWENDKVVATLLWGWSMGDCIGWLEDFLMGMDSTLEPS (SEQ ID NO:192) (ULBP2.R).

[0301] In other embodiments, the MicAbodies of the disclosure comprise an engineered α1-α2 domain comprising the amino acid sequence EPHSLSYDITVIPKFRPGPRWCAVQGQVDEKTFLHYDCGNKTVTPVSPLGKKLNVTTAWKAQN PVLREVVDILTEQLWDIQLENYTPKEPLTLQARMSCEQKAEGHSSGSWQFSFDGQIFLLFDSE KRMWTTVHPGARKMKEKWENDKVVATMFWSWSMGDCIGWLEDFLMGMDSTLEPS (SEQ ID NO:193) (ULBP2.AA).

[0302] In other embodiments, the MicAbodies of the disclosure comprise an engineered α1-α2 domain comprising the amino acid sequence EPHSLSYDITVIPKFRPGPRWCAVQGQVDEKTFLHYDCGNKTVTPVSPLGKKLNVTTAWKAQN PVLREVVDILTEQLWDIQLENYTPKEPLTLQARMSCEQKAEGHSSGSWQFSFDGQIFLLFDSE KRMWTTVHPGARKMKEKWENDKVVATLMWQWSMGDCIGWLEDFLMGMDSTLEPS (SEQ ID NO:194) (ULBP2.AB).

[0303] An exemplary engineered NKG2D receptor comprises the amino acid sequence NSLFNQEVQIPLTESYCGPCPKNWICYKNNCYQFFDESKNWYESQASCMSQNASLLKVYSKE DQDLLKLVKSYHWMGLVHIPTNGSWQWEDGSILSPNLLTIIEMQKGDCALYASSFKGYIENCST PNTYICMQRTV (SEQ ID NO:195) in which the tyrosine at position 73 has been replaced with another amino acid, for example alanine.

[0304] Another exemplary engineered NKG2D receptor comprises the amino acid sequence FLNSLFNQEVQIPLTESYCGPCPKNWICYKNNCYQFFDESKNWYESQASCMSQNASLLKVYS KEDQDLLKLVKSYHWMGLVHIPTNGSWQWEDGSILSPNLLTIIEMQKGDCALYASSFKGYIENC STPNTYICMQRTV (SEQ ID NO:196) in which the tyrosines are positions 75 and 122 have been replaced with another amino acid, for example alanine at position 75 and phenylalanine at position 122.

5.6 Nucleic Acids, Recombinant Vectors and Host Cells

[0305] The present disclosure encompasses nucleic acid molecules encoding immunoglobulin light and heavy chain genes for anti-glyco-MUC4 antibodies, vectors comprising such nucleic acids, and host cells capable of producing the anti-glyco-MUC4 antibodies of the disclosure. In certain aspects, the nucleic acid molecules encode, and the host cells are capable of expressing, the anti-glyco-MUC4 antibodies and antibody-binding fragments of the disclosure (*e.g.*, as described in Section 5.1 and numbered embodiments 1 to 414) as well as fusion proteins (*e.g.*, as described in numbered embodiments 421 to 445), chimeric antigen receptors (*e.g.*, as described in Section 5.3 and numbered embodiments 446 to 479), and chimeric T cell receptors (*e.g.*, as described in Section 5.4 and numbered embodiments 490 to 584) containing them. Exemplary nucleic acids of the disclosure are described in embodiments 585 and 586, exemplary vectors of the disclosure are described in numbered embodiments 587 to 589, and exemplary host cells of the disclosure are described in numbered embodiments 590 to 596.

[0306] An anti-glyco-MUC4 antibody of the disclosure can be prepared by recombinant expression of immunoglobulin light and heavy chain genes in a host cell. To express an antibody recombinantly, a host cell is transfected with one or more recombinant expression vectors carrying DNA fragments encoding the immunoglobulin light and heavy chains of the antibody such that the light and heavy chains are expressed in the host cell and, optionally, secreted into the medium in which the host cells are cultured, from which medium the antibodies can be recovered. Standard recombinant DNA methodologies are used to obtain

antibody heavy and light chain genes, incorporate these genes into recombinant expression vectors and introduce the vectors into host cells, such as those described in Molecular Cloning; A Laboratory Manual, Second Edition (Sambrook, Fritsch and Maniatis (eds), Cold Spring Harbor, N. Y., 1989), Current Protocols in Molecular Biology (Ausubel, F. M. *et al.*, eds., Greene Publishing Associates, 1989) and in U.S. Pat. No. 4,816,397.

[0307] To generate nucleic acids encoding such anti-glyco-MUC4 antibodies, DNA fragments encoding the light and heavy chain variable regions are first obtained. These DNAs can be obtained by amplification and modification of germline DNA or cDNA encoding light and heavy chain variable sequences, for example using the polymerase chain reaction (PCR). Germline DNA sequences for human heavy and light chain variable region genes are known in the art (see, *e.g.*, the "VBASE" human germline sequence database; see also Kabat *et al.*, 1991, Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242; Tomlinson *et al.*, 1992, J. Mol. Biol. 22T:116-198; and Cox *et al.*, 1994, Eur. J. Immunol. 24:827-836; the contents of each of which are incorporated herein by reference).

[0308] Once DNA fragments encoding anti-glyco-MUC4 antibody-related V_H and V_L segments are obtained, these DNA fragments can be further manipulated by standard recombinant DNA techniques, for example to convert the variable region genes to full-length antibody chain genes, to Fab fragment genes or to a scFv gene. In these manipulations, a V_H- or V_L -encoding DNA fragment is operatively linked to another DNA fragment encoding another protein, such as an antibody constant region or a flexible linker. The term "operatively linked," as used in this context, is intended to mean that the two DNA fragments are joined such that the amino acid sequences encoded by the two DNA fragments remain in-frame.

[0309] The isolated DNA encoding the V_H region can be converted to a full-length heavy chain gene by operatively linking the V_H-encoding DNA to another DNA molecule encoding heavy chain constant regions (CH_1, CH_2, CH_3 and, optionally, CH_4). The sequences of human heavy chain constant region genes are known in the art (see, *e.g.*, Kabat *et al.*, 1991, Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The heavy chain constant region can be an IgG_1, IgG_2, IgG_3, IgG_4, IgA, IgE, IgM or IgD constant region, but in certain embodiments is an IgG_1 or IgG_4 constant region. For a Fab fragment heavy chain gene, the V_H-encoding DNA can be operatively linked to another DNA molecule encoding only the heavy chain CH1 constant region.

[0310] The isolated DNA encoding the V_L region can be converted to a full-length light chain gene (as well as a Fab light chain gene) by operatively linking the V_L-encoding DNA to another DNA molecule encoding the light chain constant region, CL. The sequences of human light

chain constant region genes are known in the art (see, *e.g.*, Kabat *et al.*, 1991, Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The light chain constant region can be a kappa or lambda constant region, but in certain embodiments is a kappa constant region.

[0311] To create an scFv gene, the V_H- and V_L-encoding DNA fragments can be operatively linked to another fragment encoding a flexible linker, *e.g.*, encoding the amino acid sequence $(Gly_4\sim Ser)_3$, such that the V_H and V_L sequences can be expressed as a contiguous single-chain protein, with the V_H and V_L regions joined by the flexible linker (see, *e.g.*, Bird *et al.*, 1988, Science 242:423-426; Huston *et al.*, 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; McCafferty *et al.*, 1990, Nature 348:552-554).

[0312] To express the anti-glyco-MUC4 antibodies of the disclosure, DNAs encoding partial or full-length light and heavy chains, obtained as described above, are inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences. In this context, the term "operatively linked" is intended to mean that an antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be inserted into separate vectors or, more typically, both genes are inserted into the same expression vector.

[0313] The antibody genes are inserted into the expression vector by standard methods (*e.g.*, ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present). Prior to insertion of the anti-glyco-MUC4 antibody-related light or heavy chain sequences, the expression vector can already carry antibody constant region sequences. For example, one approach to converting the anti-glyco-MUC4 monoclonal antibody-related V_H and V_L sequences to full-length antibody genes is to insert them into expression vectors already encoding heavy chain constant and light chain constant regions, respectively, such that the V_H segment is operatively linked to the CH segment(s) within the vector and the V_L segment is operatively linked to the CL segment within the vector. Additionally or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene can be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (*i.e.*, a signal peptide from a non-immunoglobulin protein).

[0314] In addition to the antibody chain genes, the recombinant expression vectors of the disclosure carry regulatory sequences that control the expression of the antibody chain genes in a host cell. The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals) that control the transcription or translation of the antibody chain genes. Such regulatory sequences are described, for example, in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif., 1990. It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, *etc.* Suitable regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV) (such as the CMV promoter/enhancer), Simian Virus 40 (SV40) (such as the SV40 promoter/enhancer), adenovirus, (*e.g.*, the adenovirus major late promoter (AdMLP)) and polyoma. For further description of viral regulatory elements, and sequences thereof, see, *e.g.*, U.S. Pat. No. 5,168,062 by Stinski, U.S. Pat. No. 4,510,245 by Bell *et al.*, and U.S. Pat. No. 4,968,615 by Schaffner *et al.*

[0315] In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors of the disclosure can carry additional sequences, such as sequences that regulate replication of the vector in host cells (*e.g.*, origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see, *e.g.*, U.S. Pat. Nos. 4,399,216, 4,634,665 and 5,179,017, all by Axel *et al.*). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Suitable selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in $DHFR^-$ host cells with methotrexate selection/amplification) and the neo gene (for G418 selection). For expression of the light and heavy chains, the expression vector(s) encoding the heavy and light chains is transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, *e.g.*, electroporation, lipofection, calcium-phosphate precipitation, DEAE-dextran transfection and the like.

[0316] It is possible to express the antibodies of the disclosure in either prokaryotic or eukaryotic host cells. In certain embodiments, expression of antibodies is performed in eukaryotic cells, *e.g.*, mammalian host cells, of optimal secretion of a properly folded and immunologically active antibody. Exemplary mammalian host cells for expressing the recombinant antibodies of the disclosure include Chinese Hamster Ovary (CHO cells) (including $DHFR^-$ CHO cells, described in Urlaub and Chasin, 1980, Proc. Natl. Acad. Sci. USA 77:4216-

4220, used with a DHFR selectable marker, *e.g.*, as described in Kaufman and Sharp, 1982, Mol. Biol. 159:601-621), NSO myeloma cells, COS cells and SP2 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods. Host cells can also be used to produce portions of intact antibodies, such as Fab fragments or scFv molecules. It is understood that variations on the above procedure are within the scope of the present disclosure. For example, it can be desirable to transfect a host cell with DNA encoding either the light chain or the heavy chain (but not both) of an anti-glyco-MUC4 antibody of this disclosure.

[0317] For expression of a CAR of the disclosure, for example as described in Section 5.3 and in numbered embodiments 446 to 479, it is preferable that the host cell is a T cell, preferably a human T cell. In some embodiments, the host cell exhibits an anti-tumor immunity when the cell is cross-linked with glyco-MUC4 on a tumor cell. Detailed methods for producing the T cells of the disclosure are described in Section 5.6.1.

[0318] For expression of a chimeric TCR of the disclosure, for example as described in Section 5.4 and in numbered embodiments 490 to 584, it is preferable that the host cell is a T cell, preferably a human T cell. In some embodiments, the host cell exhibits an anti-tumor immunity when the cell is cross-linked with glyco-MUC4 on a tumor cell. Detailed methods for producing the T cells of the disclosure are described in Section 5.6.1.

[0319] Recombinant DNA technology can also be used to remove some or all of the DNA encoding either or both of the light and heavy chains that is not necessary for binding to glyco-MUC4. The molecules expressed from such truncated DNA molecules are also encompassed by the antibodies of the disclosure.

[0320] For recombinant expression of an anti-glyco-MUC4 antibody of the disclosure, the host cell can be co-transfected with two expression vectors of the disclosure, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors can contain identical selectable markers, or they can each contain a separate selectable marker. Alternatively, a single vector can be used which encodes both heavy and light chain polypeptides.

[0321] Once a nucleic acid encoding one or more portions of an anti-glyco-MUC4 antibody, further alterations or mutations can be introduced into the coding sequence, for example to generate nucleic acids encoding antibodies with different CDR sequences, antibodies with reduced affinity to the Fc receptor, or antibodies of different subclasses.

[0322] The anti-glyco-MUC4 antibodies of the disclosure can also be produced by chemical synthesis (*e.g.*, by the methods described in Solid Phase Peptide Synthesis, 2nd ed., 1984 The Pierce Chemical Co., Rockford, Ill.). Variant antibodies can also be generated using a cell-free platform (see, *e.g.*, Chu *et al.*, Biochemia No. 2, 2001 (Roche Molecular Biologicals) and Murray *et al.*, 2013, Current Opinion in Chemical Biology, 17:420-426).

[0323] Once an anti-glyco-MUC4 antibody of the disclosure has been produced by recombinant expression, it can be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (*e.g.*, ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Further, the anti-glyco-MUC4 antibodies of the present disclosure and/or binding fragments can be fused to heterologous polypeptide sequences described herein or otherwise known in the art to facilitate purification.

[0324] Once isolated, the anti-glyco-MUC4 antibody can, if desired, be further purified, *e.g.*, by high performance liquid chromatography (see, *e.g.*, Fisher, Laboratory Techniques In Biochemistry And Molecular Biology, Work and Burdon, eds., Elsevier, 1980), or by gel filtration chromatography on a Superdex™ 75 column (Pharmacia Biotech AB, Uppsala, Sweden).

5.6.1. Recombinant Production of CARs and Chimeric TCRs in T Cells

[0325] In some embodiments, nucleic acids encoding the anti-glyco-MUC4 CARs or chimeric TCRs of the disclosure are delivered into cells using a retroviral or lentiviral vector. CAR- or chimeric TCR-expressing retroviral and lentiviral vectors can be delivered into different types of eukaryotic cells as well as into tissues and whole organisms using transduced cells as carriers or cell-free local or systemic delivery of encapsulated, bound or naked vectors. The method used can be for any purpose where stable expression is required or sufficient.

[0326] In other embodiments, the CAR or chimeric TCR sequences are delivered into cells using *in vitro* transcribed mRNA. *In vitro* transcribed mRNA CAR or chimeric TCR can be delivered into different types of eukaryotic cells as well as into tissues and whole organisms using transfected cells as carriers or cell-free local or systemic delivery of encapsulated, bound or naked mRNA. The method used can be for any purpose where transient expression is required or sufficient.

[0327] In another embodiment, the desired CAR or chimeric TCR can be expressed in the cells by way of transponsons.

[0328] One advantage of RNA transfection methods of the disclosure is that RNA transfection is essentially transient and a vector-free: an RNA transgene can be delivered to a lymphocyte and expressed therein following a brief *in vitro* cell activation, as a minimal expressing cassette without the need for any additional viral sequences. Under these conditions, integration of the transgene into the host cell genome is unlikely. Cloning of cells is not necessary because of the

efficiency of transfection of the RNA and its ability to uniformly modify the entire lymphocyte population.

[0329] Genetic modification of T cells with *in vitro*-transcribed RNA (IVT-RNA) makes use of two different strategies both of which have been successively tested in various animal models. Cells are transfected with *in vitro*-transcribed RNA by means of lipofection or electroporation. Preferably, it is desirable to stabilize IVT-RNA using various modifications in order to achieve prolonged expression of transferred IVT-RNA.

[0330] Some IVT vectors are known in the literature which are utilized in a standardized manner as template for *in vitro* transcription and which have been genetically modified in such a way that stabilized RNA transcripts are produced. Currently protocols used in the art are based on a plasmid vector with the following structure: a 5' RNA polymerase promoter enabling RNA transcription, followed by a gene of interest which is flanked either 3' and/or 5' by untranslated regions (UTR), and a 3' polyadenyl cassette containing 50-70 A nucleotides. Prior to *in vitro* transcription, the circular plasmid is linearized downstream of the polyadenyl cassette by type II restriction enzymes (recognition sequence corresponds to cleavage site). The polyadenyl cassette thus corresponds to the later poly(A) sequence in the transcript. As a result of this procedure, some nucleotides remain as part of the enzyme cleavage site after linearization and extend or mask the poly (A) sequence at the 3' end. It is not clear, whether this nonphysiological overhang affects the amount of protein produced intracellularly from such a construct.

[0331] RNA has several advantages over more traditional plasmid or viral approaches. Gene expression from an RNA source does not require transcription and the protein product is produced rapidly after the transfection. Further, since the RNA has to only gain access to the cytoplasm, rather than the nucleus, and therefore typical transfection methods result in an extremely high rate of transfection. In addition, plasmid-based approaches require that the promoter driving the expression of the gene of interest be active in the cells under study.

[0332] In another aspect, the RNA construct can be delivered into the cells by electroporation. See, *e.g.*, the formulations and methodology of electroporation of nucleic acid constructs into mammalian cells as taught in US 2004/0014645, US 2005/0052630A1, US 2005/0070841A1, US 2004/0059285A1, US 2004/0092907A1. The various parameters including electric field strength required for electroporation of any known cell type are generally known in the relevant research literature as well as numerous patents and applications in the field. See *e.g.*, U.S. Pat. No. 6,678,556, U.S. Pat. No. 7,171,264, and U.S. Pat. No. 7,173,116. Apparatus for therapeutic application of electroporation are available commercially, *e.g.*, the MedPulser™ DNA Electroporation Therapy System (Inovio/Genetronics, San Diego, Calif.), and are described in patents such as U.S. Pat. No. 6,567,694; U.S. Pat. No. 6,516,223, U.S. Pat. No. 5,993,434, U.S. Pat. No. 6,181,964, U.S. Pat. No. 6,241,701, and U.S. Pat. No. 6,233,482; electroporation

may also be used for transfection of cells *in vitro* as described *e.g.,* in US20070128708A1. Electroporation may also be utilized to deliver nucleic acids into cells *in vitro*. Accordingly, electroporation-mediated administration into cells of nucleic acids including expression constructs utilizing any of the many available devices and electroporation systems known to those of skill in the art presents an exciting new means for delivering an RNA of interest to a target cell.

5.6.1.1 Sources of T Cells

[0333] Prior to expansion and genetic modification, a source of T cells is obtained from a subject. The term "subject" is intended to include living organisms in which an immune response can be elicited (*e.g.*, mammals). Examples of subjects include humans, dogs, cats, mice, rats, and transgenic species thereof. Preferably, subjects are human.

[0334] T cells can be obtained from a number of sources, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In certain embodiments of the present disclosure, any number of T cell lines available in the art, may be used. In certain embodiments of the present disclosure, T cells can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled artisan, such as Ficoll™ separation. In one preferred embodiment, cells from the circulating blood of an individual are obtained by apheresis. The apheresis product typically contains lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and platelets. In one embodiment, the cells collected by apheresis may be washed to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In one embodiment of the disclosure, the cells are washed with phosphate buffered saline (PBS). In an alternative embodiment, the wash solution lacks calcium and may lack magnesium or may lack many if not all divalent cations. Again, surprisingly, initial activation steps in the absence of calcium lead to magnified activation. As those of ordinary skill in the art would readily appreciate a washing step may be accomplished by methods known to those in the art, such as by using a semi-automated "flow-through" centrifuge (for example, the Cobe 2991 cell processor, the Baxter CytoMate, or the Haemonetics Cell Saver 5) according to the manufacturer's instructions. After washing, the cells may be resuspended in a variety of biocompatible buffers, such as, for example, Ca-free, Mg-free PBS, PlasmaLyte A, or other saline solution with or without buffer. Alternatively, the undesirable components of the apheresis sample may be removed and the cells directly resuspended in culture media.

[0335] In another embodiment, T cells are isolated from peripheral blood lymphocytes by lysing the red blood cells and depleting the monocytes, for example, by centrifugation through a PERCOLL™ gradient or by counterflow centrifugal elutriation. A specific subpopulation of T cells, such as $CD3^+$, CD28', $CD4^+$, $CD8^+$, $CD45RA^+$ and $CD45RO^+$ T cells, can be further

isolated by positive or negative selection techniques. For example, in one embodiment, T cells are isolated by incubation with anti-CD3/anti-CD28 (*i.e.*, 3 x 28)-conjugated beads, such as DYNABEADS® M-450 CD3/CD28 T, for a time period sufficient for positive selection of the desired T cells. In one embodiment, the time period is about 30 minutes. In a further embodiment, the time period ranges from 30 minutes to 36 hours or longer and all integer values there between. In a further embodiment, the time period is at least 1, 2, 3, 4, 5, or 6 hours. In yet another preferred embodiment, the time period is 10 to 24 hours. In one preferred embodiment, the incubation time period is 24 hours. For isolation of T cells from patients with leukemia, use of longer incubation times, such as 24 hours, can increase cell yield. Longer incubation times may be used to isolate T cells in any situation where there are few T cells as compared to other cell types, such in isolating tumor infiltrating lymphocytes (TIL) from tumor tissue or from immunocompromised individuals. Further, use of longer incubation times can increase the efficiency of capture of CD8+ T cells. Thus, by simply shortening or, lengthening the time T cells are allowed to bind to the CD3/CD28 beads and/or by increasing or decreasing the ratio of beads to T cells (as described further herein), subpopulations of T cells can be preferentially selected for or against at culture initiation or at other time points during the process. Additionally, by increasing or decreasing the ratio of anti-CD3 and/or anti-CD28 antibodies on the beads or other surface, subpopulations of T cells can be preferentially selected for or against at culture initiation or at other desired time points. The skilled artisan would recognize that multiple rounds of selection can also be used in the context of this disclosure. In certain embodiments, it may be desirable to perform the selection procedure and use the “unselected” cells in the activation and expansion process. “Unselected” cells can also be subjected to further rounds of selection.

[0336] Enrichment of a T cell population by negative selection can be accomplished with a combination of antibodies directed to surface markers unique to the negatively selected cells. One method is cell sorting and/or selection via negative magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the cells negatively selected. For example, to enrich for $CD4^+$ cells by negative selection, a monoclonal antibody cocktail typically includes antibodies to CD14, CD20, CD11b, CD16, HLA-DR, and CD8. In certain embodiments, it may be desirable to enrich for or positively select for regulatory T cells which typically express $CD4^+$, $CD25^+$, $CD62L^{hi}$, $GITR^+$, and $FoxP3^+$. Alternatively, in certain embodiments, T regulatory cells are depleted by anti-C25 conjugated beads or other similar method of selection.

[0337] For isolation of a desired population of cells by positive or negative selection, the concentration of cells and surface (*e.g.*, particles such as beads) can be varied. In certain embodiments, it may be desirable to significantly decrease the volume in which beads and cells are mixed together (*i.e.*, increase the concentration of cells), to ensure maximum contact of

cells and beads. For example, in one embodiment, a concentration of 2 billion cells/ml is used. In one embodiment, a concentration of 1 billion cells/ml is used. In a further embodiment, greater than 100 million cells/ml is used. In a further embodiment, a concentration of cells of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In yet another embodiment, a concentration of cells from 75, 80, 85, 90, 95, or 100 million cells/ml is used. In further embodiments, concentrations of 125 or 150 million cells/ml can be used. Using high concentrations can result in increased cell yield, cell activation, and cell expansion. Further, use of high cell concentrations allows more efficient capture of cells that may weakly express target antigens of interest, such as CD28-negative T cells, or from samples where there are many tumor cells present (*i.e.*, leukemic blood, tumor tissue, *etc.*). Such populations of cells may have therapeutic value and would be desirable to obtain. For example, using high concentration of cells allows more efficient selection of $CD8^+$ T cells that normally have weaker CD28 expression.

[0338] In a related embodiment, it may be desirable to use lower concentrations of cells. By significantly diluting the mixture of T cells and surface (*e.g.*, particles such as beads), interactions between the particles and cells are minimized. This selects for cells that express high amounts of desired antigens to be bound to the particles. For example, $CD4^+$ T cells express higher levels of CD28 and are more efficiently captured than $CD8^+$ T cells in dilute concentrations. In one embodiment, the concentration of cells used is 5×10^6/ml. In other embodiments, the concentration used can be from about 1×10^5/ml to 1×10^6/ml, and any integer value in between.

[0339] In other embodiments, the cells may be incubated on a rotator for varying lengths of time at varying speeds at either 2-10° C. or at room temperature.

[0340] T cells for stimulation can also be frozen after a washing step. Wishing not to be bound by theory, the freeze and subsequent thaw step provides a more uniform product by removing granulocytes and to some extent monocytes in the cell population. After the washing step that removes plasma and platelets, the cells may be suspended in a freezing solution. While many freezing solutions and parameters are known in the art and will be useful in this context, one method involves using PBS containing 20% DMSO and 8% human serum albumin, or culture media containing 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin and 7.5% DMSO, or 31.25% Plasmalyte-A, 31.25% Dextrose 5%, 0.45% NaCl, 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin, and 7.5% DMSO or other suitable cell freezing media containing for example, Hespan and PlasmaLyte A, the cells then are frozen to -80° C. at a rate of 1° per minute and stored in the vapor phase of a liquid nitrogen storage tank. Other methods of controlled freezing may be used as well as uncontrolled freezing immediately at -20° C. or in liquid nitrogen.

[0341] In certain embodiments, cryopreserved cells are thawed and washed as described herein and allowed to rest for one hour at room temperature prior to activation using the methods of the present disclosure.

[0342] Also contemplated in the context of the disclosure is the collection of blood samples or apheresis product from a subject at a time period prior to when the expanded cells as described herein might be needed. As such, the source of the cells to be expanded can be collected at any time point necessary, and desired cells, such as T cells, isolated and frozen for later use in T cell therapy for any number of diseases or conditions that would benefit from T cell therapy, such as those described herein. In one embodiment a blood sample or an apheresis is taken from a generally healthy subject. In certain embodiments, a blood sample or an apheresis is taken from a generally healthy subject who is at risk of developing a disease, but who has not yet developed a disease, and the cells of interest are isolated and frozen for later use. In certain embodiments, the T cells may be expanded, frozen, and used at a later time. In certain embodiments, samples are collected from a patient shortly after diagnosis of a particular disease as described herein but prior to any treatments. In a further embodiment, the cells are isolated from a blood sample or an apheresis from a subject prior to any number of relevant treatment modalities, including but not limited to treatment with agents such as natalizumab, efalizumab, antiviral agents, chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAMPATH, anti-CD3 antibodies, cytoxan, fludarabine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, and irradiation. These drugs inhibit either the calcium dependent phosphatase calcineurin (cyclosporine and FK506) or inhibit the p70S6 kinase that is important for growth factor induced signaling (rapamycin). (Liu *et al.*, Cell 66:807-815, 1991; Henderson *et al.*, Immun. 73:316-321, 1991; Bierer *et al.*, Curr. Opin. Immun. 5:763-773, 1993). In a further embodiment, the cells are isolated for a patient and frozen for later use in conjunction with (*e.g.*, before, simultaneously or following) bone marrow or stem cell transplantation or T cell ablative therapy using either chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide.

[0343] In a further embodiment of the present disclosure, T cells are obtained from a patient directly following treatment. In this regard, it has been observed that following certain cancer treatments, in particular treatments with drugs that damage the immune system, shortly after treatment during the period when patients would normally be recovering from the treatment, the quality of T cells obtained may be optimal or improved for their ability to expand ex vivo. Likewise, following *ex vivo* manipulation using the methods described herein, these cells may be in a preferred state for enhanced engraftment and *in vivo* expansion. Thus, it is contemplated within the context of the present disclosure to collect blood cells, including T cells, dendritic cells, or other cells of the hematopoietic lineage, during this recovery phase.

Further, in certain embodiments, mobilization (for example, mobilization with GM-CSF) and conditioning regimens can be used to create a condition in a subject wherein repopulation, recirculation, regeneration, and/or expansion of particular cell types is favored, especially during a defined window of time following therapy. Illustrative cell types include T cells, B cells, dendritic cells, and other cells of the immune system.

5.6.1.2 Activation and Expansion of T Cells

[0344] T cells are activated and expanded generally using methods as described, for example, in U.S. Pat. Nos. 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; 6,867,041; and U.S. Patent Application Publication No. 20060121005.

[0345] Generally, the T cells of the disclosure are expanded by contact with a surface having attached thereto an agent that stimulates a CD3/TCR complex associated signal and a ligand that stimulates a co-stimulatory molecule on the surface of the T cells. In particular, T cell populations may be stimulated as described herein, such as by contact with an anti-CD3 antibody, or antigen-binding fragment thereof, or an anti-CD2 antibody immobilized on a surface, or by contact with a protein kinase C activator (*e.g.*, bryostatin) in conjunction with a calcium ionophore. For co-stimulation of an accessory molecule on the surface of the T cells, a ligand that binds the accessory molecule is used. For example, a population of T cells can be contacted with an anti-CD3 antibody and an anti-CD28 antibody, under conditions appropriate for stimulating proliferation of the T cells. To stimulate proliferation of either $CD4^+$ T cells or $CD8^+$ T cells, an anti-CD3 antibody and an anti-CD28 antibody. Examples of an anti-CD28 antibody include 9.3, B-T3, XR-CD28 (Diaclone, Besancon, France) can be used as can other methods commonly known in the art (Berg *et al.*, Transplant Proc. 30(8):3975-3977, 1998; Haanen *et al.*, J. Exp. Med. 190(9):13191328, 1999; Garland *et al.*, J. Immunol Meth. 227(1-2):53-63, 1999).

[0346] In certain embodiments, the primary stimulatory signal and the co-stimulatory signal for the T cell may be provided by different protocols. For example, the agents providing each signal may be in solution or coupled to a surface. When coupled to a surface, the agents may be coupled to the same surface (*i.e.*, in "cis" formation) or to separate surfaces (*i.e.*, in "trans" formation). Alternatively, one agent may be coupled to a surface and the other agent in solution. In one embodiment, the agent providing the co-stimulatory signal is bound to a cell surface and the agent providing the primary activation signal is in solution or coupled to a surface. In certain embodiments, both agents can be in solution. In another embodiment, the agents may be in soluble form, and then cross-linked to a surface, such as a cell expressing Fc receptors or an antibody or other binding agent which will bind to the agents. In this regard, see for example, U.S. Patent Application Publication Nos. 20040101519 and 20060034810 for

artificial antigen presenting cells (aAPCs) that are contemplated for use in activating and expanding T cells in the present disclosure.

[0347] In one embodiment, the two agents are immobilized on beads, either on the same bead, *i.e.*, "cis," or to separate beads, *i.e.*, "trans." By way of example, the agent providing the primary activation signal is an anti-CD3 antibody or an antigen-binding fragment thereof and the agent providing the co-stimulatory signal is an anti-CD28 antibody or antigen-binding fragment thereof; and both agents are co-immobilized to the same bead in equivalent molecular amounts. In one embodiment, a 1:1 ratio of each antibody bound to the beads for $CD4^+$ T cell expansion and T cell growth is used. In certain aspects of the present disclosure, a ratio of anti CD3:CD28 antibodies bound to the beads is used such that an increase in T cell expansion is observed as compared to the expansion observed using a ratio of 1:1. In one particular embodiment an increase of from about 1 to about 3 fold is observed as compared to the expansion observed using a ratio of 1:1. In one embodiment, the ratio of CD3:CD28 antibody bound to the beads ranges from 100:1 to 1:100 and all integer values there between. In one aspect of the present disclosure, more anti-CD28 antibody is bound to the particles than anti-CD3 antibody, *i.e.*, the ratio of CD3:CD28 is less than one. In certain embodiments of the disclosure, the ratio of anti CD28 antibody to anti CD3 antibody bound to the beads is greater than 2:1. In one particular embodiment, a 1:100 CD3:CD28 ratio of antibody bound to beads is used. In another embodiment, a 1:75 CD3:CD28 ratio of antibody bound to beads is used. In a further embodiment, a 1:50 CD3:CD28 ratio of antibody bound to beads is used. In another embodiment, a 1:30 CD3:CD28 ratio of antibody bound to beads is used. In one preferred embodiment, a 1:10 CD3:CD28 ratio of antibody bound to beads is used. In another embodiment, a 1:3 CD3:CD28 ratio of antibody bound to the beads is used. In yet another embodiment, a 3:1 CD3:CD28 ratio of antibody bound to the beads is used.

[0348] Ratios of particles to cells from 1:500 to 500:1 and any integer values in between may be used to stimulate T cells or other target cells. As those of ordinary skill in the art can readily appreciate, the ratio of particles to cells may depend on particle size relative to the target cell. For example, small sized beads could only bind a few cells, while larger beads could bind many. In certain embodiments the ratio of cells to particles ranges from 1:100 to 100:1 and any integer values in-between and in further embodiments the ratio comprises 1:9 to 9:1 and any integer values in between, can also be used to stimulate T cells. The ratio of anti-CD3- and anti-CD28-coupled particles to T cells that result in T cell stimulation can vary as noted above, however certain preferred values include 1:100, 1:50, 1:40, 1:30, 1:20, 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, and 15:1 with one preferred ratio being at least 1:1 particles per T cell. In one embodiment, a ratio of particles to cells of 1:1 or less is used. In one particular embodiment, a preferred particle: cell ratio is 1:5. In further embodiments, the ratio of particles to cells can be varied depending on the day of stimulation.

For example, in one embodiment, the ratio of particles to cells is from 1:1 to 10:1 on the first day and additional particles are added to the cells every day or every other day thereafter for up to 10 days, at final ratios of from 1:1 to 1:10 (based on cell counts on the day of addition). In one particular embodiment, the ratio of particles to cells is 1:1 on the first day of stimulation and adjusted to 1:5 on the third and fifth days of stimulation. In another embodiment, particles are added on a daily or every other day basis to a final ratio of 1:1 on the first day, and 1:5 on the third and fifth days of stimulation. In another embodiment, the ratio of particles to cells is 2:1 on the first day of stimulation and adjusted to 1:10 on the third and fifth days of stimulation. In another embodiment, particles are added on a daily or every other day basis to a final ratio of 1:1 on the first day, and 1:10 on the third and fifth days of stimulation. One of skill in the art will appreciate that a variety of other ratios may be suitable for use in the present disclosure. In particular, ratios will vary depending on particle size and on cell size and type.

[0349] In further embodiments of the present disclosure, the cells, such as T cells, are combined with agent-coated beads, the beads and the cells are subsequently separated, and then the cells are cultured. In an alternative embodiment, prior to culture, the agent-coated beads and cells are not separated but are cultured together. In a further embodiment, the beads and cells are first concentrated by application of a force, such as a magnetic force, resulting in increased ligation of cell surface markers, thereby inducing cell stimulation.

[0350] By way of example, cell surface proteins may be ligated by allowing paramagnetic beads to which anti-CD3 and anti-CD28 are attached (3 x 28 beads) to contact the T cells. In one embodiment the cells (for example, 10^4 to 10^9 T cells) and beads (for example, DYNABEADS® M-450 CD3/CD28 T paramagnetic beads at a ratio of 1:1) are combined in a buffer, preferably PBS (without divalent cations such as, calcium and magnesium). Again, those of ordinary skill in the art can readily appreciate any cell concentration may be used. For example, the target cell may be very rare in the sample and comprise only 0.01% of the sample or the entire sample (*i.e.*, 100%) may comprise the target cell of interest. Accordingly, any cell number is within the context of the present disclosure. In certain embodiments, it may be desirable to significantly decrease the volume in which particles and cells are mixed together (*i.e.*, increase the concentration of cells), to ensure maximum contact of cells and particles. For example, in one embodiment, a concentration of about 2 billion cells/ml is used. In another embodiment, greater than 100 million cells/ml is used. In a further embodiment, a concentration of cells of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In yet another embodiment, a concentration of cells from 75, 80, 85, 90, 95, or 100 million cells/ml is used. In further embodiments, concentrations of 125 or 150 million cells/ml can be used. Using high concentrations can result in increased cell yield, cell activation, and cell expansion. Further, use of high cell concentrations allows more efficient capture of cells that may weakly express target antigens of interest, such as CD28-negative T cells. Such populations of cells may have

therapeutic value and would be desirable to obtain in certain embodiments. For example, using high concentration of cells allows more efficient selection of CD8+ T cells that normally have weaker CD28 expression.

[0351] In one embodiment of the present disclosure, the mixture may be cultured for several hours (about 3 hours) to about 14 days or any hourly integer value in between. In another embodiment, the mixture may be cultured for 21 days. In one embodiment of the disclosure the beads and the T cells are cultured together for about eight days. In another embodiment, the beads and T cells are cultured together for 2-3 days. Several cycles of stimulation may also be desired such that culture time of T cells can be 60 days or more. Conditions appropriate for T cell culture include an appropriate media (*e.g.*, Minimal Essential Media or RPMI Media 1640 or, X-vivo 15, (Lonza)) that may contain factors necessary for proliferation and viability, including serum (*e.g.*, fetal bovine or human serum), interleukin-2 (IL-2), insulin, IFN-γ, IL-4, IL-7, GM-CSF, IL-10, IL-12, IL-15, TGFβ, and TNF-α or any other additives for the growth of cells known to the skilled artisan. Other additives for the growth of cells include, but are not limited to, surfactant, plasmanate, and reducing agents such as N-acetyl-cysteine and 2-mercaptoethanol. Media can include RPMI 1640, AIM-V, DMEM, MEM, α-MEM, F-12, X-Vivo 15, and X-Vivo 20, Optimizer, with added amino acids, sodium pyruvate, and vitamins, either serum-free or supplemented with an appropriate amount of serum (or plasma) or a defined set of hormones, and/or an amount of cytokine(s) sufficient for the growth and expansion of T cells. Antibiotics, *e.g.*, penicillin and streptomycin, are included only in experimental cultures, not in cultures of cells that are to be infused into a subject. The target cells are maintained under conditions necessary to support growth, for example, an appropriate temperature (*e.g.*, 37° C.) and atmosphere (*e.g.*, air plus 5% CO_2).

[0352] T cells that have been exposed to varied stimulation times may exhibit different characteristics. For example, typical blood or apheresed peripheral blood mononuclear cell products have a helper T cell population (T_H, $CD4^+$) that is greater than the cytotoxic or suppressor T cell population (T_C, $CD8^+$). Ex vivo expansion of T cells by stimulating CD3 and CD28 receptors produces a population of T cells that prior to about days 8-9 consists predominately of T_H cells, while after about days 8-9, the population of T cells comprises an increasingly greater population of T_C cells. Accordingly, depending on the purpose of treatment, infusing a subject with a T cell population comprising predominately of T_H cells may be advantageous. Similarly, if an antigen-specific subset of T_C cells has been isolated it may be beneficial to expand this subset to a greater degree.

[0353] Further, in addition to CD4 and CD8 markers, other phenotypic markers vary significantly, but in large part, reproducibly during the course of the cell expansion process. Thus, such reproducibility enables the ability to tailor an activated T cell product for specific purposes.

5.7 Neuraminidase

[0354] Sialic acids are terminal sugars of glycans on either glycoproteins or glycolipids on the cell surface, and have been shown to be aberrantly expressed during tumor transformation and malignant progression. Hypersialylation frequently occurs in tumor tissues due to aberrant expression of sialytransferases/sialidases. This can result in accelerated cancer progression. Sialylation facilitates immune escape, enhances tumor proliferation and metastasis, helps tumor angiogenesis, and assists in resisting apoptosis and cancer therapy.

[0355] Host cells (*e.g.*, T cells, NK cells) expressing a CAR of the disclosure can be engineered to coexpress a cell surface or secreted neuraminidase (sialidase) along with the CAR. The cell surface neuraminidase, anchored to the cell surface via a heterologous transmembrane, gives the host cell glycoediting activity. This enhances cytotoxic effects and anti-tumor efficacy of the CAR-T cell and immune cells such as innate NK cells and monocytes. Host cells coexpressing a CAR and an engineered neuraminidase are described in PCT Publication No WO2020/236964, which is incorporated herein by reference in its entirety.

[0356] A neuraminidase can be coexpressed in a host cell along with a CAR described herein. Exemplary host cells coexpressing a neuraminidase and a CAR are described in the specific embodiments.

[0357] The neuraminidase can be included as a domain of a fusion protein described herein.

[0358] In certain embodiments, the neuraminidase is EC 3.2.1.18 or EC 3.2.1.129.

[0359] In some embodiments, the neuraminidase is derived from *Micromonospora viridifaciens*.

[0360] In some aspects, the neuraminidase comprises an amino acid sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to:

GGSPVPPGGEPLYTEQDLAVNGREGFPNYRIPALTVTPDGDLLASYDGRPTGIDAPGPNSILQ RRSTDGGRTWGEQQVVSAGQTTAPIKGFSDPSYLVDRETGTIFNFHVYSQRQGFAGSRPGTD PADPNVLHANVATSTDGGLTWSHRTITADITPDPGWRSRFAASGEGIQLRYGPHAGRLIQQYTI INAAGAFQAVSVYSDDHGRTWRAGEAVGVGMDENKTVELSDGRVLLNSRDSARSGYRKVAV STDGGHSYGPVTIDRDLPDPTNNASIIRAFPDAPAGSARAKVLLFSNAASQTSRSQGTIRMSCD DGQTWPVSKVFQPGSMSYSTLTALPDGTYGLLYEPGTGIRYANFNLAWLGG (SEQ ID NO:222).

[0361] The neuraminidase can be retained at a surface of a host cell engineered to express the neuraminidase, or can be secreted by a host cell engineered to express the neuraminidase. The hose cell engineered to express the neuraminidase can include, for example, a vector encoding the neuraminidase.

5.8 Compositions

[0362] The anti-glyco-MUC4 antibodies, fusion proteins, and/or anti-glyco-MUC4 ADCs of the disclosure may be in the form of compositions comprising the anti-glyco-MUC4 antibody, fusion protein and/or ADC and one or more carriers, excipients and/or diluents. The compositions may be formulated for specific uses, such as for veterinary uses or pharmaceutical uses in humans. The form of the composition (*e.g.*, dry powder, liquid formulation, *etc.*) and the excipients, diluents and/or carriers used will depend upon the intended uses of the antibody, fusion protein and/or ADC and, for therapeutic uses, the mode of administration.

[0363] For therapeutic uses, the compositions may be supplied as part of a sterile, pharmaceutical composition that includes a pharmaceutically acceptable carrier. This composition can be in any suitable form (depending upon the desired method of administering it to a patient). The pharmaceutical composition can be administered to a patient by a variety of routes such as orally, transdermally, subcutaneously, intranasally, intravenously, intramuscularly, intratumorally, intrathecally, topically or locally. The most suitable route for administration in any given case will depend on the particular antibody and/or ADC, the subject, and the nature and severity of the disease and the physical condition of the subject. Typically, the pharmaceutical composition will be administered intravenously or subcutaneously.

[0364] Pharmaceutical compositions can be conveniently presented in unit dosage forms containing a predetermined amount of an anti-glyco-MUC4 antibody and/or anti-glyco-MUC4 ADC of the disclosure per dose. The quantity of antibody and/or ADC included in a unit dose will depend on the disease being treated, as well as other factors as are well known in the art. Such unit dosages may be in the form of a lyophilized dry powder containing an amount of antibody and/or ADC suitable for a single administration, or in the form of a liquid. Dry powder unit dosage forms may be packaged in a kit with a syringe, a suitable quantity of diluent and/or other components useful for administration. Unit dosages in liquid form may be conveniently supplied in the form of a syringe pre-filled with a quantity of antibody and/or ADC suitable for a single administration.

[0365] The pharmaceutical compositions may also be supplied in bulk from containing quantities of ADC suitable for multiple administrations.

[0366] Pharmaceutical compositions may be prepared for storage as lyophilized formulations or aqueous solutions by mixing an antibody, fusion protein, and/or ADC having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers typically employed in the art (all of which are referred to herein as "carriers"), *i.e.*, buffering agents, stabilizing agents, preservatives, isotonifiers, non-ionic detergents, antioxidants, and other miscellaneous additives. See, Remington's Pharmaceutical Sciences, 16th edition (Osol, ed. 1980). Such additives should be nontoxic to the recipients at the dosages and concentrations employed.

[0367] Buffering agents help to maintain the pH in the range which approximates physiological conditions. They may be present at a wide variety of concentrations, but will typically be present in concentrations ranging from about 2 mM to about 50 mM. Suitable buffering agents for use with the present disclosure include both organic and inorganic acids and salts thereof such as citrate buffers (*e.g.*, monosodium citrate-disodium citrate mixture, citric acid-trisodium citrate mixture, citric acid-monosodium citrate mixture, *etc.*), succinate buffers (*e.g.*, succinic acid-monosodium succinate mixture, succinic acid-sodium hydroxide mixture, succinic acid-disodium succinate mixture, *etc.*), tartrate buffers (*e.g.*, tartaric acid-sodium tartrate mixture, tartaric acid-potassium tartrate mixture, tartaric acid-sodium hydroxide mixture, *etc.*), fumarate buffers (*e.g.*, fumaric acid-monosodium fumarate mixture, fumaric acid-disodium fumarate mixture, monosodium fumarate-disodium fumarate mixture, *etc.*), gluconate buffers (*e.g.*, gluconic acid-sodium glyconate mixture, gluconic acid-sodium hydroxide mixture, gluconic acid-potassium glyconate mixture, *etc.*), oxalate buffer (*e.g.*, oxalic acid-sodium oxalate mixture, oxalic acid-sodium hydroxide mixture, oxalic acid-potassium oxalate mixture, *etc.*), lactate buffers (*e.g.*, lactic acid-sodium lactate mixture, lactic acid-sodium hydroxide mixture, lactic acid-potassium lactate mixture, *etc.*) and acetate buffers (*e.g.*, acetic acid-sodium acetate mixture, acetic acid-sodium hydroxide mixture, *etc.*). Additionally, phosphate buffers, histidine buffers and trimethylamine salts such as Tris can be used.

[0368] Preservatives may be added to retard microbial growth, and can be added in amounts ranging from about 0.2%-1% (w/v). Suitable preservatives for use with the present disclosure include phenol, benzyl alcohol, meta-cresol, methyl paraben, propyl paraben, octadecyldimethylbenzyl ammonium chloride, benzalconium halides (*e.g.*, chloride, bromide, and iodide), hexamethonium chloride, and alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, and 3-pentanol. Isotonicifiers sometimes known as "stabilizers" can be added to ensure isotonicity of liquid compositions of the present disclosure and include polyhydric sugar alcohols, for example trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol. Stabilizers refer to a broad category of excipients which can range in function from a bulking agent to an additive which solubilizes the therapeutic agent or helps to prevent denaturation or adherence to the container wall. Typical stabilizers can be polyhydric sugar alcohols (enumerated above); amino acids such as arginine, lysine, glycine, glutamine, asparagine, histidine, alanine, ornithine, L-leucine, 2-phenylalanine, glutamic acid, threonine, *etc.*, organic sugars or sugar alcohols, such as lactose, trehalose, stachyose, mannitol, sorbitol, xylitol, ribitol, myoinisitol, galactitol, glycerol and the like, including cyclitols such as inositol; polyethylene glycol; amino acid polymers; sulfur containing reducing agents, such as urea, glutathione, thioctic acid, sodium thioglycolate, thioglycerol, α-monothioglycerol and sodium thio sulfate; low molecular weight polypeptides (*e.g.*, peptides of 10 residues or fewer); proteins such as human serum albumin, bovine serum albumin, gelatin or immunoglobulins; hydrophylic polymers, such as polyvinylpyrrolidone monosaccharides,

such as xylose, mannose, fructose, glucose; disaccharides such as lactose, maltose, sucrose and trehalose; and trisaccacharides such as raffinose; and polysaccharides such as dextran. Stabilizers may be present in amounts ranging from 0.5 to 10 wt % per wt of ADC.

[0369] Non-ionic surfactants or detergents (also known as "wetting agents") may be added to help solubilize the glycoprotein as well as to protect the glycoprotein against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stressed without causing denaturation of the protein. Suitable non-ionic surfactants include polysorbates (20, 80, *etc.*), polyoxamers (184, 188 *etc.*), and pluronic polyols. Non-ionic surfactants may be present in a range of about 0.05 mg/mL to about 1.0 mg/mL, for example about 0.07 mg/mL to about 0.2 mg/mL.

[0370] Additional miscellaneous excipients include bulking agents (*e.g.*, starch), chelating agents (*e.g.*, EDTA), antioxidants (*e.g.*, ascorbic acid, methionine, vitamin E), and cosolvents.

5.9 Methods of Use

[0371] The anti-glyco-MUC4 antibody or binding fragments described herein can be used in various diagnostic assays and therapeutic methods. In some embodiments, a patient can be diagnosed with a cancer using any method as described herein (*e.g.*, as described in Section 5.9.1) and subsequently treated using any method as described herein (*e.g.*, as described in Section 5.9.2). The diagnostic methods described herein (*e.g.*, as described in Section 5.9.1) can be utilized to monitor the patient's cancer status during or following cancer therapy (including but not limited to cancer therapy as described in Section 5.9.2).

5.9.1. Diagnostic Methods

[0372] The anti-glyco-MUC4 antibody or binding fragments (including immunoconjugates and labeled antibodies and binding fragments) can be used in diagnostic assays. For example, the antibodies and binding fragments can be employed in immunoassays, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays, including immunohistochemistry, enzyme-linked immunosorbent assay (ELISA), fluorescence-activated cell sorting (FACS), and Western blots.

[0373] The anti-glyco-MUC4 antibody or binding fragments described herein can be used in a detection assay and/or a diagnostic assay to detect a biomarker in a sample, such as, *e.g.*, a patient-derived biological sample. The biomarker may be a protein biomarker (*e.g.*, a tumor-associated glycoform of MUC4, for example a glycoform of MUC4 comprising the amino acid sequence CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) glycosylated with GalNAc on the serine and threonine residues shown in bold underlined text) present on the surface of or within, *e.g.*, a cancer cell or a cancer-derived extracellular vesicle.

[0374] An anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure can be used in a method of detecting a biomarker in a sample comprising one or more EVs (*e.g.*, a liquid

biopsy). In such embodiments, an EV surface biomarker is recognized by the anti-glyco-MUC4 antibody or antigen-binding fragment of the disclosure. Exemplary methods of detecting the biomarker include, but are not limited to, capture assays, immunoassays, such as immunoprecipitation; Western blot; ELISA; immunohistochemistry; immunocytochemistry; flow cytometry; and immuno-PCR. In some embodiments, an immunoassay can be a chemiluminescent immunoassay. In some embodiments, an immunoassay can be a high-throughput and/or automated immunoassay platform.

[0375] The anti-glyco-MUC4 antibody or binding fragments described herein also are useful for radiographic *in vivo* imaging, wherein an antibody labeled with a detectable moiety such as a radio-opaque agent or radioisotope is administered to a subject, preferably into the bloodstream, and the presence and location of the labeled antibody in the host is assayed. This imaging technique is useful in the staging and treatment of malignancies.

5.9.2. Therapeutic Methods

[0376] The anti-glyco-MUC4 antibody or binding fragments, fusion proteins, ADCs, CARs and chimeric TCRs described herein are useful for treatment of glyco-MUC4 expressing cancers, including, for example, pancreatic, lung, breast, gall bladder, salivary gland, prostate, biliary tract, esophageal, papillary thyroid carcinoma, low-grade fibromyxoid sarcoma, and ovarian cancers.

[0377] Thus, the disclosure provides anti-glyco-MUC4 antibodies, binding fragments, fusion proteins, ADCs, CARs, and chimeric TCRs as described herein for use as a medicament, for example for use in the treatment of cancer, *e.g.*, any of the cancers identified in the previous paragraph, for use in a diagnostic assay, and for use in radiographic *in vivo* imaging. The disclosure further provides for the use of the anti-glyco-MUC4 antibodies, binding fragments, fusion proteins, ADCs, CARs and chimeric TCRs as described herein in the manufacture of a medicament, for example for the treatment of cancer, *e.g.*, any of the cancers identified in the previous paragraph.

[0378] When using the CARs or chimeric TCRs of the disclosure for therapy, the therapeutic methods of the disclosure comprise administering to a subject with a glyco-MUC4-expressing tumor an effective amount of a genetically modified cell engineered to express a CAR or chimeric TCR of the disclosure, for example a CAR as described in Section 5.3 or in numbered embodiments 446 to 479, or a chimeric TCR as described in Section 5.4 or in numbered embodiments 490 to 584, or a MicAbody as described in Section 5.5 or numbered embodiments 427 to 430. Methods of modifying cells, particularly T cells, to express a CAR or chimeric TCR, are described in Section 5.6.1.

[0379] When using the MicAbodies of the disclosure for therapy, the therapeutic methods of the disclosure comprise administering to a subject with a glyco-MUC4-expressing tumor

therapeutically effective amounts of a MicAbody of the disclosure, for example a MicAbody described in Section 5.5 or numbered embodiments 427 to 430, and a genetically modified T-cell engineered to express a CAR comprising a NKG2D receptor capable of specifically binding the MicAbody.

5.10 MUC4 Peptides

[0380] Also provided are isolated MUC4 glycopeptides, or glyco-MUC4 peptides, comprising the amino acid CTIPSTAMHTRSTAAPIPILP (SEQ ID NO:155), or a fragment thereof. In some embodiments, the MUC4 glycopeptide is glycosylated with O-linked GalNAc on the serine and threonine residues at amino acid positions 12 and 13 of CTIPSTAMHTRSTAAPIPILP (SEQ ID NO:155), respectively. In some embodiments the MUC4 glycopeptide comprises the amino acid CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) or a fragment thereof, with O-linked GalNAc on the serine and threonine residues shown with bold and underlined text. Exemplary isolated MUC4 glycopeptides are described in numbered embodiments 653 to 665.

[0381] The present disclosure encompasses synthetic synthesis of the isolated MUC4 glycoproteins and recombinant methods for producing the isolated MUC4 glycoproteins.

[0382] In certain embodiments, the isolated MUC4 peptides are synthesized using a solid-phase peptide synthesis (SPPS) strategy. SPPS methods are known in the art. SPPS provides for the rapid assembly of a polypeptide through successive reactions of amino acid derivatives on a solid support. Through repeated cycles of alternating N-terminal deprotection and coupling reactions, successive amino acid derivatives are added to the polypeptide. In other embodiments, isolated MUC4 peptides are synthesized using a solution-phase peptide synthesis strategy. Solution-phase peptide synthesis methods are known in the art.

[0383] To ensure proper O-linked glycosylation with GalNAc on the serine at amino acid position 12 of SEQ ID NO:154 and the threonine at amino acid position 13 of SEQ ID NO:154, pre-synthesized glycosylated amino acids can be used in the elongation reactions.

[0384] Nucleic acid molecules encoding the isolated MUC4 glycopeptides, vectors comprising such nucleic acids, and host cells capable of producing the isolated MUC4 glycopeptides of the disclosure are provided. In certain aspects, the nucleic acid molecules encode, and the host cells are capable of expressing, the MUC4 glycopeptide as well as fusion proteins that include the MUC4 glycoproteins.

[0385] An isolated MUC4 glycopeptide of the disclosure can be prepared by recombinant expression in a host cell. To express a MUC4 glycopeptide recombinantly, a host cell is transfected with a recombinant expression vector carrying DNA encoding the glycopeptide such that the glycopeptide is expressed in the host cell and, optionally, secreted into the medium in which the host cells are cultured, from which medium the glycoproteins can be recovered (*i.e.,* isolated). Standard recombinant DNA methodologies are used to obtain a MUC4 glycoprotein

gene, incorporate the gene into recombinant expression vectors and introduce the vectors into host cells, such as those described in Molecular Cloning; A Laboratory Manual, Second Edition (Sambrook, Fritsch and Maniatis (eds), Cold Spring Harbor, N. Y., 1989), 122 Current Protocols in Molecular Biology (Ausubel, F. M. *et al.*, eds., Greene Publishing Associates, 1989) and in U.S. Pat. No. 4,816,397.

[0386] It is possible to express the MUC4 glycoproteins of the disclosure in either prokaryotic or eukaryotic host cells. In certain embodiments, expression of MUC4 glycoprotein is performed in eukaryotic cells, e.g., mammalian host cells. To produce the isolated MUC4 glycoproteins of the disclosure, a host cell is selected based on its ability to glycosylate serine at amino acid position 12 of SEQ ID NO:154 and threonine at amino acid position 13 of SEQ ID NO:154. An exemplary host cell is the COSMC HEK293 cell.

5.10.1. MUC4 Peptide Compositions

[0387] The MUC4 glycopeptides of the disclosure may be in the form of compositions comprising the MUC4 glycopeptide and one or more carriers, excipients, diluents and/or adjuvants. The compositions may be formulated for specific uses, such as for veterinary uses or pharmaceutical uses in humans. The form of the composition (e.g., dry powder, liquid formulation, etc.) and the excipients, diluents and/or carriers used will depend upon the intended uses of the MUC4 glycopeptide and, for therapeutic uses, the mode of administration.

[0388] For therapeutic uses, the compositions may be supplied as part of a sterile, pharmaceutical composition that includes a pharmaceutically acceptable carrier and/or a pharmaceutically acceptable adjuvant. This composition can be in any suitable form (depending upon the desired method of administering it to a patient). The pharmaceutical composition can be administered to a patient by a variety of routes such as orally, transdermally, subcutaneously, intranasally, intravenously, intramuscularly, intratumorally, intrathecally, topically or locally. The most suitable route for administration in any given case will depend on the particular MUC4 glycopeptide to be administered, the subject, and the nature and severity of the disease and the physical condition of the subject. Typically, the pharmaceutical composition will be administered intravenously or subcutaneously.

[0389] Pharmaceutical compositions can be conveniently presented in unit dosage forms containing a predetermined amount of an MUC4 glycopeptide of the disclosure per dose. The quantity of MUC4 glycopeptide included in a unit dose will depend on the disease being treated, as well as other factors as are well known in the art. Such unit dosages may be in the form of a lyophilized dry powder containing an amount of MUC4 glycopeptide suitable for a single administration, or in the form of a liquid. Dry powder unit dosage forms may be packaged in a kit with a syringe, a suitable quantity of diluent and/or other components useful for administration. Unit dosages in liquid form may be conveniently supplied in the form of a syringe pre-filled with a quantity of MUC4 glycopeptide suitable for a single administration.

[0390] The pharmaceutical compositions may also be supplied in bulk form containing quantities of MUC4 glycopeptide suitable for multiple administrations.

[0391] Pharmaceutical compositions may be prepared for storage as lyophilized formulations or aqueous solutions by mixing a MUC4 glycopeptide having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients, adjuvants or stabilizers typically employed in the art (all of which are referred to herein as "carriers"), *i.e.*, buffering agents, stabilizing agents, preservatives, isotonifiers, non-ionic detergents, antioxidants, and other miscellaneous additives. *See*, Remington's Pharmaceutical Sciences, 16th edition (Osol, ed. 1980). Such additives should be nontoxic to the recipients at the dosages and concentrations employed.

[0392] In some embodiments, the composition includes one or more pharmaceutically acceptable adjuvants. Adjuvants include, for example, aluminum salts (e.g., amorphous aluminum hydroxyphosphate sulfate (AAHS), aluminum hydroxide, aluminum phosphate, potassium aluminum sulfate (Alum)), dsRNA analogues, lipid A analogues, flagellin, imidazoquinolines, CpG ODN, saponins (e.g., QS21), C-type lectin ligands (e.g., TDB), CD1d ligands (a-galactosylceramide), M F59, AS01, AS02, AS03, ASO4, AS15, AF03, GLA-SE, IC31, CAF01, and virosomes. Other adjuvants known in the art, including chemical adjuvants, genetic adjuvants, protein adjuvants, and lipid adjuvants, can also be included in the compositions.

[0393] Buffering agents help to maintain the pH in the range which approximates physiological conditions. They may be present at a wide variety of concentrations, but will typically be present in concentrations ranging from about 2 mM to about 50 mM. Suitable buffering agents for use with the present disclosure include both organic and inorganic acids and salts thereof such as citrate buffers (*e.g.*, monosodium citrate-disodium citrate mixture, citric acid-trisodium citrate mixture, citric acid-monosodium citrate mixture, etc.), succinate buffers (*e.g.*, succinic acid-monosodium succinate mixture, succinic acid-sodium hydroxide mixture, succinic acid-disodium succinate mixture, etc.), tartrate buffers (*e.g.*, tartaric acid-sodium tartrate mixture, tartaric acid-potassium tartrate mixture, tartaric acid-sodium hydroxide mixture, etc.), fumarate buffers (*e.g.*, fumaric acid-monosodium fumarate mixture, fumaric acid-disodium fumarate mixture, monosodium fumarate-disodium fumarate mixture, etc.), gluconate buffers (*e.g.*, gluconic acid-sodium glyconate mixture, gluconic acid-sodium hydroxide mixture, gluconic acid-potassium glyconate mixture, etc.), oxalate buffer (*e.g.*, oxalic acid-sodium oxalate mixture, oxalic acid-sodium hydroxide mixture, oxalic acid-potassium oxalate mixture, etc.), lactate buffers (*e.g.*, lactic acid-sodium lactate mixture, lactic acid-sodium hydroxide mixture, lactic acid-potassium lactate mixture, etc.) and acetate buffers (*e.g.*, acetic acid-sodium acetate mixture, acetic acid-sodium hydroxide mixture, etc.). Additionally, phosphate buffers, histidine buffers and trimethylamine salts such as Tris can be used.

[0394] Preservatives may be added to retard microbial growth, and can be added in amounts ranging from about 0.2%-1% (w/v). Suitable preservatives for use with the present disclosure include phenol, benzyl alcohol, meta-cresol, methyl paraben, propyl paraben, octadecyldimethylbenzyl ammonium chloride, benzalconium halides (*e.g.*, chloride, bromide, and iodide), hexamethonium chloride, and alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, and 3-pentanol. Isotonicifiers sometimes known as "stabilizers" can be added to ensure isotonicity of liquid compositions of the present disclosure and include polyhydric sugar alcohols, for example trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol. Stabilizers refer to a broad category of excipients which can range in function from a bulking agent to an additive which solubilizes the therapeutic agent or helps to prevent denaturation or adherence to the container wall. Typical stabilizers can be polyhydric sugar alcohols (enumerated above); amino acids such as arginine, lysine, glycine, glutamine, asparagine, histidine, alanine, ornithine, L-leucine, 2-phenylalanine, glutamic acid, threonine, etc., organic sugars or sugar alcohols, such as lactose, trehalose, stachyose, mannitol, sorbitol, xylitol, ribitol, myoinisitol, galactitol, glycerol and the like, including cyclitols such as inositol; polyethylene glycol; amino acid polymers; sulfur containing reducing agents, such as urea, glutathione, thioctic acid, sodium thioglycolate, thioglycerol, α-monothioglycerol and sodium thio sulfate; low molecular weight polypeptides (*e.g.*, peptides of 10 residues or fewer); proteins such as human serum albumin, bovine serum albumin, gelatin or immunoglobulins; hydrophylic polymers, such as polyvinylpyrrolidone monosaccharides, such as xylose, mannose, fructose, glucose; disaccharides such as lactose, maltose, sucrose and trehalose; and trisaccacharides such as raffinose; and polysaccharides such as dextran. Stabilizers may be present in amounts ranging from 0.5 to 10 wt % per wt of MUC4 peptide. [0334] Non-ionic surfactants or detergents (also known as "wetting agents") may be added to help solubilize the glycoprotein as well as to protect the glycoprotein against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stressed without causing denaturation of the protein. Suitable non-ionic surfactants include polysorbates (20, 80, etc.), polyoxamers (184, 188 etc.), and pluronic polyols. Non-ionic surfactants may be present in a range of about 0.05 mg/mL to about 1.0 mg/mL, for example about 0.07 mg/mL to about 0.2 mg/m L.

[0395] Additional miscellaneous excipients include bulking agents (*e.g.*, starch), chelating agents (*e.g.*, EDTA), antioxidants (*e.g.*, ascorbic acid, methionine, vitamin E), and cosolvents.

[0396] Exemplary MUC4 peptide compositions of the disclosure are described in numbered embodiments 666 and 667.

5.10.2. Methods of Using MUC4 Peptides

[0397] The MUC4 peptides described herein can be used in the production of antibodies against a tumor-associated form of MUC4. The MUC4 peptide can be administered to an

animal. The amount of peptide administered can be effective to cause the animal to produce antibodies against the peptide. As used herein, "animal" refers to multicellular eukaryotic organism from the biological kingdom Animalia. In some embodiments, the animal is a mammal. In some embodiments, the animal is a mouse or a rabbit. Resulting antibodies can then be collected from the animal. The MUC4 peptide can be administered as purified peptide or as part of a composition provided herein.

[0398] The MUC4 peptides described herein can be used to elicit an immune response against a tumor-associated form of MUC4. The MUC4 peptide can be administered to an animal in an amount effective to cause the animal to mount an immune response (e.g., produce antibodies) against the peptide.

[0399] Exemplary methods for using the MUC4 peptides of the disclosure are described in numbered embodiments 668 to 671.

6. EXAMPLES

6.1 Example 1: Identification and Characterization of Anti-Glyco-MUC4 Antibodies

6.1.1. Overview

[0400] Glycans are essential membrane components and neoplastic transformation of human cells is virtually always associated with aberrant glycosylation of proteins and lipids. There are several types of protein glycosylation, including N-glycosylation and many types of O-glycosylation, but one of the most diverse types is the mucin type GalNAc type O-glycosylation (hereafter called O-glycosylation). Cancer associated changes in O-glycans are particularly interesting and the most frequently observed aberrant glycophenotype is expression of the most immature truncated O-glycan structures designated Tn (GalNAcα1-O-Ser/Thr), STn (NeuAcα2-6GalNAcα1-O-Ser/Thr), and T (Galβ1-3GalNAcα1-O-Ser/Thr) antigens. Truncated O-glycans are observed on almost all epithelial cancer cells and strongly correlated with poor prognosis. In addition, it is becoming increasingly clear that glycans also have pivotal roles in cancer development, with truncated O-glycans affecting differentiation, cell-cell and cell-matrix interactions, directly inducing oncogenic features in predisposed cells.

[0401] The inventors have identified MUC4 glycopeptide epitopes in human cancer cells and used the defined glyco-peptides to develop cancer specific anti-glyco-MUC4 monoclonal antibodies.

6.1.2. Materials and Methods

6.1.2.1 Synthesis of Tn MUC4 glycopeptide

[0402] The MUC4 glycopeptide, CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154), with O-linked GalNAc on the serine and threonine residues shown with bold and underlined text was synthesized using a standard FMOC peptide synthesis strategy. Pre-synthesized glycosylated

amino acids were coupled to the elongating peptide at specific locations using solid or solution phase peptide chemistry in a stepwise fashion. After completing the full sequence and removing all protecting groups, the resulting glycopeptide was purified by high-performance liquid chromatography (HPLC) and characterized by mass spectrometry (electrospray ionization in positive mode).

6.1.2.1 Immunization Protocol

[0403] Female Balb/c mice were immunized subcutaneously with the Tn-glycosylated MUC4 glycopeptide conjugated to KLH (keyhole limpet hemocyanin) through a maleimide linker. The mice were immunized on days 0, 14, and 35 with 50 µg, 45 µg, and 45 µg of KLH-glycopeptide, respectively. The first immunization used Freund's complete adjuvant. All subsequent immunizations used Freund's incomplete adjuvant. On Day 45, tail bleeds were evaluated for polyclonal response. On day 56 or after, mice to be fused were boosted with 15 ug of KLH-glycopeptide in Freund's incomplete adjuvant 3 to 5 days before hybridoma fusion. Splenocytes from mice were fused with SP2/0-Ag14 (ATCC, cat# CRL-1581) myeloma cells using the Electro Cell Manipulator (ECM2001) from BTX Harvard Apparatus. Hybridomas were seeded in 96-well plates, cultured, scaled, and evaluated and selected for specificity towards MUC4-Tn using a combination of selection criteria including ELISA, FLOW cytometry, and immunofluorescence to obtain monoclonal antibodies having specificity for MUC4-Tn.

6.1.3. Results

6.1.3.1 Glycopeptide specific antibodies to Tn-MUC4

[0404] Glycopeptide reactive antibodies were generated using the Tn-glycosylated MUC4 glycopeptide. Antibodies generated using MUC4 glycopeptide, including 2D5, 5B8, and 15F3, proved superior in selectivity.

6.2 Example 2: Functional characterization of 2D5.2F6.2C11, 5B8.2A11.2C7, and 15F3.2D11.1E6 antibodies by Octet and Biacore

6.2.1. Overview

[0405] 2D5.2F6.2C11 (hereinafter "2D5"), 5B8.2A11.2C7 (hereinafter "5B8"), and 15F3.2D11.1E6 (hereinafter "15F3") were characterized by Biacore to test the reactivity of anti-MUC4 mAbs to titrated MUC4 peptides. 2D5, 5B8, and 15F3 were also characterized by Octet to test the reactivity of anti-MUC4 mAbs to peptides with different glycosylated sites (including a non-glycosylated peptide) as shown in Table 6.

Table 6

Peptide	Sequence (Bold and Underlined= GalNAc Site)
MUC4-Tn	Biotin- CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154)
MUC4-Tn (S)	Biotin- CTIPSTAMHTR**S**TAAPIPILP (SEQ ID NO:197)
MUC4-Tn (T)	Biotin- CTIPSTAMHTRS**T**AAPIPILP (SEQ ID NO:198)

MUC4	Biotin- CTIPSTAMHTRSTAAPIPILP (SEQ ID NO:155)

6.2.2. Materials and Methods

6.2.2.1 Surface Plasmon Resonance

[0406] Antibody affinity assays can be carried out using surface plasmon resonance (*e.g.*, using a Biacore system (Cytiva)). In a surface plasmon resonance assay, one or more antibodies can be immobilized onto a biosensor and presented with an analyte (e.g., the glyco-MUC4 peptide CTIPSTAMHTR**ST**AAPIPILP-amide (the amino acid portion of which is SEQ ID NO:154; bold and underlined residues indicate GalNAc glycosylation sites) or a negative control analyte such as un unglycosylated MUC4 peptide (CTIPSTAMHTRSTAAPIPILP-amide (the amino acid portion of which is SEQ ID NO:155)). The antibodies are contacted with different concentrations of the analyte, for example concentrations of 2.5 nM, 7.4 nM, 22 nM, 66 nM and 200 nM. Affinity is measured using multi-cycle kinetics in triplicate for each analyte concentration, with 1 min association and 5 min dissociation. When comparing the binding affinities of two antibodies, the same concentration of both antibodies was used (*e.g.*, measured using a 1 μM concentration of each antibody). The affinity is determined by fitting the binding curve to a specific model: kinetic fit (1:1 model) or if applicable heterogenous ligand binding model.

6.2.2.2 Bio-Layer Interferometry (Octet)

[0407] Antibody affinity and epitope binning of monoclonal antibodies can be assessed against specific antigens using BLI. In a BLI assay, the antigen can be immobilized onto a biosensor (e.g., the glyco-MUC4 peptide CTIPSTAMHTR**ST**AAPIPILP-amide (the amino acid portion of which is SEQ ID NO:154) or a negative control analyte such as un unglycosylated MUC4 peptide (CTIPSTAMHTRSTAAPIPILP-amide) (the amino acid portion of which is SEQ ID NO:155)) and presented to one antibody for affinity measurements or two competing antibodies in tandem (or consecutive steps) for epitope binning. The binding to non-overlapping epitopes occurs if saturation with the first antibody does not block the binding of the second antibody. The affinity is determined by fitting the binding curve to a specific model: a 1:1 monovalent model or a 2:1 bivalent model. The error (>95% confidence) is calculated by how close the generated curve matches the model.

6.2.2.3 Flow Cytometry

[0408] Adherent cells were dissociated with TrypLE select (Gibco) and washed from the flask surface with cell culture media (RPMI w/ L-glutamine, 1% PenStrep, & 10% FBS). Cells were washed several times by centrifugation at 300*g for 5 min at 4 °C followed by resuspension in PBS with 1% BSA (PBS/1% BSA). Cells were resuspended between $5x10^5$ cells/ml to $2x10^6$ cell/ml and then distributed into a 96 well U-bottom plate. Diluted commercial antibody (0.25-2 μg/ml), or hybridoma supernatants, or blood serum for polyclonal responses, were added to cells and incubated for 1 hr on ice. Following several washes with PBS/1% BSA, cells were

incubated for 30 min on ice with a 1:1600 dilution of AlexaFluor647 conjugated $F(ab)_2$ goat anti-mouse IgG Fcγ (JacksonImmunoResearch). Cells were washed again with PBS/1% BSA and then fixed in 1% formaldehyde in PBS/1% BSA. Cells were analysed on either a 2 or 4 laser Attune NXT flow cytometer. Data was processed in FlowJo Software.

6.2.2.4 Immunofluorescence

[0409] Cells were seeded to 50% confluency in glass chamber slides (nunc) and incubated 12-18 hours at 37 °C, 5% CO_2. Following overnight growth, media from slides was removed and cells were fixed with 4% formaldehyde in PBS (pH 7.4) for 10 min at room temperature. Slides were washed in PBS. Diluted commercial antibody (1-4 µg/ml), or hybridoma supernatants, or blood serum for polyclonal responses, were added to the slides and the slides were incubated overnight at 4 °C. The slides were washed in PBS and stained with a 1:800 dilution of AlexaFluor488 conjugated $F(ab)_2$ rabbit anti-mouse IgG (H+L) (Invitrogen) for 45 min at room temperature. The slides were washed in PBS and mounted using Prolong Gold Antifade Mountant with DAPI (Thermofisher) and examined using an Olympus FV3000 confocal microscope.

6.2.3. Results

6.2.3.1 Binding specificities of mAbs 2D5, 5B8, and 15F3

[0410] To characterize the binding specificities of 2D5, 5B8, and 15F3for non-glycosylated and Tn-glycosylated MUC4, flow cytometry analysis of the MUC4 mouse antibodies on T3M4 COSMC-KO and T3M4 cells was performed. It was found that 2D5, 5B8, and 15F3 only reacted with Tn-glycosylated MUC4 (*i.e.*, T3M4 COSMC-KO cells) and not with its non-glycosylated counterpart (*i.e.*, T3M4 cells) (FIGS. 1A-1E). The affinities of 2D5, 5B8, and 15F3 against the MUC4 glycopeptides were determined by Biacore and Octet. Table 7 summarizes dissociation constants (Kd) for 2D5, 5B8, and 15F3, along with Mab 6E3 (US Pat. No. 10,139,414) as a comparator, against different glycoforms of MUC4 peptide, as well as unglycosylated MUC4 and MUC1-Tn with +/- error at 95% confidence. Table 8 provides the dissociation constants (Kd) for 2D5 and mAb 6E3 (US Pat. No. 10,139,414), an earlier anti Tn-MUC4 antibody, as a comparator. Included is the +/- error at 95% confidence.

Table 7

	Affinity (Biacore)			***Apparent Affinity (Octet)***				
Antibody	***MUC4-Tn***	***MUC4***	***MUC1-Tn***	***MUC4-Tn***	***MUC4-Tn (S)***	***MUC4-Tn (T)***	***MUC4***	***MUC1-Tn***
2D5	3.96 nM	>400 nM	>400 nM	2 nM +/- 0.251 nM	> 10 µM	0.4.3 µM +/- 5.5 µM	>10 µM	2.9 µM +/-5.9 µM

5B8	3.93 nM	>400 nM	>400 nM	2.32 nM +/- 2.32 nM	> 10 µM	.56 µM +/- 1 pM	>10 µM	2.8 µM +/-7.6 µM
15F3	3.56 nM	>400 nM	>400 nM	50.2 nM +/- 10nM	> 10 µM	79 µM +/- 66 µM	>10 µM	2.5 µM +/-3.7 µM

Table 8

	Apparent Affinity (Octet)	
Antibody	***MUC4-Tn***	***MUC4***
2D5	2 nM +/- 0.251 nM	>10 µM
mAb 6E3	700 nM +/- 19.7 nM	>10 µM

[0411] To further assess the specificities of 2D5, 5B8, and 15F3 in a more natural conformational context, 2D5, 5B8, and 15F3 were used to stain T47D cells for flow cytometry and immunofluorescence. T47D cell line is inherently Tn-negative but can be induced to express the Tn-antigen by KO of the COSMC chaperone. When using 2D5, 5B8, and 15F3 to stain for flow cytometry, it was found that each selectively stained COSMC KO T47D cells but not their wildtype counterpart, despite both cells staining positive for MUC4 (see FIG. 2). In agreement with these results, immunofluorescence showed that only $MUC4^+$ Tn^+ T47D COSMC KO cells stained with 2D5, 5B8, and 15F3, whereas $MUC4^+$ Tn^- T47D WT cells were not stained (FIG. 2).

6.3 Example 3: Sequence Analysis of Anti-Glyco-MUC4 Antibodies

[0412] Rapid Amplification of cDNA Ends (RACE) was performed to determine the heavy chain and light chain nucleotide sequences for 2D5, 5B8, and 15F3. The nucleotide sequences encoding the heavy and light chain variable regions of 2D5 are set forth in SEQ ID NO:21 and SEQ ID NO:22, respectively. The heavy and light chain variable regions encoded by SEQ ID NO:21 and SEQ ID NO:22 are set forth in SEQ ID NO:1 and SEQ ID NO:2, respectively. The predicted heavy chain CDR sequences (IMGT definition) are set forth in SEQ ID NOS:3-5, respectively, and the predicted light chain CDR sequences (IMGT definition) are set forth in SEQ ID NOS:6-8, respectively. The predicted heavy chain CDR sequences (Kabat definition) are set forth in SEQ ID NO:9-11, respectively, and the predicted light chain CDR sequences (Kabat definition) are set forth in SEQ ID NO:12-14, respectively. The predicted heavy chain CDR sequences (Chothia definition) are set forth in SEQ ID NO:15-17, respectively, and the predicted light chain CDR sequences (Chothia definition) are set forth in SEQ ID NO:18-20, respectively.

[0413] The nucleotide sequences encoding the heavy and light chain variable regions of 5B8 are set forth in SEQ ID NO:43 and SEQ ID NO:44, respectively. The heavy and light chain variable regions encoded by SEQ ID NO:43 and SEQ ID NO:44 are set forth in SEQ ID NO:23 and SEQ ID NO:24, respectively. The predicted heavy chain CDR sequences (IMGT definition) are set forth in SEQ ID NOS:25-27, respectively, and the predicted light chain CDR sequences (IMGT definition) are set forth in SEQ ID NOS:28-30, respectively. The predicted heavy chain CDR sequences (Kabat definition) are set forth in SEQ ID NOS:31-33, respectively, and the predicted light chain CDR sequences (Kabat definition) are set forth in SEQ ID NOS:34-36, respectively. The predicted heavy chain CDR sequences (Chothia definition) are set forth in SEQ ID NOS:37-39, respectively, and the predicted light chain CDR sequences (Chothia definition) are set forth in SEQ ID NOS:40-42, respectively.

[0414] The nucleotide sequences encoding the heavy and light chain variable regions of 15F3 are set forth in SEQ ID NO:65 and SEQ ID NO:66, respectively. The heavy and light chain variable regions encoded by SEQ ID NO:65 and SEQ ID NO:66 are set forth in SEQ ID NO:45 and SEQ ID NO:46, respectively. The predicted heavy chain CDR sequences (IMGT definition) are set forth in SEQ ID NOS:47-49, respectively, and the predicted light chain CDR sequences (IMGT definition) are set forth in SEQ ID NOS:50-52, respectively. The predicted heavy chain CDR sequences (Kabat definition) are set forth in SEQ ID NOS:53-55, respectively, and the predicted light chain CDR sequences (Kabat definition) are set forth in SEQ ID NOS:56-58, respectively. The predicted heavy chain CDR sequences (Chothia definition) are set forth in SEQ ID NOS:59-61, respectively, and the predicted light chain CDR sequences (Chothia definition) are set forth in SEQ ID NOS:62-64, respectively.

6.4 Example 4: Tissue expression of Tn-glycosylated MUC4 epitope recognized by 2D5, 5B8, and 15F3.

6.4.1. Overview

[0415] 2D5, 5B8, and 15F3 were characterized by Immunohistochemistry on various normal and cancer tissue.

6.4.2. Materials and methods

[0416] Paraffin embedded tissue micro arrays (TMAs) or tissue sections were de-paraffinized with xylene and ethanol, following antigen retrieval with citrate buffer (pH 6.0) and heated in a microwave for 18 min. TMAs obtained from USBIOMAX and were stained with Ultra Vison Quanto Detection System HRP DAB. Briefly, TMAs were washed in TBS, incubated with mAb supernatant for 2 hours. After wash in TBS x 2, the TMAs was incubated with Primary Antibody Amplifier Quanto for 10 min. After wash in TBS, TMAs were incubated with HRP polymer quanto (10 min) followed by DAB chromogen. Slides were counterstained with hematoxylin, were dehydrated, and mounted.

6.4.3. Results

[0417] When staining formalin-fixed paraffin embedded tissue sections for immunohistochemistry, positive staining was observed with 2D5, 5B8, and 15F3 with strong staining in 5/6 prostate (*see* FIG. 3A). This staining pattern correlated with staining for normal MUC4 expression, showing that MUC4 expression in these carcinomas predicted reactivity to 2D5, 5B8, and 15F3. Importantly, no reactivity when using 2D5, 5B8, and 15F3 to stain healthy adjacent tissues was observed (FIG. 3A).

[0418] Formalin-fixed paraffin embedded tissue sections of multi-normal human tissues array, representing FDA guidelines for antibody cross-reactivity testing showed no positive cellular surface stain for 2D5 (FIGS. 3C-3E) by IHC despite the presence of positive surface stain for MUC4 on the following tissues: larynx, bladder, cervix, uterus, colon, small intestine, and stomach (FIGS. 3C-3E) The data show that while MUC4 is present on the cellular surface of normal tissue, Tn modified MUC4 is absent.

[0419] Formalin-fixed paraffin embedded tissue sections of multiple organ tumor tissues array show specific cellular surface stain for 2D5 on the following tissues: 2/3 rectum, 2/3 ovary, 3/3 ovary (FIGS. 3F-3G). Importantly, no specific cellular surface stain was observed when using 2D5 to stain healthy adjacent tissues (FIGS. 3F-3G).

[0420] In conclusion, 2D5, 5B8, and 15F3 were found to show specific cell surface staining on cancer tissue sections, but not their healthy counterparts.

[0421] The identity of each tissue in the TMAs is set forth in Tables 9, 10, 11, and 12, with each table representing a unique TMA.

TABLE 9

Position	No.	Age	Sex	Organ/Anatomic Site	Pathology diagnosis	TNM	Grade	
A1	1	47	M	Pancreas	Adenocarcinoma	T2N0M0	1	Malignant
A2	2	47	M	Pancreas	Adenocarcinoma	T2N0M0	1	Malignant
A3	3	47	M	Pancreas	Adjacent normal pancreas tissue	-	-	NAT
A4	4	47	M	Pancreas	Adjacent normal pancreas tissue	-	-	NAT
A5	5	54	F	Pancreas	Adenocarcinoma	T3N0M0	2	Malignant
A6	6	54	F	Pancreas	Adenocarcinoma	T3N0M0	2	Malignant
A7	7	54	F	Pancreas	Cancer adjacent pancreas tissue	-	-	AT
A8	8	54	F	Pancreas	Cancer adjacent pancreas tissue	-	-	AT
B1	9	44	M	Pancreas	Adenocarcinoma	T3N0M0	2	Malignant
B2	10	44	M	Pancreas	Adenocarcinoma	T3N0M0	2	Malignant
B3	11	44	M	Pancreas	Cancer adjacent pancreas tissue	-	-	AT
B4	12	44	M	Pancreas	Cancer adjacent pancreas tissue	-		AT
B5	13	50	M	Pancreas	Adenocarcinoma	-	1	Malignant
B6	14	50	M	Pancreas	Adenocarcinoma	-	1	Malignant

TABLE 9								
Position	No.	Age	Sex	Organ/Anatomic Site	Pathology diagnosis	TNM	Grade	
B7	15	50	M	Pancreas	Adjacent normal pancreas tissue	-	-	NAT
B8	16	50	M	Pancreas	Adjacent normal pancreas tissue	-	-	NAT
C1	17	47	F	Pancreas	Adenocarcinoma	T2N0M0	3	Malignant
C2	18	47	F	Pancreas	Adenocarcinoma	T2N0M0	3	Malignant
C3	19	47	F	Pancreas	Adjacent chronic pancreatitis tissue	-	-	AT
C4	20	47	F	Pancreas	Cancer adjacent pancreas tissue	-	-	AT
C5	21	44	M	Pancreas	Adenocarcinoma	T3N0M0	3	Malignant
C6	22	44	M	Pancreas	Adenocarcinoma	T3N0M0	3	Malignant
C7	23	44	M	Pancreas	Cancer adjacent pancreas tissue	-	-	AT
C8	24	44	M	Pancreas	Cancer adjacent pancreas tissue	-	-	AT

TABLE 10						
Position	No.	Age	Sex	Organ/Anatomic Site	Pathology diagnosis	TNM
A1	1	2	F	Liver	Normal liver tissue	normal
A2	2	50	F	Liver	Normal liver tissue	normal
A3	3	14	F	Liver	Normal liver tissue	normal
A4	4	35	F	Liver	Normal liver tissue	normal
A5	5	24	M	Liver	Normal liver tissue	normal
A6	6	21	F	Liver	Normal liver tissue	normal
A7	7	Fetus	F	Liver	Normal fetal liver tissue	normal
A8	8	35	M	Liver	Normal liver tissue	normal
B1	9	35	M	Liver	Normal liver tissue	normal
B2	10	40	M	Liver	Normal liver tissue	normal
B3	11	40	M	Liver	Normal liver tissue	normal
B4	12	38	M	Liver	Normal liver tissue	normal
B5	13	34	M	Liver	Normal liver tissue	normal
B6	14	27	M	Liver	Normal liver tissue	normal
B7	15	25	F	Liver	Normal liver tissue	normal
B8	16	42	F	Pancreas	Normal pancreas tissue	normal
C1	17	35	F	Pancreas	Normal pancreas tissue	normal
C2	18	1 mon.	M	Pancreas	Normal pancreas tissue	normal
C3	19	35	M	Pancreas	Normal pancreas tissue	normal
C4	20	38	F	Pancreas	Normal pancreas tissue	normal
C5	21	56	M	Stomach	Normal stomach tissue	normal
C6	22	35	F	Stomach	Normal stomach tissue	normal
C7	23	35	M	Stomach	Normal stomach tissue	normal
C8	24	22	M	Stomach	Normal gastric mucosa tissue	normal
D1	25	40	M	Stomach	Normal stomach tissue	normal
D2	26	38	F	Stomach	Normal stomach tissue	normal
D3	27	35	M	Stomach	Normal stomach tissue	normal
D4	28	48	M	Stomach	Normal stomach tissue	normal
D5	29	52	F	Stomach	Normal stomach tissue	normal
D6	30	24	M	Esophagus	Normal esophagus tissue	normal

TABLE 10

Position	No.	Age	Sex	Organ/Anatomic Site	Pathology diagnosis	TNM
D7	31	21	F	Esophagus	Normal esophagus tissue (fibrous and connective tissue)	normal
D8	32	26	M	Esophagus	Normal esophagus tissue	normal
E1	33	22	M	Esophagus	Normal esophagus tissue	normal
E2	34	48	M	Esophagus	Normal esophagus tissue	normal
E3	35	59	M	Esophagus	Normal esophagus tissue	normal
E4	36	50	F	Colon	Normal colon tissue	normal
E5	37	49	M	Colon	Normal colon tissue	normal
E6	38	21	F	Colon	Normal colon tissue (fibrous and smooth muscle tissue)	normal
E7	39	35	M	Colon	Normal colon tissue	normal
E8	40	49	M	Intestine	Normal small intestine tissue (sparse)	normal
F1	41	35	F	Intestine	Normal small intestine tissue	normal
F2	42	40	M	Intestine	Normal small intestine tissue	normal
F3	43	38	F	Intestine	Normal small intestine tissue	normal
F4	44	42	F	Intestine	Normal small intestine tissue with necrosis	normal
F5	45	57	F	Intestine	Normal small intestine tissue	normal
F6	46	37	M	Intestine	Normal small intestine tissue	normal
F7	47	61	F	Intestine	Normal small intestine tissue	normal
F8	48	27	M	Intestine	Normal small intestine tissue	normal

TABLE 11

Position	No.	Age	Sex	Organ/Anatomic Site	Pathology diagnosis	TNM
A1	1	2	F	Cerebrum	Cerebrum gray matter tissue	Normal
A2	2	50	F	Cerebrum	Cerebrum gray matter tissue	Normal
A3	3	24	F	Cerebrum	Cerebrum gray matter tissue	Normal
A4	4	21	F	Cerebrum	Cerebrum gray matter and white matter tissue	Normal
A5	5	35	M	Cerebrum	Cerebrum white matter tissue	Normal
A6	6	35	F	Cerebrum	Cerebrum gray matter and white matter tissue	Normal
A7	7	24	F	Cerebellum	Cerebellum tissue	Normal
A8	8	35	M	Cerebellum	Cerebellum tissue	Normal
A9	9	35	F	Cerebellum	Cerebellum tissue	Normal
B1	10	41	F	Adrenal gland	Adrenal gland tissue	Normal
B2	11	18	F	Adrenal gland	Adrenal gland tissue	Normal
B3	12	43	F	Adrenal gland	Adrenal gland tissue	Normal
B4	13	35	M	Ovary	Adjacent normal ovary tissue	NAT
B5	14	61	M	Ovary	Adjacent normal ovary tissue	NAT
B6	15	52	F	Ovary	Adjacent normal ovary tissue	NAT
B7	16	35	M	Pancreas	Pancreas tissue	Normal
B8	17	35	M	Pancreas	Pancreas tissue	Normal
B9	18	16	F	Pancreas	Pancreas tissue	Normal
C1	19	27	F	Lymph node	Lymph node tissue	Normal
C2	20	30	M	Lymph node	Lymph node tissue	Normal
C3	21	35	F	Lymph node	Lymph node tissue	Normal
C4	22	54	F	Hypophysis	Neurohypophysis tissue	Normal
C5	23	54	F	Hypophysis	Adenohypophysis tissue	Normal

TABLE 11

Position	No.	Age	Sex	Organ/Anatomic Site	Pathology diagnosis	TNM
C6	24	54	M	Hypophysis	Adenohypophysis tissue	Normal
C7	25	45	F	Testis	Testis tissue	Normal
C8	26	30	F	Testis	Testis tissue	Normal
C9	27	33	M	Testis	Testis tissue	Normal
D1	28	45	M	Thyroid gland	Thyroid gland tissue	Normal
D2	29	18	M	Thyroid gland	Thyroid gland tissue	Normal
D3	30	50	F	Thyroid gland	Thyroid gland tissue	Normal
D4	31	41	F	Breast	Adjacent normal breast tissue (fibrous tissue)	NAT
D5	32	42	F	Breast	Breast tissue	Normal
D6	33	27	M	Breast	Breast tissue	Normal
D7	34	21	M	Spleen	Spleen tissue	Normal
D8	35	22	M	Spleen	Spleen tissue	Normal
D9	36	37	M	Spleen	Spleen tissue	Normal
E1	37	50	F	Tonsil	Cancer adjacent lingual tonsil tissue	AT
E2	38	50	M	Tonsil	Cancer adjacent lingual tonsil tissue	AT
E3	39	50	F	Tonsil	Cancer adjacent lingual tonsil tissue	AT
E4	40	15	F	Thymus gland	Thymus gland tissue	Normal
E5	41	21	F	Thymus gland	Thymus gland tissue	Normal
E6	42	21 Days	F	Thymus gland	Thymus gland tissue	Normal
E7	43	30	M	Bone marrow	Bone marrow tissue	Normal
E8	44	40	M	Bone marrow	Bone marrow tissue	Normal
E9	45	33	M	Bone marrow	Bone marrow tissue	Normal
F1	46	48	M	Lung	Lung tissue	Normal
F2	47	35	M	Lung	Lung tissue	Normal
F3	48	30	F	Lung	Lung tissue	Normal
F4	49	40	F	Heart	Cardiac muscle tissue	Normal
F5	50	35	M	Heart	Cardiac muscle tissue	Normal
F6	51	35	M	Heart	Cardiac muscle tissue	Normal
F7	52	45	M	Esophagus	Esophagus tissue	Normal
F8	53	23	M	Esophagus	Esophagus tissue	Normal
F9	54	43	M	Esophagus	Esophagus tissue	Normal
G1	55	42	M	Stomach	Stomach tissue	Normal
G2	56	35	M	Stomach	Stomach tissue	Normal
G3	57	39	M	Stomach	Stomach tissue	Normal
G4	58	45	F	Small intestine	Small intestine tissue	Normal
G5	59	40	M	Small intestine	Small intestine tissue	Normal
G6	60	21	M	Small intestine	Small intestine tissue	Normal
G7	61	35	M	Colon	Colon tissue	Normal
G8	62	32	M	Colon	Colon tissue	Normal
G9	63	35	F	Colon	Colon tissue (sparse)	Normal
H1	64	38	M	Liver	Liver tissue	Normal
H2	65	23	F	Liver	Liver tissue	Normal
H3	66	50	M	Liver	Liver tissue	Normal
H4	67	42	M	Salivary gland	Adjacent normal salivary gland tissue	NAT
H5	68	22	F	Salivary gland	Salivary gland tissue	Normal

TABLE 11						
Position	No.	Age	Sex	Organ/Anatomic Site	Pathology diagnosis	TNM
H6	69	43	M	Salivary gland	Salivary gland tissue	Normal
H7	70	16	M	Kidney	Kidney tissue	Normal
H8	71	38	M	Kidney	Kidney tissue	Normal
H9	72	21	M	Kidney	Kidney tissue	Normal
I1	73	30	M	Prostate	Prostate tissue	Normal
I2	74	31	F	Prostate	Prostate tissue	Normal
I3	75	30	M	Prostate	Prostate tissue	Normal
I4	76	18	M	Uterus	Endometrium tissue	Normal
I5	77	41	M	Uterus	Endometrium tissue (smooth muscle)	Normal
I6	78	54	M	Uterus	Adjacent normal endometrium tissue	NAT
I7	79	47	M	Cervix	Adjacent normal cervix tissue	NAT
I8	80	45	F	Cervix	Adjacent normal cervix tissue	AT
I9	81	52	M	Cervix	Adjacent normal cervix tissue	NAT
J1	82	30	M	Skeletal muscle	Skeletal muscle tissue	Normal
J2	83	40	M	Skeletal muscle	Skeletal muscle tissue	Normal
J3	84	50	F	Skeletal muscle	Skeletal muscle tissue	Normal
J4	85	50	F	Skin	Skin tissue	Normal
J5	86	21 Days	F	Skin	Skin tissue	Normal
J6	87	50	F	Skin	Skin tissue	Normal
J7	88	35	F	Nerve	Peripheral nerve tissue	Normal
J8	89	25	F	Nerve	Peripheral nerve tissue	Normal
J9	90	50	M	Nerve	Peripheral nerve tissue	Normal
K1	91	47	M	Pericardium	Pericardial mesothelial tissue	Normal
K2	92	49	M	Diaphragm	Diaphragm and pleural mesothelial tissue	Normal
K3	93	33	M	Pericardium	Pericardial mesothelial tissue	Normal
K4	94	62	M	Eye	Adjacent normal choroid and sclera tissue	NAT
K5	95	55	F	Eye	Adjacent normal sclera tissue	NAT
K6	96	42	M	Eye	Adjacent normal choroid and sclera tissue	NAT
K7	97	49	M	Larynx	Larynx tissue（submucosal glands）	Normal
K8	98	39	M	Larynx	Larynx tissue	Normal
K9	99	21	M	Larynx	Laryngeal cartilage tissue	Normal

TABLE 12						
Position	No.	Age	Sex	Organ/Anatomic Site	Pathology diagnosis	TNM
A1	1	62	M	Esophagus	Squamous cell carcinoma	Malignant
A2	2	56	M	Esophagus	Squamous cell carcinoma	Malignant
A3	3	72	F	Esophagus	Squamous cell carcinoma	Malignant
A4	4	74	M	Stomach	Adenocarcinoma	Malignant
A5	5	56	M	Stomach	Adenocarcinoma	Malignant
A6	6	55	F	Stomach	Adenocarcinoma	Malignant
A7	7	67	M	Colon	Adenocarcinoma	Malignant
A8	8	58	M	Colon	Adenocarcinoma	Malignant
A9	9	37	M	Colon	Adenocarcinoma	Malignant

TABLE 12						
Position	No.	Age	Sex	Organ/Anatomic Site	Pathology diagnosis	TNM
B1	10	38	M	Esophagus	Adjacent normal esophagus tissue	NAT
B2	11	64	M	Esophagus	Adjacent normal esophagus tissue	NAT
B3	12	48	M	Esophagus	Adjacent normal esophagus tissue	NAT
B4	13	54	M	Stomach	Adjacent normal stomach tissue	NAT
B5	14	54	M	Stomach	Adjacent normal stomach tissue	NAT
B6	15	64	F	Stomach	Adjacent normal stomach tissue	NAT
B7	16	56	F	Colon	Adjacent normal colon tissue	NAT
B8	17	70	F	Colon	Adjacent normal colon tissue	NAT
B9	18	64	F	Colon	Adjacent normal colon tissue	NAT
C1	19	55	M	Rectum	Adenocarcinoma	Malignant
C2	20	67	M	Rectum	Adenocarcinoma	Malignant
C3	21	44	F	Rectum	Adenocarcinoma	Malignant
C4	22	32	F	Liver	Hepatocellular carcinoma	Malignant
C5	23	40	M	Liver	Hepatocellular carcinoma	Malignant
C6	24	55	F	Liver	Hepatocellular carcinoma	Malignant
C7	25	66	M	Lung	Squamous cell carcinoma	Malignant
C8	26	55	M	Lung	Squamous cell carcinoma	Malignant
C9	27	55	M	Lung	Squamous cell carcinoma	Malignant
D1	28	43	F	Rectum	Cancer adjacent rectum tissue	AT
D2	29	52	M	Rectum	Adjacent normal rectum tissue	NAT
D3	30	67	M	Rectum	Adjacent normal rectum tissue	NAT
D4	31	63	M	Liver	Adjacent normal liver tissue	NAT
D5	32	55	M	Liver	Adjacent normal liver tissue	NAT
D6	33	56	M	Liver	Adjacent normal liver tissue	NAT
D7	34	68	F	Lung	Adjacent normal lung tissue	NAT
D8	35	65	M	Lung	Adjacent normal lung tissue	NAT
D9	36	68	M	Lung	Adjacent normal lung tissue	NAT
E1	37	70	M	Kidney	Clear cell carcinoma	Malignant
E2	38	46	M	Kidney	Clear cell carcinoma	Malignant
E3	39	82	M	Kidney	Clear cell carcinoma	Malignant
E4	40	29	F	Breast	Invasive carcinoma of no special type	Malignant
E5	41	51	F	Breast	Invasive carcinoma of no special type	Malignant
E6	42	63	F	Breast	Invasive carcinoma of no special type	Malignant
E7	43	45	F	Cervix	Squamous cell carcinoma	Malignant
E8	44	76	F	Cervix	Squamous cell carcinoma	Malignant
E9	45	47	F	Cervix	Squamous cell carcinoma	Malignant
F1	46	54	M	Kidney	Adjacent normal kidney tissue	NAT
F2	47	56	M	Kidney	Adjacent normal kidney tissue	NAT
F3	48	61	F	Kidney	Adjacent normal kidney tissue	NAT
F4	49	43	F	Breast	Adjacent normal breast tissue	NAT
F5	50	38	F	Breast	Adjacent normal breast tissue	NAT
F6	51	41	F	Breast	Adjacent normal breast tissue	NAT

TABLE 12						
Position	No.	Age	Sex	Organ/Anatomic Site	Pathology diagnosis	TNM
F7	52	39	F	Cervix	Adjacent normal cervical canals tissue	NAT
F8	53	25	F	Cervix	Adjacent normal cervical tissue	NAT
F9	54	49	F	Cervix	Adjacent normal cervical tissue	NAT
G1	55	55	F	Ovary	High grade serous carcinoma	Malignant
G2	56	49	F	Ovary	High grade serous carcinoma	Malignant
G3	57	48	F	Ovary	High grade serous carcinoma	Malignant
G4	58	76	M	Prostate	Adenocarcinoma	Malignant
G5	59	80	M	Prostate	Adenocarcinoma	Malignant
G6	60	82	M	Prostate	Adenocarcinoma	Malignant
G7	61	55	M	Pancreas	Duct adenocarcinoma	Malignant
G8	62	65	M	Pancreas	Duct adenocarcinoma	Malignant
G9	63	68	F	Pancreas	Duct adenocarcinoma	Malignant
H1	64	53	F	Ovary	Adjacent normal ovary tissue	NAT
H2	65	45	F	Ovary	Adjacent normal ovary tissue	NAT
H3	66	40	F	Ovary	Adjacent normal ovary tissue	NAT
H4	67	63	M	Prostate	Adjacent normal prostate tissue	NAT
H5	68	52	M	Prostate	Adjacent normal prostate tissue	NAT
H6	69	35	M	Prostate	Adjacent normal prostate tissue	NAT
H7	70	69	F	Pancreas	Adjacent normal pancreas tissue	NAT
H8	71	64	M	Pancreas	Adjacent normal pancreas tissue	NAT
H9	72	70	M	Pancreas	Adjacent normal pancreas tissue	NAT

6.5 Example 5: Tn-MUC4 based CARs

6.5.1. Overview

[0422] Chimeric antigen receptors (CARs) having VH and VL domains of 2D5, 5B8, and 15F3 were designed. CARs were then evaluated in a target-specific cytotoxicity assay.

6.5.2. Materials and Methods

6.5.2.1 Vector Design

[0423] Various CAR constructs having scFvs having VH and VL domains of 2D5, 5B8, and 15F3 were designed (FIGS. 4A-4C). In the constructs, the VH and VL are attached together with one long linker $(GGGGS)_3$ (SEQ ID NO:160) to the CD8a hinge followed by a CD28 transmembrane domain and a second generation CAR (CD28 intracellular signal domain, and a CD3-zeta intracellular chain). The N-terminus of the scFvs was attached to a CD8a signal sequence. The MUC4 CARs were subcloned into the Virapower lentivirus vector pLENTI6.3-V5-DEST (Invitrogen).

[0424] Nucleotide sequences encoding the CARs are shown in Table 13. Amino acid sequences of the CARs are shown in Table 14.

Table 13
Nucleotide sequences encoding CARs

Construct	Nucleic acid sequence	Nucleic Acid Description	SEQ ID NO:
1 (2D5-CART)	ATGGCTCTGCCCGTTACAGCTCTGCTGCTGCCTCTGGCTCT GCTTCTGCATGCCGCCAGACCTAACATCATGCTGACACAGA GCCCTAGCAGCCTGGCTGTGTCTGCCGGCGAGAAAGTGAC CATGAGCTGCAAGAGCAGCCAGAGCGTGCTGTACTCCAGC GACCAGAAGAACTACCTGGCCTGGTATCAGCAGAAGCCCGG ACAGTCTCCCAAGCTGCTGATCTACTGGGCCAGCACCAGAG AAAGCGGCGTGCCCGATAGATTCACAGGCAGCGGCTCTGG CACCGACTTCACCCTGACAATCAGCAACGTGCAGGCCGAGG ATCTGGCCGTGTACTACTGTCACCAGTACCTGAGCAGCTAC ACCTTCGGCGGAGGCACCAAGCTGGAAATCAAAGGCGGAG GCGGATCTGGCGGCGGAGGTAGCGGTGGCGGAGGATCTCA AGTTCAGCTGCAGCAGTCCGATGCCGAGCTGGTTAAGCCTG GCGCCTCTGTGCGGATCAGCTGTAAAGCCTACGGCTACACA TTCACCGACCACGCCATCCACTGGGTCAAGCAGAAACCTGA ACAGGGCCTCGAGTGGCTGGGCTACATCAGCCCTGGCAAC GACGACATCCAGTACAACGCCAAGTTCAAGGGCAAAGCCAC ACTGACCGCCGACAAGTCTAGCAGCACAGCCTACATGCAGC TCAACAGCCTGACCAGCGACGACAGCGCCGTGTATTTCTGC AAGCGGAGCATGGCCAACAGCTTCGACTATTGGGGCCAGG GCACAACCCTGACCGTGTCCTCTACAACAACCCCTGCTCCT CGGCCACCTACACCAGCTCCTACAATTGCCTCTCAACCTCT GTCTCTGCGGCCCGAGGCTTGTAGACCTGCTGCTGGCGGA GCTGTGCACACAAGAGGACTGGATTTCGCCTGCGACTTCTG GGTGCTCGTGGTTGTTGGCGGAGTGCTGGCCTGTTACTCTC TGCTGGTCACCGTGGCCTTCATCATCTTTTGGGTCCGAAGC AAGAGAAGCCGGCTGCTGCACAGCGACTACATGAACATGAC CCCTAGACGGCCCGGACCTACCAGAAAGCACTACCAGCCTT ACGCTCCTCCTAGAGACTTCGCCGCCTACCGGTCCAGAGTG AAGTTCAGCAGATCCGCTGATGCCCCTGCCTATCAGCAGGG CCAGAACCAGCTGTACAACGAGCTGAACCTGGGGAGAAGA GAAGAGTACGACGTGCTGGACAAGCGGAGAGGCAGAGATC CTGAGATGGGCGGCAAGCCCAGACGGAAGAATCCTCAAGA GGGCCTGTACAATGAACTGCAGAAAGACAAGATGGCCGAGG CCTACAGCGAGATCGGAATGAAGGGCGAGCGCAGAAGAGG CAAGGGACACGATGGACTGTACCAGGGCCTGAGCACCGCC ACCAAGGATACCTATGATGCCCTGCACATGCAGGCCCTGCC TCCAAGAAGAAAGAGAGGCTCTGGCGAAGGCAGAGGCTCC CTGCTTACATGTGGCGACGTGGAAGAGAACCCCGGACCAAT GGTGTCCAAGGGCGAAGAGGACAACATGGCCATCATCAAAG AATTCATGCGGTTCAAGGTGCACATGGAAGGCAGCGTGAAC GGCCACGAGTTCGAGATTGAAGGCGAAGGCGAGGGCAGAC CTTACGAGGGAACACAGACCGCCAAGCTGAAAGTGACAAAA GGCGGCCCACTGCCTTTCGCCTGGGACATCCTGTCTCCACA GTTTATGTACGGCAGCAAGGCCTACGTGAAGCACCCCGCCG ATATTCCCGACTACCTGAAGCTGAGCTTCCCCGAGGGCTTC AAGTGGGAGAGAGTGATGAACTTCGAGGACGGCGGCGTCG TGACCGTGACTCAAGATAGCTCTCTGCAGGACGGCGAGTTC ATCTACAAAGTGAAGCTGCGGGGCACCAACTTTCCCTCTGA TGGCCCCGTGATGCAGAAAAAGACCATGGGCTGGGAAGCC AGCAGCGAGAGAATGTACCCTGAAGATGGCGCCCTGAAAG GCGAGATCAAGCAGCGGCTGAAACTGAAGGATGGCGGCCA	1-63 = CD8A signal sequence 64-399 = 2D5 LC 400-444 = Linker 445-795 = 2D5 HC 796-930 = CD8a hinge 931-1011 = CD28 transmembrane domain 1012-1134 = intracellular signal domain 1135-1470 = CD3-zeta intracellular chain 14718-2253 = T2A-mCherry	203

Table 13
Nucleotide sequences encoding CARs

Construct	Nucleic acid sequence	Nucleic Acid Description	SEQ ID NO:
	CTACGACGCCGAAGTGAAAACCACCTACAAGGCCAAGAAAC CCGTGCAGCTGCCAGGCGCCTACAACGTGAACATCAAGCTG GACATTACCAGCCACAACGAGGACTACACCATCGTGGAACA GTACGAGAGAGCCGAAGGCAGGCACTCTACAGGCGGAATG GACGAGCTGTATAAGTAG		
2 (15F3-CART)	ATGGCTCTGCCCGTTACAGCTCTGCTGCTGCCTCTGGCTCT GCTTCTGCATGCCGCCAGACCTAACATCATGCTGACACAGA GCCCTAGCAGCCTGGCTGTGTCTGCCGGCGAGAAAGTGAC CATGAGCTGCAAGAGCAGCCAGAGCGTGCTGTACTCCAGC GACCAGAAGAACTACCTGGCCTGGTATCAGCAGAAGCCCGG ACAGTCTCCCAAGCTGCTGATCTACTGGGCCAGCACCAGAG AAAGCGGCGTGCCCGATAGATTCACAGGCAGCGGCTCTGG CACCGACTTCACCCTGACAATCTCTAACGTGCGCGCCGAGG ATCTGGCCGTGTACTACTGTCACCAGTACCTGAGCAGCTAC ACCTTCGGCGGAGGCACCAAGCTGGAAATCAAAGGCGGAG GCGGATCTGGCGGCGGAGGTAGCGGTGGCGGAGGATCTCA AGTTCAGCTGCAGCAGTCTGACGCCGAGCTGGTTGAACCTG GCGCCTCTGTGAAGATCAGCTGCAAGGCCTACGGCTACACA TTCACCGACCACGCCATCCACTGGGTCAAGCAGAAACCTGA ACAGGGCCTCGAGTGGCTGGGCTACATCAGCCCTGGCAAC GACGACATCCAGTACAACGCCAAGTTCAAGGGCAGAGCTAC CCTGACCGCCGACAAGTCTAGCAGCACAGCCTACATGCAGC TCAACAGCCTGACCAGCGACGACAGCGCCGTGTATTTCTGC AAGCGGAGCATGGCCAACAGCTTCGACTTTTGGGGCCAGG GCACCACACTGACCGTGTCCTCTACAACAACCCCTGCTCCT CGGCCACCTACACCAGCTCCTACAATTGCCTCTCAACCTCT GTCTCTGCGGCCCGAGGCTTGTAGACCTGCTGCTGGCGGA GCTGTGCACACAAGAGGACTGGATTTCGCCTGCGACTTCTG GGTGCTCGTGGTTGTTGGCGGAGTGCTGGCCTGTTACTCTC TGCTGGTCACCGTGGCCTTCATCATCTTTTGGGTCCGAAGC AAGAGAAGCCGGCTGCTGCACAGCGACTACATGAACATGAC CCCTAGACGGCCCGGACCTACCAGAAAGCACTACCAGCCTT ACGCTCCTCCTAGAGACTTCGCCGCCTACCGGTCCAGAGTG AAGTTCAGCAGATCCGCTGATGCCCCTGCCTATCAGCAGGG CCAGAACCAGCTGTACAACGAGCTGAACCTGGGGAGAAGA GAAGAGTACGACGTGCTGGACAAGCGGAGAGGCAGAGATC CTGAGATGGGCGGCAAGCCCAGACGGAAGAATCCTCAAGA GGGCCTGTACAATGAACTGCAGAAAGACAAGATGGCCGAGG CCTACAGCGAGATCGGAATGAAGGGCGAGCGCAGAAGAGG CAAGGGACACGATGGACTGTACCAGGGCCTGAGCACCGCC ACCAAGGATACCTATGATGCCCTGCACATGCAGGCCCTGCC TCCAAGAAGAAAGAGAGGCTCTGGCGAAGGCAGAGGCTCC CTGCTTACATGTGGCGACGTGGAAGAGAACCCCGGACCAAT GGTGTCCAAGGGCGAAGAGGACAACATGGCCATCATCAAAG AATTCATGCGGTTCAAGGTGCACATGGAAGGCAGCGTGAAC GGCCACGAGTTCGAGATTGAAGGCGAAGGCGAGGGCAGAC CTTACGAGGGAACACAGACCGCCAAGCTGAAAGTGACAAAA GGCGGCCCACTGCCTTTCGCCTGGGACATCCTGTCTCCACA GTTTATGTACGGCTCCAAGGCCTATGTGAAGCACCCCGCCG ACATTCCCGACTACCTGAAGCTGAGCTTCCCCGAGGGCTTC AAGTGGGAGAGAGTGATGAACTTCGAGGACGGCGGCGTCG TGACCGTGACTCAAGATAGCTCTCTGCAGGACGGCGAGTTC ATCTACAAAGTGAAGCTGCGGGGCACCAACTTTCCCTCTGA	1-63 = CD8A signal sequence 64-399 = 15F3 LC 400-444 = Linker 445-795 = 15F3 HC 796-930 = CD8a hinge 931-1011 = CD28 transmembrane domain 1012-1134 = intracellular signal domain 1135-1470 = CD3-zeta intracellular chain 1471-2253 = T2A-mCherry	204

Table 13 Nucleotide sequences encoding CARs			
Construct	**Nucleic acid sequence**	**Nucleic Acid Description**	**SEQ ID NO:**
	TGGCCCCGTGATGCAGAAAAAGACCATGGGCTGGGAAGCC AGCAGCGAGAGAATGTACCCTGAAGATGGCGCCCTGAAAG GCGAGATCAAGCAGCGGCTGAAACTGAAGGATGGCGGCCA CTACGACGCCGAAGTGAAAACCACCTACAAGGCCAAGAAAC CCGTGCAGCTGCCAGGCGCCTACAACGTGAACATCAAGCTG GACATTACCAGCCACAACGAGGACTACACCATCGTGGAACA GTACGAGAGAGCCGAAGGCAGGCACTCTACAGGCGGAATG GACGAGCTGTATAAGTAG		
3 (5B8-CART)	ATGGCTCTGCCCGTTACAGCTCTGCTGCTGCCTCTGGCTCT GCTTCTGCATGCCGCCAGACCTAATATCATGATGACACAGA GCCCCAGCAGCCTGGTGGTGTCTGCTGGCGAGAAAGTGAC CATGAGCTGCAAGAGCAGCCACAGCGTGCTGTACTCCAGCA ACCAGAAGAACTACCTGGCCTGGTATCAGCAGAAGCCCGGC CAGTCTCCTAAGCTGCTGATCTACTGGGCCAGCACCAAGAA TAGCGGCGTGCCCGATAGATTCACAGGCAGCGGCTCTGGC ACCGACTTCACCCTGACAATCAGCTCTGTGCAGGCCGAGGA TCTGGCCGTGTACTACTGTCACCAGTACCTGAGCAGCTACA CCTTCGGCGGAGGCACCAAGCTGGAAATCAAAGGCGGAGG CGGATCTGGCGGCGGAGGTAGCGGTGGCGGAGGATCTCAA GTTCAGCTGCAGCAGTCCGATGCCGAGCTGGTTAAGCCTGG CGCCTCTGTGAAGATCAGCTGCAAGGCCAGCGGCTACACAT TCACCGATCACGCCATCCACTGGGTCAAGCAGAAACCAGAG CAGGGCCTCGAGTGGATCGGCTACTTTTCTCCCGGCAACGG CGACATCAAGTACAACGAGAAGTTCAAGGGCAAAGCCACAC TGACCGCCGACAGAAGCAGCTCCACAGCCAACATGCACCTG AACAGCCTGACCAGCGAGGACAGCGCCGTGTATTTCTGCAA GCGGAGCATGGCCAACTACTTCGACTATTGGGGCCAGGGC ACAACCCTGACCGTGTCCTCTACAACAACCCCTGCTCCTCG GCCACCTACACCAGCTCCTACAATTGCCTCTCAACCTCTGTC TCTGCGGCCCGAGGCTTGTAGACCTGCTGCTGGCGGAGCT GTGCACACAAGAGGACTGGATTTCGCCTGCGACTTCTGGGT GCTCGTGGTTGTTGGCGGAGTGCTGGCCTGTTACTCTCTGC TGGTCACCGTGGCCTTCATCATCTTTTGGGTCCGAAGCAAG AGAAGCCGGCTGCTGCACAGCGACTACATGAACATGACCCC TAGACGGCCCGGACCTACCAGAAAGCACTACCAGCCTTACG CTCCTCCTAGAGACTTCGCCGCCTACCGGTCCAGAGTGAAG TTCAGCAGATCCGCTGATGCCCCTGCCTATCAGCAGGGCCA GAACCAGCTGTACAATGAGCTGAACCTGGGGCGCAGAGAA GAGTACGACGTGCTGGACAAGAGAAGAGGCAGGGACCCTG AGATGGGCGGCAAGCCCAGAAGAAAGAACCCTCAAGAGGG CCTGTATAACGAGCTGCAGAAAGACAAGATGGCCGAGGCCT ACAGCGAGATCGGAATGAAGGGCGAACGCAGAAGAGGAAA GGGCCACGACGGACTGTATCAGGGCCTGAGCACAGCCACC AAGGACACCTATGATGCCCTGCACATGCAGGCCCTGCCTCC AAGAAGAAAAAGAGGCTCCGGCGAAGGCAGAGGCTCCCTG CTTACATGCGGAGATGTGGAAGAGAACCCCGGACCAATGGT GTCCAAGGGCGAAGAGGACAACATGGCCATCATCAAAGAAT TCATGCGGTTCAAGGTGCACATGGAAGGCAGCGTGAACGG CCACGAGTTCGAGATTGAAGGCGAAGGCGAGGGCAGACCT TACGAGGGAACACAGACCGCCAAGCTGAAAGTGACAAAAGG CGGCCCACTGCCTTTCGCCTGGGACATCCTGTCTCCACAGT TTATGTACGGCAGCAAGGCCTACGTGAAGCACCCCGCCGAT ATTCCCGACTACCTGAAGCTGAGCTTCCCCGAGGGCTTCAA	1-63 = CD8A signal sequence 64-399 = 5B8 LC 400-444 = Linker 445-795 = 5B8 HC 796-930 = CD8a hinge 931-1011 = CD28 transmembrane domain 1012-1134 = intracellular signal domain 1135-1470 = CD3-zeta intracellular chain 1471-2253 = T2A-mCherry	205

Table 13 Nucleotide sequences encoding CARs			
Construct	**Nucleic acid sequence**	**Nucleic Acid Description**	**SEQ ID NO:**
	GTGGGAGAGAGTGATGAACTTCGAGGACGGCGGCGTCGTG ACCGTGACTCAAGATAGCTCTCTGCAGGACGGCGAGTTCAT CTACAAAGTGAAGCTGCGGGGCACCAACTTTCCCTCTGATG GCCCCGTGATGCAGAAAAAGACCATGGGCTGGGAAGCCAG CAGCGAGAGAATGTACCCTGAAGATGGCGCCCTGAAAGGC GAGATCAAGCAGCGGCTGAAACTGAAGGATGGCGGCCACT ACGACGCCGAAGTGAAAACCACCTACAAGGCCAAGAAACCC GTGCAGCTGCCAGGCGCCTACAACGTGAACATCAAGCTGGA CATTACCAGCCACAACGAGGACTACACCATCGTGGAACAGT ACGAGAGAGCCGAAGGCAGGCACTCTACAGGCGGAATGGA CGAGCTGTATAAGTAG		

Table 14 CAR Amino Acid Sequences			
Construct	**Amino acid sequence**	**Amino Acid Description**	**SEQ ID NO:**
1 (2D5-CART)	MALPVTALLLPLALLLHAARPNIMLTQSPSSLAVSAGE KVTMSCKSSQSVLYSSDQKNYLAWYQQKPGQSPKL LIYWASTRESGVPDRFTGSGSGTDFTLTISNVQAEDL AVYYCHQYLSSYTFGGGTKLEIKGGGGSGGGGSGG GGSQVQLQQSDAELVKPGASVRISCKAYGYTFTDHA IHWVKQKPEQGLEWLGYISPGNDDIQYNAKFKGKAT LTADKSSSTAYMQLNSLTSDDSAVYFCKRSMANSFD YWGQGTTLTVSSTTTPAPRPPTPAPTIASQPLSLRPE ACRPAAGGAVHTRGLDFACDFWVLVVVGGVLACYS LLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRK HYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQ LYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP QEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL YQGLSTATKDTYDALHMQALPPRRKRGSGEGRGSL LTCGDVEENPGPMVSKGEEDNMAIIKEFMRFKVHME GSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLP FAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFK WERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGT NFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIK QRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIK LDITSHNEDYTIVEQYERAEGRHSTGGMDELYK	1-21=CD8a signal sequence 22-133= 2D5 LC 134-148 = Linker 149-265 = HC 2D5 266 -310 = CD8a hinge 311-337 CD28 transmembrane 338-378 CD28 intracellular domain 379-490 CD3z intracellular domain 491-750 = T2A mcherry	206
2 (15F3-CART)	MALPVTALLLPLALLLHAARPNIMLTQSPSSLAVSAGE KVTMSCKSSQSVLYSSDQKNYLAWYQQKPGQSPKL LIYWASTRESGVPDRFTGSGSGTDFTLTISNVRAEDL AVYYCHQYLSSYTFGGGTKLEIKGGGGSGGGGSGG GGSQVQLQQSDAELVEPGASVKISCKAYGYTFTDHA IHWVKQKPEQGLEWLGYISPGNDDIQYNAKFKGRAT LTADKSSSTAYMQLNSLTSDDSAVYFCKRSMANSFD FWGQGTTLTVSSTTTPAPRPPTPAPTIASQPLSLRPE ACRPAAGGAVHTRGLDFACDFWVLVVVGGVLACYS LLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRK HYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQ LYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP QEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL	1-21=CD8a signal sequence 22-133= 15F3 LC 134-148 = Linker 149-265 = HC 15F3 266 -310 = CD8a hinge 311-337 CD28 transmembrane	207

Table 14 CAR Amino Acid Sequences			
Construct	**Amino acid sequence**	**Amino Acid Description**	**SEQ ID NO:**
	YQGLSTATKDTYDALHMQALPPRRKRGSGEGRGSL LTCGDVEENPGPMVSKGEEDNMAIIKEFMRFKVHME GSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLP FAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFK WERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGT NFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIK QRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIK LDITSHNEDYTIVEQYERAEGRHSTGGMDELYK*	338-378 CD28 intracellular domain 379-490 CD3z intracellular domain 491-750 = T2A mcherry	
3 (5B8-CART)	MALPVTALLLPLALLLHAARPNIMMTQSPSSLVVSAG EKVTMSCKSSHSVLYSSNQKNYLAWYQQKPGQSPK LLIYWASTKNSGVPDRFTGSGSGTDFTLTISSVQAED LAVYYCHQYLSSYTFGGGTKLEIKGGGGSGGGGSG GGGSQVQLQQSDAELVKPGASVKISCKASGYTFTDH AIHWVKQKPEQGLEWIGYFSPGNGDIKYNEKFKGKA TLTADRSSSTANMHLNSLTSEDSAVYFCKRSMANYF DYWGQGTTLTVSSTTTPAPRPPTPAPTIASQPLSLRP EACRPAAGGAVHTRGLDFACDFWVLVVVGGVLACY SLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTR KHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQN QLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDG LYQGLSTATKDTYDALHMQALPPRRKRGSGEGRGS LLTCGDVEENPGPMVSKGEEDNMAIIKEFMRFKVHM EGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPL PFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGF KWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGT NFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIK QRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIK LDITSHNEDYTIVEQYERAEGRHSTGGMDELYK*	1-21=CD8a signal sequence 22-133= 5B8 LC 134-148 = Linker 149-265 = HC 5B8 266 -310 = CD8a hinge 311-337 CD28 transmembrane 338-378 CD28 intracellular domain 379-490 CD3z intracellular domain 491-750 = T2A mcherry	208

6.5.2.2 Transduction and expansion

[0425] Lentivirus was produced in HEK293T cells transfected with lipofectamine (Thermofisher) overnight following standard protocols. The lentiviral supernatant was harvested after 48-72 hours. Healthy donor PBMCs were isolated using Lymphoprep density centrifugation followed by plastic adherence to get rid of adherent cells. The non-adherent PBMCs were cultured in RPMI-1640 Dutch modification with 10% FBS, 50μM 2-mercaptoethanol, and 20ng/ml rIL-2 and were activated using human T-activator CD3/CD28 Dynabeads. Following activation, the T cells were transduced twice with viral supernatant for 24 hours with a multiplicity of infection (MOI) of at least 5:1. Additionally, 1ul per 1.5x10^7 cells of transplus virus transduction enhancer (Alstembio) was added to enhance infection efficiency. Transduced CAR T cells were expanded in culture medium at densities between $0.5x10^6$ cells/mL and $1x10^6$ cells/mL until used for studies.

6.5.2.3 Cytotoxicity assay

[0426] HaCaT WT and COSMC KO cells were seeded at a density of 20,000 cells per well in 96-well E-plates and allowed to adhere overnight. One day later, CAR T cells were added at effector-target cell ratios of 5:1 or 3:1 and were incubated for 2-3 days. Cytotoxicity of target cells co-cultured with CAR T cells was evaluated by electric conductivity using iCelligence plate reader. For 100% cell death controls, 1% tween in PBS or 1uM staurosporine was used. To assess IFN-γ production by the CAR T cells, supernatant was harvested from the co-cultures, and ELISA was performed according to manufacturer's instructions (Abcam).

6.5.2.4 In vivo tumor assay

[0427] A cell line-based xenograft solid tumor model was established by subcutaneous flank injection of T3M4 COSMC-KO cells. When tumor volume reached 200 mm^3, mice were randomized and treated intravenously with 2nd generation 2D5-CART (10^7 cells per injection) on days 1 and 5. The effect of each treatment on the growth of tumors was measured by volume (measured by caliper on days 7, 14, 21, 32, and 46) and by body weight. There were no clinical signs indicating adverse events in treated mice.

6.5.3. Results

[0428] CAR constructs were expressed in human T cells. Surface expression of CART constructs was confirmed by flow cytometry using either Alexa488-ProteinL or Biotin-MUC4 glycopeptide antigen. 2D5-CART and 5B8-CART specifically killed Tn+ COSMC-KO T3M4, but not Tn- T3M4 at either 5 to 1 or 10 to 1 ratios of T cells to T3M4s (FIGS. 5A-5B, Table 15). The time to kill 50% Tn+ COSMC-KO T3M4 was 4.25 hrs for 2D5-CART at the 5:1 ratio and 1.5 hrs for the 10:1 ratio. The time to kill 50% Tn+ COSMC-KO T3M4 was 5 hrs for 5B8-CART at the 10:1 ratio. The data indicate that 2D5-CART and 5B8-CART selectively target cells expressing MUC4-Tn.

Table 15
KT50 (Time to kill 50% of cells)

Target Cell	T Cell Ratio	2D5 (KT50)	5B8 (KT50)	T cells (KT50)
T3M4	5:1	4.25 hrs	N/A	N/A
COSMC-KO	10:1	1.5 hrs	5 hrs	N/A
T cells incubated for 7 hrs with T3M4 cells				

[0429] A cell line-based xenograft solid tumor model was established by subcutaneous flank injection of T3M4 COSMC-KO cells. When tumor volume reached 200 mm^3, mice were randomized and treated intravenously with 2nd generation 2D5-CART (10^7 cells per injection) on days 1 and 5. The effect of each treatment on the growth of tumors was measured by volume (measured by caliper on days 7, 14, 21, 32, and 46). We observed a 67% decrease in tumor growth in the treatment condition (2D5-CART) vs control.

6.6 Example 6: Tn-MUC4 based CrossMabs

6.6.1. Overview

[0430] CrossMabs having VH and VL domains of 2D5 were designed.

6.6.2. Materials and Methods

6.6.2.1 Vector Design

[0431] Nucleotide sequences encoding the CrossMAbs are shown in Table 16. Amino acid sequences of the CrossMAbs are shown in Table 17. Briefly, CrossMabs were created using a 2×1 format (2 2D5 FABs and 1 CD3 FAB) by co-expressing 4 constructs. The first construct (Long HC-2D5/CD3) is composed of the variable heavy chain sequence of 2D5 attached to the human CH1 domain, which is attached to a linker and a CD3 FAB with human CL-kappa domain followed by a linker, hCH2, hCH3, and CHS domains. The second construct (Short HC-2D5) is composed of the variable heavy chain sequence of 2D5 attached to the human CH1 domain, which is attached to a hinge followed by hCH2, hCH3, CHS domains. The hCH2 domains contain the LALA-PG mutations (L234A, L235A, P329G), while the hCH3 have the appropriate CrossMAb mutations (Long HC-2D5/CD3 the “knob” mutations S354C, T366W, while Short HC-2D5 has the “hole” mutations Y349C/T366S/L368A/Y407V). The third construct (Cross VL CD3) is composed of the variable light chain sequence of a commercial anti-CD3 antibody followed by a short linker and human CH1 domain and a hinge. The fourth construct (VL-2D5) is composed of the variable light chain sequence of 2D5 attached to the human CL-kappa domain.

Table 16
Nucleotide sequences encoding CrossMabs

Construct	Nucleic acid sequence	Nucleic Acid Description	SEQ ID NO:
1 (Long HC-2D5/CD3)	CAGGTGCAGCTGCAGCAGAGCGACGCCGAGCTGGTGA AGCCCGGCGCCAGCGTGAGGATCAGCTGCAAGGCCTA CGGCTACACCTTCACCGACCACGCCATCCACTGGGTGA AGCAGAAGCCCGAGCAGGGCCTGGAGTGGCTGGGCTA CATCAGCCCCGGCAACGACGACATCCAGTACAACGCCA AGTTCAAGGGCAAGGCCACCCTGACCGCCGACAAGAG CAGCAGCACCGCCTACATGCAGCTGAACAGCCTGACCA GCGACGACAGCGCCGTGTACTTCTGCAAGAGGAGCATG GCCAACAGCTTCGACTACTGGGGCCAGGGCACCACCCT GACCGTGAGCAGCGCCAGCACCAAGGGCCCCAGCGTG TTCCCCCTGGCCCCCAGCAGCAAGAGCACCAGCGGCG GCACCGCCGCCCTGGGCTGCCTGGTGAAGGACTACTTC CCCGAGCCCGTGACCGTGAGCTGGAACAGCGGCGCCC TGACCAGCGGCGTGCACACCTTCCCCGCCGTGCTGCA GAGCAGCGGCCTGTACAGCCTGAGCAGCGTGGTGACC GTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATCTG CAACGTGAACCACAAGCCCAGCAACACCAAGGTGGACA AGAAGGTGGAGCCCAAGAGCTGCGACGGCGGCGGCGG CAGCGGCGGCGGCGGCAGCGAGGTGCAGCTGCTGGA GAGCGGCGGCGGCCTGGTGCAGCCCGGCGGCAGCCT GAGGCTGAGCTGCGCCGCCAGCGGCTTCACCTTCAGC	1-351 = 2D5 HC 352-645 = hCH1 646-693 = LINKER 694-1068 = CD3 HC 1069-1389 = CL 1390-1419 = Linker 1420-2070 = CH2, CH3, CHS	209

Table 16
Nucleotide sequences encoding CrossMabs

Construct	Nucleic acid sequence	Nucleic Acid Description	SEQ ID NO:
	ACCTACGCCATGAACTGGGTGAGGCAGGCCCCCGGCA AGGGCCTGGAGTGGGTGAGCAGGATCAGGAGCAAGTA CAACAACTACGCCACCTACTACGCCGACAGCGTGAAGG GCAGGTTCACCATCAGCAGGGACGACAGCAAGAACACC CTGTACCTGCAGATGAACAGCCTGAGGGCCGAGGACAC CGCCGTGTACTACTGCGTGAGGCACGGCAACTTCGGCA ACAGCTACGTGAGCTGGTTCGCCTACTGGGGCCAGGG CACCCTGGTGACCGTGAGCAGCGCCAGCGTGGCCGCC CCCAGCGTGTTCATCTTCCCCCCCAGCGACGAGCAGCT GAAGAGCGGCACCGCCAGCGTGGTGTGCCTGCTGAAC AACTTCTACCCCAGGGAGGCCAAGGTGCAGTGGAAGGT GGACAACGCCCTGCAGAGCGGCAACAGCCAGGAGAGC GTGACCGAGCAGGACAGCAAGGACAGCACCTACAGCCT GAGCAGCACCCTGACCCTGAGCAAGGCCGACTACGAG AAGCACAAGGTGTACGCCTGCGAGGTGACCCACCAGG GCCTGAGCAGCCCCGTGACCAAGAGCTTCAACAGGGG CGAGTGCGACAAGACCCACACCTGCCCCCCCTGCCCC GCCCCCGAGGCCGCCGGCGGCCCCAGCGTGTTCCTGT TCCCCCCCAAGCCCAAGGACACCCTGATGATCAGCAGG ACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCC ACGAGGACCCCGAGGTGAAGTTCAACTGGTACGTGGAC GGCGTGGAGGTGCACAACGCCAAGACCAAGCCCAGGG AGGAGCAGTACAACAGCACCTACAGGGTGGTGAGCGTG CTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAGG AGTACAAGTGCAAGGTGAGCAACAAGGCCCTGGGCGC CCCCATCGAGAAGACCATCAGCAAGGCCAAGGGCCAG CCCAGGGAGCCCCAGGTGTACACCCTGCCCCCCTGCA GGGACGAGCTGACCAAGAACCAGGTGAGCCTGTGGTG CCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGG AGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAA GACCACCCCCCCCGTGCTGGACAGCGACGGCAGCTTC TTCCTGTACAGCAAGCTGACCGTGGACAAGAGCAGGTG GCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCAC GAGGCCCTGCACAACCACTACACCCAGAAGAGCCTGAG CCTGAGCCCCGGCAAG		
2 (Short HC-2D5)	CAGGTGCAGCTGCAGCAGAGCGACGCCGAGCTGGTGA AGCCCGGCGCCAGCGTGAGGATCAGCTGCAAGGCCTA CGGCTACACCTTCACCGACCACGCCATCCACTGGGTGA AGCAGAAGCCCGAGCAGGGCCTGGAGTGGCTGGGCTA CATCAGCCCCGGCAACGACGACATCCAGTACAACGCCA AGTTCAAGGGCAAGGCCACCCTGACCGCCGACAAGAG CAGCAGCACCGCCTACATGCAGCTGAACAGCCTGACCA GCGACGACAGCGCCGTGTACTTCTGCAAGAGGAGCATG GCCAACAGCTTCGACTACTGGGGCCAGGGCACCACCCT GACCGTGAGCAGCGCCAGCACCAAGGGCCCCAGCGTG TTCCCCCTGGCCCCCAGCAGCAAGAGCACCAGCGGCG GCACCGCCGCCCTGGGCTGCCTGGTGAAGGACTACTTC CCCGAGCCCGTGACCGTGAGCTGGAACAGCGGCGCCC TGACCAGCGGCGTGCACACCTTCCCCGCCGTGCTGCA GAGCAGCGGCCTGTACAGCCTGAGCAGCGTGGTGACC GTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATCTG CAACGTGAACCACAAGCCCAGCAACACCAAGGTGGACA	1-351 = 2D5 HC 352-645 = hCH1 646-690 = hinge 691-1341 = CH2, CH3, CHS	210

Table 16
Nucleotide sequences encoding CrossMabs

Construct	Nucleic acid sequence	Nucleic Acid Description	SEQ ID NO:
	AGAAGGTGGAGCCCAAGAGCTGCGACAAGACCCACAC CTGCCCCCCCTGCCCCGCCCCCGAGGCCGCCGGCGGC CCCAGCGTGTTCCTGTTCCCCCCCAAGCCCAAGGACAC CCTGATGATCAGCAGGACCCCCGAGGTGACCTGCGTG GTGGTGGACGTGAGCCACGAGGACCCCGAGGTGAAGT TCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGC CAAGACCAAGCCCAGGGAGGAGCAGTACAACAGCACCT ACAGGGTGGTGAGCGTGCTGACCGTGCTGCACCAGGA CTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTGAGCA ACAAGGCCCTGGGCGCCCCCATCGAGAAGACCATCAG CAAGGCCAAGGGCCAGCCCAGGGAGCCCCAGGTGTGC ACCCTGCCCCCCAGCAGGGACGAGCTGACCAAGAACC AGGTGAGCCTGAGCTGCGCCGTGAAGGGCTTCTACCCC AGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGC CCGAGAACAACTACAAGACCACCCCCCCCGTGCTGGAC AGCGACGGCAGCTTCTTCCTGGTGAGCAAGCTGACCGT GGACAAGAGCAGGTGGCAGCAGGGCAACGTGTTCAGC TGCAGCGTGATGCACGAGGCCCTGCACAACCACTACAC CCAGAAGAGCCTGAGCCTGAGCCCCGGCAAG		
3 (Cross VL CD3)	CAGGCCGTGGTGACCCAGGAGCCCAGCCTGACCGTGA GCCCCGGCGGCACCGTGACCCTGACCTGCGGCAGCAG CACCGGCGCCGTGACCACCAGCAACTACGCCAACTGG GTGCAGGAGAAGCCCGGCCAGGCCTTCAGGGGCCTGA TCGGCGGCACCAACAAGAGGGCCCCCGGCACCCCCGC CAGGTTCAGCGGCAGCCTGCTGGGCGGCAAGGCCGCC CTGACCCTGAGCGGCGCCCAGCCCGAGGACGAGGCCG AGTACTACTGCGCCCTGTGGTACAGCAACCTGTGGGTG TTCGGCGGCGGCACCAAGCTGACCGTGCTGAGCAGCG CCAGCACCAAGGGCCCCAGCGTGTTCCCCCTGGCCCC CAGCAGCAAGAGCACCAGCGGCGGCACCGCCGCCCTG GGCTGCCTGGTGAAGGACTACTTCCCCGAGCCCGTGAC CGTGAGCTGGAACAGCGGCGCCCTGACCAGCGGCGTG CACACCTTCCCCGCCGTGCTGCAGAGCAGCGGCCTGTA CAGCCTGAGCAGCGTGGTGACCGTGCCCAGCAGCAGC CTGGGCACCCAGACCTACATCTGCAACGTGAACCACAA GCCCAGCAACACCAAGGTGGACAAGAAGGTGGAGCCC AAGAGCTGC	1-327 = CD3 light chain 328-333 = Linker 334-627 = hCH1 628-642 = Hinge	211
4 (VL-2D5)	AACATCATGCTGACCCAGAGCCCCAGCAGCCTGGCCGT GAGCGCCGGCGAGAAGGTGACCATGAGCTGCAAGAGC AGCCAGAGCGTGCTGTACAGCAGCGACCAGAAGAACTA CCTGGCCTGGTACCAGCAGAAGCCCGGCCAGAGCCCC AAGCTGCTGATCTACTGGGCCAGCACCAGGGAGAGCG GCGTGCCCGACAGGTTCACCGGCAGCGGCAGCGGCAC CGACTTCACCCTGACCATCAGCAACGTGCAGGCCGAGG ACCTGGCCGTGTACTACTGCCACCAGTACCTGAGCAGC TACACCTTCGGCGGCGGCACCAAGCTGGAGATCAAGAG GACCGTGGCCGCCCCCAGCGTGTTCATCTTCCCCCCCA GCGACGAGCAGCTGAAGAGCGGCACCGCCAGCGTGGT GTGCCTGCTGAACAACTTCTACCCCAGGGAGGCCAAGG TGCAGTGGAAGGTGGACAACGCCCTGCAGAGCGGCAA CAGCCAGGAGAGCGTGACCGAGCAGGACAGCAAGGAC AGCACCTACAGCCTGAGCAGCACCCTGACCCTGAGCAA	1-336 = 2D5 LC 337-657 = hCL-kappa	212

Table 16 Nucleotide sequences encoding CrossMabs			
Construct	**Nucleic acid sequence**	**Nucleic Acid Description**	**SEQ ID NO:**
	GGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAG GTGACCCACCAGGGCCTGAGCAGCCCCGTGACCAAGA GCTTCAACAGGGGCGAGTGC		

Table 17 CrossMab Amino Acid Sequences			
Construct	**Amino acid sequence**	**Amino Acid Description**	**SEQ ID NO:**
1 (Long HC-2D5/CD3)	QVQLQQSDAELVKPGASVRISCKAYGYTFTDHAIHWVKQ KPEQGLEWLGYISPGNDDIQYNAKFKGKATLTADKSSST AYMQLNSLTSDDSAVYFCKRSMANSFDYWGQGTTLTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCDGGGGSGGGGSE VQLLESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQ APGKGLEWVSRIRSKYNNYATYYADSVKGRFTISRDDSK NTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYW GQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTIS KAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	1-117 = 2D5 HC 118-215 = hCH1 216-231 = LINKER 232-356 = CD3 HC 357-463 = CL 464-473 = Linker 474-690 = CH2, CH3, CHS	213
2 (Short HC-2D5)	QVQLQQSDAELVKPGASVRISCKAYGYTFTDHAIHWVKQ KPEQGLEWLGYISPGNDDIQYNAKFKGKATLTADKSSST AYMQLNSLTSDDSAVYFCKRSMANSFDYWGQGTTLTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK	1-117 = 2D5 HC 118-215 = hCH1 216-230 = hinge 231-447 = CH2, CH3, CHS	214
3 (Cross VL CD3)	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWV QEKPGQAFRGLIGGTNKRAPGTPARFSGSLLGGKAALTL SGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC NVNHKPSNTKVDKKVEPKSC	1-109 = CD3 light chain 110-111 = Linker 112-209 = hCH1 210-214 = Hinge	215

Table 17 CrossMab Amino Acid Sequences			
Construct	**Amino acid sequence**	**Amino Acid Description**	**SEQ ID NO:**
4 (VL-2D5	NIMLTQSPSSLAVSAGEKVTMSCKSSQSVLYSSDQKNYL AWYQQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDF TLTISNVQAEDLAVYYCHQYLSSYTFGGGTKLEIKRTVAA PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC	1-112 = 2D5 LC 113-219 = hCL-kappa	216

6.6.2.2 Generation of CrossMabs

[0432] CrossMabs were produced by transient transfection of EXPI-CHO cells. IL2 signal sequences were added to each construct. CrossMabs were harvested from the supernatant after 6 days of expression. CrossMabs were purified by conventional methods using ProteinA agarose beads.

6.6.2.3 Cytotoxicity assay

[0433] MCF7 WT and HCT116, and HaCaT WT and COSMC KO cells were seeded at a density of 20,000 cells per well in 96-well E-plates and allowed to adhere overnight. One day later, CD4+ Tcells or PBMCs were added at effector-target cell ratios of 5:1 or 10:1 and incubated for 2-3 days. Cytotoxicity of target cells was evaluated by electric conductivity using iCelligence plate reader. For 100% cell death controls, 1% tween in PBS or 1uM staurosporine was used.

6.6.2.4 *In vivo* tumor assay

[0434] A patient-derived xenograft solid tumor model (Champions (CTG-2823) was established by subcutaneous flank injection. Tumor volume at TCB injection was 200 mm^3. TCB was delivered by IV injection. PBMCs were injected at day 0 and at day 17. TCB was dosed on day 0, 1, 2, 3,4, 20, and 22. Tumor volumes were measured by calliper twice weekly (days 2, 5, 10, 12, 18, 20, 28, and 30). There were no clinical signs indicating adverse events in treated mice.

6.6.3. Results

[0435] 2D5-CrossMAb can actively kill cells in vitro with high Muc4-Tn expression (COSMC-KO HaCaTs and HCT-116s) at sub-nM concentrations (100-300pM; FIGs. 8 and 9A-9B). 2D5-TCB can also kill cells in vitro with lower MUC4-Tn expression (FIGS. 9A-9B and Table 18). The data indicates that 2D5-CrossMab selectively target cells expressing MUC4-Tn.

Table 18 Cytotoxicity EC50s for CrossMab TCB-2D5		
Cell Line	**EC50**	**Receptor count**
HaCaT (COSMC-KO)	100pM	Tn high (~11,000 receptors per cell)
MCF7	1 nM	Tn low (~1,000 receptors per cell)
HCT116 (COSMC-KO)	300pM	Tn high (~12,000 receptors per cell)

6.7 Example 7: Humanized Antibodies and Antigen-Binding Fragments

6.7.1. Overview

[0436] The murine antibody 2D5 was humanized using standard CDR-grafting technology. For the heavy chain, four templates, IGHV-1*01, IGHV1-69*06, IGHV5-78*01, and IGHV7-4-1*02 were employed in order to generate CDR-grafted versions containing successively aggressive levels of humanization, *i.e.*, identity to the human acceptor germline. Similarly for the light chain, three templates, IGKV4-1*01, IGKV2-40*01, and IGKV3-20*01, were employed to generate CDR-grafted versions containing successively aggressive levels of humanization.

[0437] Expression constructs were designed for expression in Expi-293 cells. IL2 secretion signals were added to both heavy and light chain constructs. Antibodies were purified with ProteinA beads using conventional methods. Humanized candidates were evaluated for their ability to binding to the non-glycosylated and Tn-glycosylate MUC4 peptides using ELISA. The humanized candidates were also compared to the parental antibody by: size exclusion chromatography; flow cytometry to detect binding affinity to target-positive cells; and Octet to determine binding affinity to the peptide antigen.

6.7.2. Materials and Methods

6.7.2.1 Vector Design

[0438] For each germline, three humanize versions were created: a conservative “A” sequence, a less conservative “B” sequence, and an “aggressive” “C” sequence (*see* Tables 4A-4G). Consensus sequences of all three of the A, B, and C sequences for each germline were also created that reflect the most common amino acid residue at each position.

[0439] These humanized templates are assembled and assayed for optimal biophysical and functional properties in two phases. In the first phase, up to 12 pairs of the conservative “A” designs are constructed and assayed for binding to the MUC4 glycopeptide. After selection of the most optimal combination based upon the “A” designs, the conservative “A” designs are iteratively replaced with the less conservative “B” designs and ultimately with the least conservative “C” designs.

6.7.2.2 ELISA

[0440] 96-well Corning high bind ELISA microplates plates were coated with MUC4 peptides titrated in 0.2 M bicarbonate buffer, pH 9.4 overnight at 4 °C in concentrations ranging from 0.08 µg/ml to 10 µg/ml. BSA was used as a control/measure of background. The plates were then blocked with SuperBlock™ (Thermo Fisher) for 1 hr at room temperature. After plate washing, the humanized variants of 2D5 were incubated on the ELISA plate for 1 hour. All tested variants were expressed and purified using conventional methods. Briefly, Expi-293 cells were transiently transfected with heavy and light chain constructs, antibodies were secreted

into supernatant and purified using Protein A agarose beads. The plates were then washed, and then incubated with secondary antibody (1/3000 Goat Anti-mouse IgG (H+L) HRP (Abcam 62-6520)) for 1 hour. The plate was then washed and color was developed with 1-Step™ Ultra TMB (Thermo Fisher) for 2 minutes. Color development was then stopped with 2 N Sulfuric Acid. Absorbance at 450 nm was then measured.

6.7.2.3 Bio-Layer Interferometry (Octet)

[0441] Antibody affinity of the humanized candidates of 2D5 can be assessed against specific antigens using BLI. In a BLI assay, the antigen can be immobilized onto a biosensor (e.g., the glyco-MUC4 peptide CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) or a negative control analyte such as unglycosylated MUC4 peptide (CTIPSTAMHTRSTAAPIPILP (SEQ ID NO:155)).and presented to one antibody candidate for affinity measurements or two competing antibodies in tandem (or consecutive steps) for epitope binning. The binding to non-overlapping epitopes occurs if saturation with the first antibody does not block the binding of the second antibody. The affinity is determined by fitting the binding curve to a specific model: a 1:1 monovalent model or a 2:1 bivalent model. The error (>95% confidence) is calculated by how close the generated curve matches the model.

6.7.2.4 Flow Cytometry

[0442] Adherent cells were dissociated with TrypLE select (Gibco) and washed from flask surface with cell culture media (RPMI w/ L-glutamine, 1% PenStrep, & 10% FBS). Cells were washed several times by centrifugation at 300*g for 5 min at 4 °C followed by resuspension in PBS with 1% BSA (PBS/1%BSA). Cells were resuspended between $5x10^5$ cells/ml to $2x10^6$ cell/ml and then distributed into a 96 well U-bottom plate. Diluted commercial antibody (0.25-2 ug/ml), or purified humanized 2D5 candidates were added to T3M4 COSMC-KO cells and incubated for 1 hr on ice. Following several washes with PBS/1% BSA, cells were incubated for 30 min on ice with a 1:1600 dilution of AlexaFluor647 conjugated $F(ab)_2$ goat anti-human IgG Fcγ (JacksonImmunoResearch). Cells were washed again with PBS/1% BSA and then fixed in 1% formaldehyde in PBS/1% BSA. Cells were analysed on either a 2 or 4 laser Attune NXT flow cytometer. Data was processed in FlowJo Software.

6.7.2.5 Size Exclusion Chromatography

[0443] The humanized candidates for 2D5 were tested for the presence of soluble protein aggregates using size exclusion chromatography (SEC). Briefly, purified antibodies were loaded on an HPLC silica TSK-GEL G3000SW column (TOSOH Biosciences, Montgomeryville, PA) and associated UV detector (166 Detector). The mobile phase composition was PBS and flow rate was 1.0 mL/min. Concentrations of protein species were determined by monitoring the absorbance of column eluate at 280 nm.

6.7.3. Results

[0444] To characterize the 2D5 humanized candidates, affinities were measured by flow cytometry on T3M4 COSMC-KO cells, and by Octet against Tn-glycosylated MUC4.

[0445] ELISA against non-glycosylated and Tn-glycosylated MUC4 was performed. It was found that in the context of ELISA, all candidates only reacted with Tn-glycosylated MUC4 and not with its non-glycosylated counterpart (data not shown).

[0446] The affinities of the humanized candidates against the MUC4 glycopeptides were determined by Octet. Table 19 summarizes dissociation constants (Kd) with +/- error at 95% confidence. The binding to T3M4-COSMC KO (or T3M4-M) cells was determined by flow cytometry for each candidate. The EC50s for all the candidates are listed in Table 19. Finally, size exclusion chromatography was used to quantify quality of the purified antibody by measuring the presence of soluble protein aggregates (*see* Table 19). Candidate 2D5-HV1-69-B/ KV4B was the least aggregated and had >99% soluble antibody and less than 0.5% soluble aggregates. Based the affinity measurements, protein purity, and highest possible humanization percentage, 2D5-HV1-69-C/KV4B, 2D5-HV1-69-B/ KV2B, 2D5-HV1-69-C/KV4A, 2D5-HV1-69-B/ KV4B, 2D5-HV1-69-B/ KV4A exhibits the most favorable profiles, although all candidates were functional.

Table 19

Molecules	Humanization %		OCTET	SEC	Flow
	% identity		Muc4-GP	Peak area%	T3M4-M (ng/ml)
	HC	LC	Affinity	Main peak	Affinity
2D5-HV1-69-C/KV2B	**86.7**	**85.3**	**5.3 nM**	**96.79**	**19.44**
2D5-HV1-69-C/KV4B	**86.7**	**95.1**	**6.8 nM**	**98.4**	**11.94**
2D5-HV1-69-B/ KV2B	**82.7**	**85.3**	**3.3nM**	**98.68**	**24.84**
2D5-HV1-69-C/KV4A	**86.7**	**94.1**	**2.3 nM**	**96.96**	**12.72**
2D5-HV1-69-A/ KV2A	78.6	82.4	4.1 nM	98.53	17.9
2D5-HV1-69-B/ KV4B	**82.7**	**95.1**	**1.6 nM**	**99.54**	**15.89**
2D5-HV1-69-B/ KV4A	**82.7**	**94.1**	**3.6 nM**	**98.54**	**30.95**
2D5-HV1-3B/ KV2B	**84.7**	**85.3**	**7.7 nM**	**97.97**	**16.28**
2D5-cAb Chimeric antibody (human Fc domain + murine variable domain)			3.2 nM	98.94	17.74

7. SPECIFIC EMBODIMENTS, CITATION OF REFERENCES

[0447] While various specific embodiments have been illustrated and described, it will be appreciated that various changes can be made without departing from the spirit and scope of the disclosure(s). The present disclosure is exemplified by the numbered embodiments set forth below.

1. An anti-glyco-MUC4 antibody or antigen binding fragment that specifically binds to a MUC4 peptide CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) that has been glycosylated with GalNAc on the serine and threonine residues shown with bold and underlined text ("the MUC4 glycopeptide").

2. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLQQSDAELVKPGASVRISCKAYGYTFTDHAIHWVKQKPEQGLEWLGYISPGNDDIQYNAK FKGKATLTADKSSSTAYMQLNSLTSDDSAVYFCKRSMANSFDYWGQGTTLTVSS (SEQ ID NO:1) and a light chain variable (VL) sequence of NIMLTQSPSSLAVSAGEKVTMSCKSSQSVLYSSDQKNYLAWYQQKPGQSPKLLIYWASTRES GVPDRFTGSGSGTDFTLTISNVQAEDLAVYYCHQYLSSYTFGGGTKLEIK (SEQ ID NO:2) for binding to the MUC4 glycopeptide.

3. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLQQSDAELVKPGASVKISCKASGYTFTDHAIHWVKQKPEQGLEWIGYFSPGNGDIKYNEK FKGKATLTADRSSSTANMHLNSLTSEDSAVYFCKRSMANYFDYWGQGTTLTVSS (SEQ ID NO:23) and a light chain variable (VL) sequence of NIMMTQSPSSLVVSAGEKVTMSCKSSHSVLYSSNQKNYLAWYQQKPGQSPKLLIYWASTKNS GVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCHQYLSSYTFGGGTKLEIK (SEQ ID NO:24) for binding to the MUC4 glycopeptide.

4. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLQQSDAELVEPGASVKISCKAYGYTFTDHAIHWVKQKPEQGLEWLGYISPGNDDIQYNAK FKGRATLTADKSSSTAYMQLNSLTSDDSAVYFCKRSMANSFDFWGQGTTLTVSS (SEQ ID NO:45) and a light chain variable (VL) sequence of NIMLTQSPSSLAVSAGEKVTMSCKSSQSVLYSSDQKNYLAWYQQKPGQSPKLLIYWASTRES GVPDRFTGSGSGTDFTLTISNVRAEDLAVYYCHQYLSSYTFGGGTKLEIK (SEQ ID NO:46) for binding to the MUC4 glycopeptide.

5. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody

or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRAVLSADKSVSTAYLQISSLKAEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:133) and a light chain variable (VL) sequence of DIVLTQSPDSLAVSLGERATINCKSSQSVLYSSDQKNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:145) for binding to the MUC4 glycopeptide.

6. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRAVLSADKSVSTAYLQISSLKAEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:133) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNLRNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:146) for binding to the MUC4 glycopeptide.

7. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRAVLSADKSVSTAYLQISSLKAEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:133) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNERNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:147) for binding to the MUC4 glycopeptide.

8. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRAVLSADKSVSTAYLQISSLKAEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:133) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCKSSQSVLYSSDQKNYLAWYQQKPGQAPRLLIYWASTRESG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:148) for binding to the MUC4 glycopeptide.

9. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA

KFKGRAVLSADKSVSTAYLQISSLKAEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:133) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRSSQSVLYSSDQKSYLAWYQQKPGQAPRLLIYWASTRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:149) for binding to the MUC4 glycopeptide.

10. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRAVLSADKSVSTAYLQISSLKAEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:133) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRASQSVSYSSDQKSYLAWYQQKPGQAPRLLIYWASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:150) for binding to the MUC4 glycopeptide.

11. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRAVLSADKSVSTAYLQISSLKAEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:133) and a light chain variable (VL) sequence of DIVLTQTPLSLPVTPGEPASISCKSSQSVLYSSDQKNYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:151) for binding to the MUC4 glycopeptide.

12. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRAVLSADKSVSTAYLQISSLKAEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:133) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSVLYSSDEKTYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:152) for binding to the MUC4 glycopeptide.

13. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRAVLSADKSVSTAYLQISSLKAEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:133) and a light chain variable (VL) sequence of

DIVMTQTPLSLPVTPGEPASISCRSSQSLLYSSDERTYLAWYLQKPGQSPQLLIYWASTRASGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:153) for binding to the MUC4 glycopeptide.

14. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNDDIQYNQ KFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:134) and a light chain variable (VL) sequence of DIVLTQSPDSLAVSLGERATINCKSSQSVLYSSDQKNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:145) for binding to the MUC4 glycopeptide.

15. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNDDIQYNQ KFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:134) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNLRNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:146) for binding to the MUC4 glycopeptide.

16. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNDDIQYNQ KFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:134) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNERNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:147) for binding to the MUC4 glycopeptide.

17. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNDDIQYNQ KFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:134) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCKSSQSVLYSSDQKNYLAWYQQKPGQAPRLLIYWASTRESG

IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:148) for binding to the MUC4 glycopeptide.

18. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNDDIQYNQ KFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:134) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRSSQSVLYSSDQKSYLAWYQQKPGQAPRLLIYWASTRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:149) for binding to the MUC4 glycopeptide.

19. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNDDIQYNQ KFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:134) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRASQSVSYSSDQKSYLAWYQQKPGQAPRLLIYWASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:150) for binding to the MUC4 glycopeptide.

20. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNDDIQYNQ KFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:134) and a light chain variable (VL) sequence of DIVLTQTPLSLPVTPGEPASISCKSSQSVLYSSDQKNYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:151) for binding to the MUC4 glycopeptide.

21. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNDDIQYNQ KFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:134) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSVLYSSDEKTYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:152) for binding to the MUC4 glycopeptide.

22. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNDDIQYNQ KFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:134) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSLLYSSDERTYLAWYLQKPGQSPQLLIYWASTRASGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:153) for binding to the MUC4 glycopeptide.

23. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of SEQ ID NO:135 and a light chain variable (VL) sequence of DIVLTQSPDSLAVSLGERATINCKSSQSVLYSSDQKNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:145) for binding to the MUC4 glycopeptide.

24. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNANITYAQ GFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:135) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNLRNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:146) for binding to the MUC4 glycopeptide.

25. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNANITYAQ GFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:135) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNERNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:147) for binding to the MUC4 glycopeptide.

26. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNANITYAQ

GFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:135) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCKSSQSVLYSSDQKNYLAWYQQKPGQAPRLLIYWASTRESG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:148) for binding to the MUC4 glycopeptide.

27. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNANITYAQ GFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:135) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRSSQSVLYSSDQKSYLAWYQQKPGQAPRLLIYWASTRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:149) for binding to the MUC4 glycopeptide.

28. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNANITYAQ GFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:135) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRASQSVSYSSDQKSYLAWYQQKPGQAPRLLIYWASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:150) for binding to the MUC4 glycopeptide.

29. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNANITYAQ GFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:135) and a light chain variable (VL) sequence of DIVLTQTPLSLPVTPGEPASISCKSSQSVLYSSDQKNYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:151) for binding to the MUC4 glycopeptide.

30. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNANITYAQ GFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:135) and a light chain variable (VL) sequence of

DIVMTQTPLSLPVTPGEPASISCRSSQSVLYSSDEKTYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:152) for binding to the MUC4 glycopeptide.

31. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNANITYAQ GFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:135) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSLLYSSDERTYLAWYLQKPGQSPQLLIYWASTRASGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:153) for binding to the MUC4 glycopeptide.

32. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIQYNAK FKGHATLSADKSSSTAYLQWSSLKASDAAMYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:136) and a light chain variable (VL) sequence of DIVLTQSPDSLAVSLGERATINCKSSQSVLYSSDQKNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:145) for binding to the MUC4 glycopeptide.

33. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIQYNAK FKGHATLSADKSSSTAYLQWSSLKASDAAMYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:136) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNLRNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:146) for binding to the MUC4 glycopeptide.

34. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIQYNAK FKGHATLSADKSSSTAYLQWSSLKASDAAMYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:136) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNERNYLAWYQQKPGQPPKLLIYWASTRESG

VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:147) for binding to the MUC4 glycopeptide.

35. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIQYNAK FKGHATLSADKSSSTAYLQWSSLKASDAAMYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:136) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCKSSQSVLYSSDQKNYLAWYQQKPGQAPRLLIYWASTRESG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:148) for binding to the MUC4 glycopeptide.

36. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIQYNAK FKGHATLSADKSSSTAYLQWSSLKASDAAMYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:136) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRSSQSVLYSSDQKSYLAWYQQKPGQAPRLLIYWASTRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:149) for binding to the MUC4 glycopeptide.

37. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIQYNAK FKGHATLSADKSSSTAYLQWSSLKASDAAMYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:136) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRASQSVSYSSDQKSYLAWYQQKPGQAPRLLIYWASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:150) for binding to the MUC4 glycopeptide.

38. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIQYNAK FKGHATLSADKSSSTAYLQWSSLKASDAAMYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:136) and a light chain variable (VL) sequence of DIVLTQTPLSLPVTPGEPASISCKSSQSVLYSSDQKNYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:151) for binding to the MUC4 glycopeptide.

39. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIQYNAK FKGHATLSADKSSSTAYLQWSSLKASDAAMYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:136) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSVLYSSDEKTYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:152) for binding to the MUC4 glycopeptide.

40. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIQYNAK FKGHATLSADKSSSTAYLQWSSLKASDAAMYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:136) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSLLYSSDERTYLAWYLQKPGQSPQLLIYWASTRASGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:153) for binding to the MUC4 glycopeptide.

41. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIRYNAK FKGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:137) and a light chain variable (VL) sequence of DIVLTQSPDSLAVSLGERATINCKSSQSVLYSSDQKNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:145) for binding to the MUC4 glycopeptide.

42. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIRYNAK FKGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:137) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNLRNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:146) for binding to the MUC4 glycopeptide.

43. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody

or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIRYNAK FKGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:137) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNERNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:147) for binding to the MUC4 glycopeptide.

44. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIRYNAK FKGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:137) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCKSSQSVLYSSDQKNYLAWYQQKPGQAPRLLIYWASTRESG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:148) for binding to the MUC4 glycopeptide.

45. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIRYNAK FKGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:137) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRSSQSVLYSSDQKSYLAWYQQKPGQAPRLLIYWASTRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:149) for binding to the MUC4 glycopeptide.

46. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIRYNAK FKGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:137) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRASQSVSYSSDQKSYLAWYQQKPGQAPRLLIYWASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:150) for binding to the MUC4 glycopeptide.

47. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIRYNAK

FKGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:137) and a light chain variable (VL) sequence of DIVLTQTPLSLPVTPGEPASISCKSSQSVLYSSDQKNYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:151) for binding to the MUC4 glycopeptide.

48. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIRYNAK FKGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:137) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSVLYSSDEKTYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:152) for binding to the MUC4 glycopeptide.

49. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIRYNAK FKGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:137) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSLLYSSDERTYLAWYLQKPGQSPQLLIYWASTRASGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:153) for binding to the MUC4 glycopeptide.

50. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNADTRYSAS FQGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:138) and a light chain variable (VL) sequence of DIVLTQSPDSLAVSLGERATINCKSSQSVLYSSDQKNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:145) for binding to the MUC4 glycopeptide.

51. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNADTRYSAS FQGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:138) and a light chain variable (VL) sequence of

DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNLRNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:146) for binding to the MUC4 glycopeptide.

52. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNADTRYSAS FQGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:138) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNERNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:147) for binding to the MUC4 glycopeptide.

53. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNADTRYSAS FQGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:138) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCKSSQSVLYSSDQKNYLAWYQQKPGQAPRLLIYWASTRESG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:148) for binding to the MUC4 glycopeptide.

54. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNADTRYSAS FQGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:138) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRSSQSVLYSSDQKSYLAWYQQKPGQAPRLLIYWASTRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:149) for binding to the MUC4 glycopeptide.

55. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNADTRYSAS FQGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:138) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRASQSVSYSSDQKSYLAWYQQKPGQAPRLLIYWASSRATG

IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:150) for binding to the MUC4 glycopeptide.

56. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNADTRYSAS FQGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:138) and a light chain variable (VL) sequence of DIVLTQTPLSLPVTPGEPASISCKSSQSVLYSSDQKNYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:151) for binding to the MUC4 glycopeptide.

57. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNADTRYSAS FQGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:138) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSVLYSSDEKTYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:152) for binding to the MUC4 glycopeptide.

58. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNADTRYSAS FQGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:138) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSLLYSSDERTYLAWYLQKPGQSPQLLIYWASTRASGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:153) for binding to the MUC4 glycopeptide.

59. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRATLTADKSTSTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:139) and a light chain variable (VL) sequence of DIVLTQSPDSLAVSLGERATINCKSSQSVLYSSDQKNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:145) for binding to the MUC4 glycopeptide.

60. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRATLTADKSTSTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:139) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNLRNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:146) for binding to the MUC4 glycopeptide.

61. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRATLTADKSTSTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:139) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNERNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:147) for binding to the MUC4 glycopeptide.

62. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRATLTADKSTSTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:139) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCKSSQSVLYSSDQKNYLAWYQQKPGQAPRLLIYWASTRESG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:148) for binding to the MUC4 glycopeptide.

63. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRATLTADKSTSTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:139) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRSSQSVLYSSDQKSYLAWYQQKPGQAPRLLIYWASTRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:149) for binding to the MUC4 glycopeptide.

64. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody

or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRATLTADKSTSTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:139) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRASQSVSYSSDQKSYLAWYQQKPGQAPRLLIYWASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:150) for binding to the MUC4 glycopeptide.

65. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRATLTADKSTSTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:139) and a light chain variable (VL) sequence of DIVLTQTPLSLPVTPGEPASISCKSSQSVLYSSDQKNYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:151) for binding to the MUC4 glycopeptide.

66. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRATLTADKSTSTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:139) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSVLYSSDEKTYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:152) for binding to the MUC4 glycopeptide.

67. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRATLTADKSTSTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:139) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSLLYSSDERTYLAWYLQKPGQSPQLLIYWASTRASGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:153) for binding to the MUC4 glycopeptide.

68. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNQ

KFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:140) and a light chain variable (VL) sequence of DIVLTQSPDSLAVSLGERATINCKSSQSVLYSSDQKNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:145) for binding to the MUC4 glycopeptide.

69. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNQ KFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:140) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNLRNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:146) for binding to the MUC4 glycopeptide.

70. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNQ KFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:140) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNERNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:147) for binding to the MUC4 glycopeptide.

71. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNQ KFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:140) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCKSSQSVLYSSDQKNYLAWYQQKPGQAPRLLIYWASTRESG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:148) for binding to the MUC4 glycopeptide.

72. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNQ KFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:140) and a light chain variable (VL) sequence of

EIVLTQSPGTLSLSPGERATLSCRSSQSVLYSSDQKSYLAWYQQKPGQAPRLLIYWASTRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:149) for binding to the MUC4 glycopeptide.

73. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNQ KFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:140) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRASQSVSYSSDQKSYLAWYQQKPGQAPRLLIYWASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:150) for binding to the MUC4 glycopeptide.

74. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNQ KFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:140) and a light chain variable (VL) sequence of DIVLTQTPLSLPVTPGEPASISCKSSQSVLYSSDQKNYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:151) for binding to the MUC4 glycopeptide.

75. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNQ KFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:140) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSVLYSSDEKTYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:152) for binding to the MUC4 glycopeptide.

76. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNQ KFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:140) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSLLYSSDERTYLAWYLQKPGQSPQLLIYWASTRASGV

PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:153) for binding to the MUC4 glycopeptide.

77. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFSDHAIHWVRQAPGQGLEWLGYISPGNADINYAQ KFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:141) and a light chain variable (VL) sequence of DIVLTQSPDSLAVSLGERATINCKSSQSVLYSSDQKNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:145) for binding to the MUC4 glycopeptide.

78. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFSDHAIHWVRQAPGQGLEWLGYISPGNADINYAQ KFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:141) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNLRNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:146) for binding to the MUC4 glycopeptide.

79. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFSDHAIHWVRQAPGQGLEWLGYISPGNADINYAQ KFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:141) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNERNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:147) for binding to the MUC4 glycopeptide.

80. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFSDHAIHWVRQAPGQGLEWLGYISPGNADINYAQ KFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:141) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCKSSQSVLYSSDQKNYLAWYQQKPGQAPRLLIYWASTRESG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:148) for binding to the MUC4 glycopeptide.

81. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFSDHAIHWVRQAPGQGLEWLGYISPGNADINYAQ KFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:141) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRSSQSVLYSSDQKSYLAWYQQKPGQAPRLLIYWASTRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:149) for binding to the MUC4 glycopeptide.

82. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFSDHAIHWVRQAPGQGLEWLGYISPGNADINYAQ KFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:141) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRASQSVSYSSDQKSYLAWYQQKPGQAPRLLIYWASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:150) for binding to the MUC4 glycopeptide.

83. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFSDHAIHWVRQAPGQGLEWLGYISPGNADINYAQ KFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:141) and a light chain variable (VL) sequence of DIVLTQTPLSLPVTPGEPASISCKSSQSVLYSSDQKNYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:151) for binding to the MUC4 glycopeptide.

84. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFSDHAIHWVRQAPGQGLEWLGYISPGNADINYAQ KFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:141) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSVLYSSDEKTYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:152) for binding to the MUC4 glycopeptide.

85. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody

or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFSDHAIHWVRQAPGQGLEWLGYISPGNADINYAQ KFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:141) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSLLYSSDERTYLAWYLQKPGQSPQLLIYWASTRASGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:153) for binding to the MUC4 glycopeptide.

86. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYNA KFKGRATLTADKSASTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:142) and a light chain variable (VL) sequence of DIVLTQSPDSLAVSLGERATINCKSSQSVLYSSDQKNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:145) for binding to the MUC4 glycopeptide.

87. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYNA KFKGRATLTADKSASTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:142) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNLRNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:146) for binding to the MUC4 glycopeptide.

88. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYNA KFKGRATLTADKSASTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:142) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNERNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:147) for binding to the MUC4 glycopeptide.

89. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYNA

KFKGRATLTADKSASTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:142) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCKSSQSVLYSSDQKNYLAWYQQKPGQAPRLLIYWASTRESG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:148) for binding to the MUC4 glycopeptide.

90. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYNA KFKGRATLTADKSASTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:142) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRSSQSVLYSSDQKSYLAWYQQKPGQAPRLLIYWASTRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:149) for binding to the MUC4 glycopeptide.

91. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYNA KFKGRATLTADKSASTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:142) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRASQSVSYSSDQKSYLAWYQQKPGQAPRLLIYWASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:150) for binding to the MUC4 glycopeptide.

92. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYNA KFKGRATLTADKSASTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:142) and a light chain variable (VL) sequence of DIVLTQTPLSLPVTPGEPASISCKSSQSVLYSSDQKNYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:151) for binding to the MUC4 glycopeptide.

93. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYNA KFKGRATLTADKSASTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:142) and a light chain variable (VL) sequence of

DIVMTQTPLSLPVTPGEPASISCRSSQSVLYSSDEKTYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:152) for binding to the MUC4 glycopeptide.

94. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYNA KFKGRATLTADKSASTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:142) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSLLYSSDERTYLAWYLQKPGQSPQLLIYWASTRASGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:153) for binding to the MUC4 glycopeptide.

95. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYSQ KFKGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:143) and a light chain variable (VL) sequence of DIVLTQSPDSLAVSLGERATINCKSSQSVLYSSDQKNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:145) for binding to the MUC4 glycopeptide.

96. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYSQ KFKGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:143) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNLRNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:146) for binding to the MUC4 glycopeptide.

97. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYSQ KFKGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:143) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNERNYLAWYQQKPGQPPKLLIYWASTRESG

VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:147) for binding to the MUC4 glycopeptide.

98. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYSQ KFKGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:143) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCKSSQSVLYSSDQKNYLAWYQQKPGQAPRLLIYWASTRESG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:148) for binding to the MUC4 glycopeptide.

99. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYSQ KFKGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:143) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRSSQSVLYSSDQKSYLAWYQQKPGQAPRLLIYWASTRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:149) for binding to the MUC4 glycopeptide.

100. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYSQ KFKGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:143) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRASQSVSYSSDQKSYLAWYQQKPGQAPRLLIYWASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:150) for binding to the MUC4 glycopeptide.

101. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYSQ KFKGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:143) and a light chain variable (VL) sequence of DIVLTQTPLSLPVTPGEPASISCKSSQSVLYSSDQKNYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:151) for binding to the MUC4 glycopeptide.

102. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYSQ KFKGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:143) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSVLYSSDEKTYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:152) for binding to the MUC4 glycopeptide.

103. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYSQ KFKGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:143) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSLLYSSDERTYLAWYLQKPGQSPQLLIYWASTRASGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:153) for binding to the MUC4 glycopeptide.

104. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNADTQYS QKFQGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:144) and a light chain variable (VL) sequence of DIVLTQSPDSLAVSLGERATINCKSSQSVLYSSDQKNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:145) for binding to the MUC4 glycopeptide.

105. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNADTQYS QKFQGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:144) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNLRNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:146) for binding to the MUC4 glycopeptide.

106. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody

or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNADTQYS QKFQGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:144) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNERNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:147) for binding to the MUC4 glycopeptide.

107. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNADTQYS QKFQGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:144) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCKSSQSVLYSSDQKNYLAWYQQKPGQAPRLLIYWASTRESG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:148) for binding to the MUC4 glycopeptide.

108. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNADTQYS QKFQGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:144) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRSSQSVLYSSDQKSYLAWYQQKPGQAPRLLIYWASTRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:149) for binding to the MUC4 glycopeptide.

109. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNADTQYS QKFQGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:144) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRASQSVSYSSDQKSYLAWYQQKPGQAPRLLIYWASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:150) for binding to the MUC4 glycopeptide.

110. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNADTQYS

QKFQGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:144) and a light chain variable (VL) sequence of DIVLTQTPLSLPVTPGEPASISCKSSQSVLYSSDQKNYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:151) for binding to the MUC4 glycopeptide.

111. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNADTQYS QKFQGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:144) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSVLYSSDEKTYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:152) for binding to the MUC4 glycopeptide.

112. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNADTQYS QKFQGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:144) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSLLYSSDERTYLAWYLQKPGQSPQLLIYWASTRASGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:153) for binding to the MUC4 glycopeptide.

113. The anti-glyco-MUC4 antibody or antigen binding fragment of any one of embodiments 1 to 112, which specifically binds to COSMC knock-out T3M4 cells.

114. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLQQSDAELVKPGASVRISCKAYGYTFTDHAIHWVKQKPEQGLEWLGYISPGNDDIQYNAK FKGKATLTADKSSSTAYMQLNSLTSDDSAVYFCKRSMANSFDYWGQGTTLTVSS (SEQ ID NO:1) and a light chain variable (VL) sequence of NIMLTQSPSSLAVSAGEKVTMSCKSSQSVLYSSDQKNYLAWYQQKPGQSPKLLIYWASTRES GVPDRFTGSGSGTDFTLTISNVQAEDLAVYYCHQYLSSYTFGGGTKLEIK (SEQ ID NO:2) for binding to COSMC knock-out T3M4 cells.

115. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLQQSDAELVKPGASVKISCKASGYTFTDHAIHWVKQKPEQGLEWIGYFSPGNGDIKYNEK

FKGKATLTADRSSSTANMHLNSLTSEDSAVYFCKRSMANYFDYWGQGTTLTVSS (SEQ ID NO:23) and a light chain variable (VL) sequence of NIMMTQSPSSLVVSAGEKVTMSCKSSHSVLYSSNQKNYLAWYQQKPGQSPKLLIYWASTKNS GVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCHQYLSSYTFGGGTKLEIK (SEQ ID NO:24) for binding to COSMC knock-out T3M4 cells.

116. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLQQSDAELVEPGASVKISCKAYGYTFTDHAIHWVKQKPEQGLEWLGYISPGNDDIQYNAK FKGRATLTADKSSSTAYMQLNSLTSDDSAVYFCKRSMANSFDFWGQGTTLTVSS (SEQ ID NO:45) and a light chain variable (VL) sequence of NIMLTQSPSSLAVSAGEKVTMSCKSSQSVLYSSDQKNYLAWYQQKPGQSPKLLIYWASTRES GVPDRFTGSGSGTDFTLTISNVRAEDLAVYYCHQYLSSYTFGGGTKLEIK (SEQ ID NO:46) for binding to COSMC knock-out T3M4 cells.

117. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRAVLSADKSVSTAYLQISSLKAEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:133) and a light chain variable (VL) sequence of DIVLTQSPDSLAVSLGERATINCKSSQSVLYSSDQKNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:145) for binding to COSMC knock-out T3M4 cells.

118. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRAVLSADKSVSTAYLQISSLKAEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:133) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNLRNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:146) for binding to COSMC knock-out T3M4 cells.

119. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRAVLSADKSVSTAYLQISSLKAEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:133) and a light chain variable (VL) sequence of

DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNERNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:147) for binding to COSMC knock-out T3M4 cells.

120. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRAVLSADKSVSTAYLQISSLKAEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:133) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCKSSQSVLYSSDQKNYLAWYQQKPGQAPRLLIYWASTRESG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:148) for binding to COSMC knock-out T3M4 cells.

121. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRAVLSADKSVSTAYLQISSLKAEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:133) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRSSQSVLYSSDQKSYLAWYQQKPGQAPRLLIYWASTRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:149) for binding to COSMC knock-out T3M4 cells.

122. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRAVLSADKSVSTAYLQISSLKAEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:133) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRASQSVSYSSDQKSYLAWYQQKPGQAPRLLIYWASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:150) for binding to COSMC knock-out T3M4 cells.

123. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRAVLSADKSVSTAYLQISSLKAEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:133) and a light chain variable (VL) sequence of DIVLTQTPLSLPVTPGEPASISCKSSQSVLYSSDQKNYLAWYLQKPGQSPQLLIYWASTRESGV

PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:151) for binding to COSMC knock-out T3M4 cells.

124. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRAVLSADKSVSTAYLQISSLKAEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:133) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSVLYSSDEKTYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:152) for binding to COSMC knock-out T3M4 cells.

125. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRAVLSADKSVSTAYLQISSLKAEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:133) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSLLYSSDERTYLAWYLQKPGQSPQLLIYWASTRASGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:153) for binding to COSMC knock-out T3M4 cells.

126. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNDDIQYNQ KFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:134) and a light chain variable (VL) sequence of DIVLTQSPDSLAVSLGERATINCKSSQSVLYSSDQKNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:145) for binding to COSMC knock-out T3M4 cells.

127. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNDDIQYNQ KFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:134) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNLRNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:146) for binding to COSMC knock-out T3M4 cells.

128. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNDDIQYNQ KFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:134) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNERNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:147) for binding to COSMC knock-out T3M4 cells.

129. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNDDIQYNQ KFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:134) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCKSSQSVLYSSDQKNYLAWYQQKPGQAPRLLIYWASTRESG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:148) for binding to COSMC knock-out T3M4 cells.

130. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNDDIQYNQ KFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:134) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRSSQSVLYSSDQKSYLAWYQQKPGQAPRLLIYWASTRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:149) for binding to COSMC knock-out T3M4 cells.

131. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNDDIQYNQ KFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:134) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRASQSVSYSSDQKSYLAWYQQKPGQAPRLLIYWASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:150) for binding to COSMC knock-out T3M4 cells.

132. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody

or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNDDIQYNQ KFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:134) and a light chain variable (VL) sequence of DIVLTQTPLSLPVTPGEPASISCKSSQSVLYSSDQKNYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:151) for binding to COSMC knock-out T3M4 cells.

133. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNDDIQYNQ KFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:134) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSVLYSSDEKTYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:152) for binding to COSMC knock-out T3M4 cells.

134. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNDDIQYNQ KFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:134) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSLLYSSDERTYLAWYLQKPGQSPQLLIYWASTRASGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:153) for binding to COSMC knock-out T3M4 cells.

135. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNANITYAQ GFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:135) and a light chain variable (VL) sequence of DIVLTQSPDSLAVSLGERATINCKSSQSVLYSSDQKNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:145) for binding to COSMC knock-out T3M4 cells.

136. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNANITYAQ

GFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:135) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNLRNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:146) for binding to COSMC knock-out T3M4 cells.

137. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNANITYAQ GFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:135) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNERNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:147) for binding to COSMC knock-out T3M4 cells.

138. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNANITYAQ GFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:135) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCKSSQSVLYSSDQKNYLAWYQQKPGQAPRLLIYWASTRESG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:148) for binding to COSMC knock-out T3M4 cells.

139. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNANITYAQ GFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:135) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRSSQSVLYSSDQKSYLAWYQQKPGQAPRLLIYWASTRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:149) for binding to COSMC knock-out T3M4 cells.

140. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNANITYAQ GFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:135) and a light chain variable (VL) sequence of

EIVLTQSPGTLSLSPGERATLSCRASQSVSYSSDQKSYLAWYQQKPGQAPRLLIYWASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:150) for binding to COSMC knock-out T3M4 cells.

141. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNANITYAQ GFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:135) and a light chain variable (VL) sequence of DIVLTQTPLSLPVTPGEPASISCKSSQSVLYSSDQKNYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:151) for binding to COSMC knock-out T3M4 cells.

142. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNANITYAQ GFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:135) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSVLYSSDEKTYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:152) for binding to COSMC knock-out T3M4 cells.

143. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNANITYAQ GFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:135) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSLLYSSDERTYLAWYLQKPGQSPQLLIYWASTRASGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:153) for binding to COSMC knock-out T3M4 cells.

144. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIQYNAK FKGHATLSADKSSSTAYLQWSSLKASDAAMYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:136) and a light chain variable (VL) sequence of DIVLTQSPDSLAVSLGERATINCKSSQSVLYSSDQKNYLAWYQQKPGQPPKLLIYWASTRESG

VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:145) for binding to COSMC knock-out T3M4 cells.

145. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIQYNAK FKGHATLSADKSSSTAYLQWSSLKASDAAMYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:136) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNLRNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:146) for binding to COSMC knock-out T3M4 cells.

146. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIQYNAK FKGHATLSADKSSSTAYLQWSSLKASDAAMYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:136) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNERNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:147) for binding to COSMC knock-out T3M4 cells.

147. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIQYNAK FKGHATLSADKSSSTAYLQWSSLKASDAAMYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:136) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCKSSQSVLYSSDQKNYLAWYQQKPGQAPRLLIYWASTRESG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:148) for binding to COSMC knock-out T3M4 cells.

148. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIQYNAK FKGHATLSADKSSSTAYLQWSSLKASDAAMYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:136) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRSSQSVLYSSDQKSYLAWYQQKPGQAPRLLIYWASTRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:149) for binding to COSMC knock-out T3M4 cells.

149. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIQYNAK FKGHATLSADKSSSTAYLQWSSLKASDAAMYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:136) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRASQSVSYSSDQKSYLAWYQQKPGQAPRLLIYWASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:150) for binding to COSMC knock-out T3M4 cells.

150. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIQYNAK FKGHATLSADKSSSTAYLQWSSLKASDAAMYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:136) and a light chain variable (VL) sequence of DIVLTQTPLSLPVTPGEPASISCKSSQSVLYSSDQKNYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:151) for binding to COSMC knock-out T3M4 cells.

151. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIQYNAK FKGHATLSADKSSSTAYLQWSSLKASDAAMYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:136) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSVLYSSDEKTYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:152) for binding to COSMC knock-out T3M4 cells.

152. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIQYNAK FKGHATLSADKSSSTAYLQWSSLKASDAAMYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:136) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSLLYSSDERTYLAWYLQKPGQSPQLLIYWASTRASGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:153) for binding to COSMC knock-out T3M4 cells.

153. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody

or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIRYNAK FKGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:137) and a light chain variable (VL) sequence of DIVLTQSPDSLAVSLGERATINCKSSQSVLYSSDQKNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:145) for binding to COSMC knock-out T3M4 cells.

154. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIRYNAK FKGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:137) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNLRNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:146) for binding to COSMC knock-out T3M4 cells.

155. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIRYNAK FKGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:137) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNERNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:147) for binding to COSMC knock-out T3M4 cells.

156. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIRYNAK FKGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:137) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCKSSQSVLYSSDQKNYLAWYQQKPGQAPRLLIYWASTRESG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:148) for binding to COSMC knock-out T3M4 cells.

157. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIRYNAK

FKGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:137) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRSSQSVLYSSDQKSYLAWYQQKPGQAPRLLIYWASTRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:149) for binding to COSMC knock-out T3M4 cells.

158. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIRYNAK FKGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:137) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRASQSVSYSSDQKSYLAWYQQKPGQAPRLLIYWASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:150) for binding to COSMC knock-out T3M4 cells.

159. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIRYNAK FKGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:137) and a light chain variable (VL) sequence of DIVLTQTPLSLPVTPGEPASISCKSSQSVLYSSDQKNYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:151) for binding to COSMC knock-out T3M4 cells.

160. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIRYNAK FKGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:137) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSVLYSSDEKTYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:152) for binding to COSMC knock-out T3M4 cells.

161. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIRYNAK FKGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:137) and a light chain variable (VL) sequence of

DIVMTQTPLSLPVTPGEPASISCRSSQSLLYSSDERTYLAWYLQKPGQSPQLLIYWASTRASGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:153) for binding to COSMC knock-out T3M4 cells.

162. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNADTRYSAS FQGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:138) and a light chain variable (VL) sequence of DIVLTQSPDSLAVSLGERATINCKSSQSVLYSSDQKNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:145) for binding to COSMC knock-out T3M4 cells.

163. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNADTRYSAS FQGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:138) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNLRNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:146) for binding to COSMC knock-out T3M4 cells.

164. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNADTRYSAS FQGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:138) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNERNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:147) for binding to COSMC knock-out T3M4 cells.

165. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNADTRYSAS FQGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:138) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCKSSQSVLYSSDQKNYLAWYQQKPGQAPRLLIYWASTRESG

IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:148) for binding to COSMC knock-out T3M4 cells.

166. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNADTRYSAS FQGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:138) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRSSQSVLYSSDQKSYLAWYQQKPGQAPRLLIYWASTRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:149) for binding to COSMC knock-out T3M4 cells.

167. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNADTRYSAS FQGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:138) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRASQSVSYSSDQKSYLAWYQQKPGQAPRLLIYWASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:150) for binding to COSMC knock-out T3M4 cells.

168. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNADTRYSAS FQGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:138) and a light chain variable (VL) sequence of DIVLTQTPLSLPVTPGEPASISCKSSQSVLYSSDQKNYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:151) for binding to COSMC knock-out T3M4 cells.

169. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNADTRYSAS FQGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:138) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSVLYSSDEKTYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:152) for binding to COSMC knock-out T3M4 cells.

170. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNADTRYSAS FQGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:138) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSLLYSSDERTYLAWYLQKPGQSPQLLIYWASTRASGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:153) for binding to COSMC knock-out T3M4 cells.

171. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRATLTADKSTSTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:139) and a light chain variable (VL) sequence of DIVLTQSPDSLAVSLGERATINCKSSQSVLYSSDQKNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:145) for binding to COSMC knock-out T3M4 cells.

172. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRATLTADKSTSTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:139) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNLRNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:146) for binding to COSMC knock-out T3M4 cells.

173. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRATLTADKSTSTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:139) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNERNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:147) for binding to COSMC knock-out T3M4 cells.

174. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody

or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRATLTADKSTSTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:139) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCKSSQSVLYSSDQKNYLAWYQQKPGQAPRLLIYWASTRESG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:148) for binding to COSMC knock-out T3M4 cells.

175. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRATLTADKSTSTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:139) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRSSQSVLYSSDQKSYLAWYQQKPGQAPRLLIYWASTRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:149) for binding to COSMC knock-out T3M4 cells.

176. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRATLTADKSTSTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:139) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRASQSVSYSSDQKSYLAWYQQKPGQAPRLLIYWASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:150) for binding to COSMC knock-out T3M4 cells.

177. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRATLTADKSTSTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:139) and a light chain variable (VL) sequence of DIVLTQTPLSLPVTPGEPASISCKSSQSVLYSSDQKNYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:151) for binding to COSMC knock-out T3M4 cells.

178. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA

KFKGRATLTADKSTSTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:139) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSVLYSSDEKTYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:152) for binding to COSMC knock-out T3M4 cells.

179. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRATLTADKSTSTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:139) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSLLYSSDERTYLAWYLQKPGQSPQLLIYWASTRASGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:153) for binding to COSMC knock-out T3M4 cells.

180. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNQ KFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:140) and a light chain variable (VL) sequence of DIVLTQSPDSLAVSLGERATINCKSSQSVLYSSDQKNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:145) for binding to COSMC knock-out T3M4 cells.

181. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNQ KFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:140) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNLRNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:146) for binding to COSMC knock-out T3M4 cells.

182. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNQ KFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:140) and a light chain variable (VL) sequence of

DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNERNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:147) for binding to COSMC knock-out T3M4 cells.

183. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNQ KFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:140) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCKSSQSVLYSSDQKNYLAWYQQKPGQAPRLLIYWASTRESG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:148) for binding to COSMC knock-out T3M4 cells.

184. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNQ KFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:140) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRSSQSVLYSSDQKSYLAWYQQKPGQAPRLLIYWASTRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:149) for binding to COSMC knock-out T3M4 cells.

185. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNQ KFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:140) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRASQSVSYSSDQKSYLAWYQQKPGQAPRLLIYWASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:150) for binding to COSMC knock-out T3M4 cells.

186. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNQ KFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:140) and a light chain variable (VL) sequence of DIVLTQTPLSLPVTPGEPASISCKSSQSVLYSSDQKNYLAWYLQKPGQSPQLLIYWASTRESGV

PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:151) for binding to COSMC knock-out T3M4 cells.

187. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNQ KFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:140) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSVLYSSDEKTYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:152) for binding to COSMC knock-out T3M4 cells.

188. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNQ KFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:140) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSLLYSSDERTYLAWYLQKPGQSPQLLIYWASTRASGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:153) for binding to COSMC knock-out T3M4 cells.

189. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFSDHAIHWVRQAPGQGLEWLGYISPGNADINYAQ KFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:141) and a light chain variable (VL) sequence of DIVLTQSPDSLAVSLGERATINCKSSQSVLYSSDQKNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:145) for binding to COSMC knock-out T3M4 cells.

190. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFSDHAIHWVRQAPGQGLEWLGYISPGNADINYAQ KFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:141) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNLRNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:146) for binding to COSMC knock-out T3M4 cells.

191. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFSDHAIHWVRQAPGQGLEWLGYISPGNADINYAQ KFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:141) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNERNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:147) for binding to COSMC knock-out T3M4 cells.

192. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFSDHAIHWVRQAPGQGLEWLGYISPGNADINYAQ KFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:141) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCKSSQSVLYSSDQKNYLAWYQQKPGQAPRLLIYWASTRESG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:148) for binding to COSMC knock-out T3M4 cells.

193. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFSDHAIHWVRQAPGQGLEWLGYISPGNADINYAQ KFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:141) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRSSQSVLYSSDQKSYLAWYQQKPGQAPRLLIYWASTRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:149) for binding to COSMC knock-out T3M4 cells.

194. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFSDHAIHWVRQAPGQGLEWLGYISPGNADINYAQ KFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:141) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRASQSVSYSSDQKSYLAWYQQKPGQAPRLLIYWASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:150) for binding to COSMC knock-out T3M4 cells.

195. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody

or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFSDHAIHWVRQAPGQGLEWLGYISPGNADINYAQ KFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:141) and a light chain variable (VL) sequence of DIVLTQTPLSLPVTPGEPASISCKSSQSVLYSSDQKNYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:151) for binding to COSMC knock-out T3M4 cells.

196. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFSDHAIHWVRQAPGQGLEWLGYISPGNADINYAQ KFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:141) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSVLYSSDEKTYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:152) for binding to COSMC knock-out T3M4 cells.

197. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFSDHAIHWVRQAPGQGLEWLGYISPGNADINYAQ KFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:141) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSLLYSSDERTYLAWYLQKPGQSPQLLIYWASTRASGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:153) for binding to COSMC knock-out T3M4 cells.

198. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYNA KFKGRATLTADKSASTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:142) and a light chain variable (VL) sequence of DIVLTQSPDSLAVSLGERATINCKSSQSVLYSSDQKNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:145) for binding to COSMC knock-out T3M4 cells.

199. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYNA

KFKGRATLTADKSASTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:142) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNLRNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:146) for binding to COSMC knock-out T3M4 cells.

200. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYNA KFKGRATLTADKSASTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:142) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNERNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:147) for binding to COSMC knock-out T3M4 cells.

201. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYNA KFKGRATLTADKSASTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:142) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCKSSQSVLYSSDQKNYLAWYQQKPGQAPRLLIYWASTRESG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:148) for binding to COSMC knock-out T3M4 cells.

202. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYNA KFKGRATLTADKSASTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:142) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRSSQSVLYSSDQKSYLAWYQQKPGQAPRLLIYWASTRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:149) for binding to COSMC knock-out T3M4 cells.

203. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYNA KFKGRATLTADKSASTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:142) and a light chain variable (VL) sequence of

EIVLTQSPGTLSLSPGERATLSCRASQSVSYSSDQKSYLAWYQQKPGQAPRLLIYWASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:150) for binding to COSMC knock-out T3M4 cells.

204. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYNA KFKGRATLTADKSASTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:142) and a light chain variable (VL) sequence of DIVLTQTPLSLPVTPGEPASISCKSSQSVLYSSDQKNYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:151) for binding to COSMC knock-out T3M4 cells.

205. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYNA KFKGRATLTADKSASTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:142) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSVLYSSDEKTYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:152) for binding to COSMC knock-out T3M4 cells.

206. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYNA KFKGRATLTADKSASTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:142) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSLLYSSDERTYLAWYLQKPGQSPQLLIYWASTRASGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:153) for binding to COSMC knock-out T3M4 cells.

207. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYSQ KFKGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:143) and a light chain variable (VL) sequence of DIVLTQSPDSLAVSLGERATINCKSSQSVLYSSDQKNYLAWYQQKPGQPPKLLIYWASTRESG

VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:145) for binding to COSMC knock-out T3M4 cells.

208. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYSQ KFKGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:143) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNLRNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:146) for binding to COSMC knock-out T3M4 cells.

209. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYSQ KFKGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:143) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNERNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:147) for binding to COSMC knock-out T3M4 cells.

210. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYSQ KFKGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:143) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCKSSQSVLYSSDQKNYLAWYQQKPGQAPRLLIYWASTRESG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:148) for binding to COSMC knock-out T3M4 cells.

211. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYSQ KFKGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:143) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRSSQSVLYSSDQKSYLAWYQQKPGQAPRLLIYWASTRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:149) for binding to COSMC knock-out T3M4 cells.

212. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYSQ KFKGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:143) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRASQSVSYSSDQKSYLAWYQQKPGQAPRLLIYWASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:150) for binding to COSMC knock-out T3M4 cells.

213. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYSQ KFKGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:143) and a light chain variable (VL) sequence of DIVLTQTPLSLPVTPGEPASISCKSSQSVLYSSDQKNYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:151) for binding to COSMC knock-out T3M4 cells.

214. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYSQ KFKGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:143) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSVLYSSDEKTYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:152) for binding to COSMC knock-out T3M4 cells.

215. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYSQ KFKGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:143) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSLLYSSDERTYLAWYLQKPGQSPQLLIYWASTRASGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:153) for binding to COSMC knock-out T3M4 cells.

216. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody

or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNADTQYS QKFQGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:144) and a light chain variable (VL) sequence of DIVLTQSPDSLAVSLGERATINCKSSQSVLYSSDQKNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:145) for binding to COSMC knock-out T3M4 cells.

217. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNADTQYS QKFQGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:144) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNLRNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:146) for binding to COSMC knock-out T3M4 cells.

218. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNADTQYS QKFQGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:144) and a light chain variable (VL) sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNERNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:147) for binding to COSMC knock-out T3M4 cells.

219. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNADTQYS QKFQGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:144) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCKSSQSVLYSSDQKNYLAWYQQKPGQAPRLLIYWASTRESG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:148) for binding to COSMC knock-out T3M4 cells.

220. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNADTQYS

QKFQGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:144) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRSSQSVLYSSDQKSYLAWYQQKPGQAPRLLIYWASTRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:149) for binding to COSMC knock-out T3M4 cells.

221. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNADTQYS QKFQGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:144) and a light chain variable (VL) sequence of EIVLTQSPGTLSLSPGERATLSCRASQSVSYSSDQKSYLAWYQQKPGQAPRLLIYWASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:150) for binding to COSMC knock-out T3M4 cells.

222. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNADTQYS QKFQGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:144) and a light chain variable (VL) sequence of DIVLTQTPLSLPVTPGEPASISCKSSQSVLYSSDQKNYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:151) for binding to COSMC knock-out T3M4 cells.

223. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNADTQYS QKFQGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:144) and a light chain variable (VL) sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSVLYSSDEKTYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:152) for binding to COSMC knock-out T3M4 cells.

224. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 113, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNADTQYS QKFQGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:144) and a light chain variable (VL) sequence of

DIVMTQTPLSLPVTPGEPASISCRSSQSLLYSSDERTYLAWYLQKPGQSPQLLIYWASTRASGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:153) for binding to the MUC4 glycopeptide.

225. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1 to 224, comprising:

(a) a complementarity determining region (CDR) H1 comprising the amino acid sequence of a CDR-H1 of any one of Tables 1D, 1E, 1F, 2D, and 3D (*e.g.*, SEQ ID NO:67, SEQ ID NO:73, SEQ ID NO:79, SEQ ID NO:103, or SEQ ID NO:127);

(b) a CDR-H2 comprising the amino acid sequence of a CDR-H2 of any one of Tables 1D, 1E, 1F, 2D, and 3D (*e.g.*, SEQ ID NO:68, SEQ ID NO:74, SEQ ID NO:80, SEQ ID NO:104, or SEQ ID NO:128);

(c) a CDR-H3 comprising the amino acid sequence of a CDR-H3 of any one of Tables 1D, 1E, 1F, 2D, and 3D (*e.g.*, SEQ ID NO:69, SEQ ID NO:75, SEQ ID NO:81, SEQ ID NO:105, or SEQ ID NO:129);

(d) a CDR-L1 comprising the amino acid sequence of a CDR-L1 of any one of Tables 1D, 1E, 1F, 2D, and 3D (*e.g.*, SEQ ID NO:70, SEQ ID NO:76, SEQ ID NO:82, SEQ ID NO:106, or SEQ ID NO:130);

(e) a CDR-L2 comprising the amino acid sequence of a CDR-L1 of any one of Tables 1D, 1E, 1F, 2D, and 3D (*e.g.*, SEQ ID NO:71, SEQ ID NO:77, SEQ ID NO:83, SEQ ID NO:107, or SEQ ID NO:131); and

(f) a CDR-L3 comprising the amino acid sequence of a CDR-L1 of any one of Tables 1D, 1E, 1F, 2D, and 3D (*e.g.*, SEQ ID NO:72, SEQ ID NO:78, SEQ ID NO:84, SEQ ID NO:108, or SEQ ID NO:132).

226. The anti-glyco-MUC4 antibody or antigen-binding fragment of embodiment 225, wherein the amino acid designated X_1 in a CDR sequence of any one of Tables 1D, 1E, 1F, 2D, and 3D (*e.g.,* SEQ ID NO:68, SEQ ID NO:74, and/or SEQ ID NO:104) is I.

227. The anti-glyco-MUC4 antibody or antigen-binding fragment of embodiment 225, wherein the amino acid designated X_1 in a CDR sequence of any one of Tables 1D, 1E, 1F, 2D, and 3D (*e.g.,* SEQ ID NO:68, SEQ ID NO:74, and/or SEQ ID NO:104) is F.

228. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 227, wherein the amino acid designated X_2 in a CDR sequence of any one of Tables 1D, 1E, 1F, 2D, and 3D (*e.g.,* SEQ ID NO:68, SEQ ID NO:74, SEQ ID NO:80, SEQ ID NO:104, and/or SEQ ID NO:128) is D.

229. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 227, wherein the amino acid designated X_2 in a CDR sequence of any one

of Tables 1D, 1E, 1F, 2D, and 3D (*e.g.,* SEQ ID NO:68, SEQ ID NO:74, SEQ ID NO:80, SEQ ID NO:104, and/or SEQ ID NO:128) is G.

230. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 229, wherein the amino acid designated X_3 in a CDR sequence of any one of Tables 1D, 1E, 1F, 2D, and 3D (*e.g.,* SEQ ID NO:74 and/or SEQ ID NO:104) is Q.

231. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 229, wherein the amino acid designated X_3 in a CDR sequence of any one of Tables 1D, 1E, 1F, 2D, and 3D (*e.g.,* SEQ ID NO:74 and/or SEQ ID NO:104) is K.

232. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 231, wherein the amino acid designated X_4 in a CDR sequence of any one of Tables 1D, 1E, 1F, 2D, and 3D (*e.g.,* SEQ ID NO:74 and/or SEQ ID NO:104) is A.

233. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 231, wherein the amino acid designated X_4 in a CDR sequence of any one of Tables 1D, 1E, 1F, 2D, and 3D (*e.g.,* SEQ ID NO:74 and/or SEQ ID NO:104) is E.

234. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 233, wherein the amino acid designated X_5 in a CDR sequence of any one of Tables 1D, 1E, 1F, 2D, and 3D (*e.g.,* SEQ ID NO:69, SEQ ID NO:75, SEQ ID NO:81, SEQ ID NO:105, and/or SEQ ID NO:129) is S.

235. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 233, wherein the amino acid designated X_5 in a CDR sequence of any one of Tables 1D, 1E, 1F, 2D, and 3D (*e.g.,* SEQ ID NO:69, SEQ ID NO:75, SEQ ID NO:81, SEQ ID NO:105, and/or SEQ ID NO:129) is Y.

236. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 235, wherein the amino acid designated X_6 in a CDR sequence of any one of Tables 1D, 1E, 1F, 2D, and 3D (*e.g.,* SEQ ID NO:69, SEQ ID NO:75, SEQ ID NO:81, SEQ ID NO:105, and/or SEQ ID NO:129) is Y.

237. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 235, wherein the amino acid designated X_6 in a CDR sequence of any one of Tables 1D, 1E, 1F, 2D, and 3D (*e.g.,* SEQ ID NO:69, SEQ ID NO:75, SEQ ID NO:81, SEQ ID NO:105, and/or SEQ ID NO:129) is F.

238. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 237, wherein the amino acid designated X_7 in a CDR sequence of any one of Tables 1D, 1E, 1F, 2D, and 3D (*e.g.,* SEQ ID NO:70, SEQ ID NO:76, SEQ ID NO:82, SEQ ID NO:106, and/or SEQ ID NO:130) is Q.

239. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 237, wherein the amino acid designated X_7 in a CDR sequence of any one of Tables 1D, 1E, 1F, 2D, and 3D (*e.g.,* SEQ ID NO:70, SEQ ID NO:76, SEQ ID NO:82, SEQ ID NO:106, and/or SEQ ID NO:130) is H.

240. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 239, wherein the amino acid designated X_8 in a CDR sequence of any one of Tables 1D, 1E, 1F, 2D, and 3D (*e.g.,* SEQ ID NO:70, SEQ ID NO:76, SEQ ID NO:82, SEQ ID NO:106, and/or SEQ ID NO:130) is D.

241. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 239, wherein the amino acid designated X_8 in a CDR sequence of any one of Tables 1D, 1E, 1F, 2D, and 3D (*e.g.,* SEQ ID NO:70, SEQ ID NO:76, SEQ ID NO:82, SEQ ID NO:106, and/or SEQ ID NO:130) is N.

242. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 241, wherein the amino acid designated X_9 in a CDR sequence of any one of Tables 1D, 1E, 1F, 2D, and 3D (*e.g.,* SEQ ID NO:77, SEQ ID NO:83, and/or SEQ ID NO:107) is R.

243. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 241, wherein the amino acid designated X_9 in a CDR sequence of any one of Tables 1D, 1E, 1F, 2D, and 3D (*e.g.,* SEQ ID NO:77, SEQ ID NO:83, and/or SEQ ID NO:107) is K.

244. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 243, wherein the amino acid designated X_{10} in a CDR sequence of any one of Tables 1D, 1E, 1F, 2D, and 3D (*e.g.,* SEQ ID NO:77, SEQ ID NO:83, and/or SEQ ID NO:107) is E.

245. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 243, wherein the amino acid designated X_{10} in a CDR sequence of any one of Tables 1D, 1E, 1F, 2D, and 3D (*e.g.,* SEQ ID NO:77, SEQ ID NO:83, and SEQ ID NO:107) is N.

246. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 245, wherein CDR-H1 comprises the amino acid sequence of GYTFTDHA (SEQ ID NO:67).

247. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 245, wherein CDR-H1 comprises the amino acid sequence of DHAIH (SEQ ID NO:73).

248. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 245, wherein CDR-H1 comprises the amino acid sequence of GYTFTDH SEQ ID NO:79).

249. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 245, wherein CDR-H1 comprises the amino acid sequence of GYTFTDHAIH (SEQ ID NO:103).

250. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 245, wherein CDR-H1 comprises the amino acid sequence of DH (SEQ ID NO:127).

251. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 250, wherein CDR-H2 comprises the amino acid sequence of X_1SPGNX_2DI (SEQ ID NO:68).

252. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 250, wherein CDR-H2 comprises the amino acid sequence of YX_1SPGNX_2DIX_3YNX_4KFKG (SEQ ID NO:74).

253. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 250, wherein CDR-H2 comprises the amino acid sequence of SPGNX_2D (SEQ ID NO:80).

254. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 250, wherein CDR-H2 comprises the amino acid sequence of YX_1SPGNX_2DIX_3YNX_4KFKG (SEQ ID NO:104).

255. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 250, wherein CDR-H2 comprises the amino acid sequence of SPGNX_2 (SEQ ID NO:128).

256. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 255, wherein CDR-H3 comprises the amino acid sequence of KRSMANX_5FDX_6 (SEQ ID NO:69).

257. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 255, wherein CDR-H3 comprises the amino acid sequence of SMANX_5FDX_6 (SEQ ID NO:75).

258. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 255, wherein CDR-H3 comprises the amino acid sequence of SMANX_5FDX_6 (SEQ ID NO:81).

259. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 255, wherein CDR-H3 comprises the amino acid sequence of KRSMANX_5FDX_6 (SEQ ID NO:105).

260. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 255, wherein CDR-H3 comprises the amino acid sequence of SMANX_5FDX_6 (SEQ ID NO:129).

261. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 260, wherein CDR-L1 comprises the amino acid sequence of X_7SVLYSSX_8QKNY (SEQ ID NO:70).

262. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 260, wherein CDR-L1 comprises the amino acid sequence of $KSSX_7SVLYSSX_8QKNYLA$ (SEQ ID NO:76).

263. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 260, wherein CDR-L1 comprises the amino acid sequence of $KSSX_7SVLYSSX_8QKNYLA$ (SEQ ID NO:82).

264. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 260, wherein CDR-L1 comprises the amino acid sequence of $KSSX_7SVLYSSX_8QKNYLA$ (SEQ ID NO:106).

265. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 260, wherein CDR-L1 comprises the amino acid sequence of $X_7SVLYSSX_8QKNY$ (SEQ ID NO:130).

266. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 266, wherein CDR-L2 comprises the amino acid sequence of WAS (SEQ ID NO:71).

267. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 266, wherein CDR-L2 comprises the amino acid sequence of $WASTX_9X_{10}S$ (SEQ ID NO:77).

268. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 266, wherein CDR-L2 comprises the amino acid sequence of $WASTX_9X_{10}S$ (SEQ ID NO:83).

269. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 266, wherein CDR-L2 comprises the amino acid sequence of $WASTX_9X_{10}S$ (SEQ ID NO:107).

270. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 266, wherein CDR-L2 comprises the amino acid sequence of WAS (SEQ ID NO:131).

271. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 270, wherein CDR-L3 comprises the amino acid sequence of HQYLSSYT (SEQ ID NO:72).

272. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 270, wherein CDR-L3 comprises the amino acid sequence of HQYLSSYT (SEQ ID NO:78).

273. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 270, wherein CDR-L3 comprises the amino acid sequence of HQYLSSYT (SEQ ID NO:84).

274. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 270, wherein CDR-L3 comprises the amino acid sequence of HQYLSSYT (SEQ ID NO:108).

275. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 225 to 270, wherein CDR-L3 comprises the amino acid sequence of HQYLSSYT SEQ ID NO:132.

276. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 1 to 224, which comprises a VH comprising CDRs of 2D5 as defined by IMGT (*e.g.*, SEQ ID NOs:3-5) and a VL comprising CDRs of 2D5 as defined by IMGT (*e.g.*, SEQ ID NOs:6-8).

277. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1 to 224, which comprises a VH comprising CDRs of 2D5 as defined by Kabat (*e.g.*, SEQ ID NOs:9-11) and a VL comprising CDRs of 2D5 as defined by Kabat (*e.g.*, SEQ ID NOs:12-14).

278. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1 to 224, which comprises a VH comprising CDRs of 2D5 as defined by Chothia (*e.g.*, SEQ ID NOs:15-17) and a VL comprising CDRs of 2D5 as defined by Chothia (*e.g.*, SEQ ID NOs:18-20).

279. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1 to 224, which comprises a VH comprising CDRs of 5B8 as defined by IMGT (*e.g.*, SEQ ID NOs:25-27) and a VL comprising CDRs of 5B8 as defined by IMGT (*e.g.*, SEQ ID NOs:28-30).

280. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1 to 224, which comprises a VH comprising CDRs of 5B8 as defined by Kabat (*e.g.*, SEQ ID NOs:31-33) and a VL comprising CDRs of 5B8 as defined by Kabat (*e.g.*, SEQ ID NOs:34-36).

281. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1 to 224, which comprises a VH comprising CDRs of 5B8 as defined by Chothia (*e.g.*, SEQ ID NOs:37-39) and a VL comprising CDRs of 5B8 as defined by Chothia (*e.g.*, SEQ ID NOs:40-42).

282. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1 to 224, which comprises a VH comprising CDRs of 15F3 as defined by IMGT (*e.g.*, SEQ ID NOs:47-49) and a VL comprising CDRs of 15F3 as defined by IMGT (*e.g.*, SEQ ID NOs:50-52).

283. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1

to 224, which comprises a VH comprising CDRs of 15F3 as defined by Kabat (*e.g.*, SEQ ID NOs:53-55) and a VL comprising CDRs of 15F3 as defined by Kabat (*e.g.*, SEQ ID NOs:56-58).

284. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1 to 224, which comprises a VH comprising CDRs of 15F3 as defined by Chothia (*e.g.*, SEQ ID NOs:59-61) and a VL comprising CDRs of 15F3 as defined by Chothia (*e.g.*, SEQ ID NOs:62-64.

285. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1 to 224, which comprises a VH comprising CDRs of GYTFTDHAIH (SEQ ID NO:85), YISPGNDDIQYNAKFKG (SEQ ID NO:86), and KRSMANSFDY (SEQ ID NO:87); and a VL comprising CDRs of KSSQSVLYSSDQKNYLA (SEQ ID NO:88), WASTRES (SEQ ID NO:89), and HQYLSSYT (SEQ ID NO:90).

286. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1 to 224, which comprises a VH comprising CDRs of GYTFTDHAIH (SEQ ID NO:91), YFSPGNGDIKYNEKFKG (SEQ ID NO:92), and KRSMANYFDY (SEQ ID NO:93); and a VL comprising CDRs of KSSHSVLYSSNQKNYLA (SEQ ID NO:94), WASTKNS (SEQ ID NO:95), and HQYLSSYT (SEQ ID NO:96).

287. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1 to 224, which comprises a VH comprising CDRs of GYTFTDHAIH (SEQ ID NO:97), YISPGNDDIQYNAKFKG (SEQ ID NO:98), and KRSMANSFDF (SEQ ID NO:99); and a VL comprising CDRs of KSSQSVLYSSDQKNYLA (SEQ ID NO:100), WASTRES (SEQ ID NO:101), and HQYLSSYT (SEQ ID NO:102).

288. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1 to 224, which comprises a VH comprising CDRs of DH (SEQ ID NO:109), SPGNDD (SEQ ID NO:110), and SMANSFDY (SEQ ID NO:111); and a VL comprising CDRs of QSVLYSSDQKNY (SEQ ID NO:112), WAS (SEQ ID NO:113), and HQYLSSYT (SEQ ID NO:114).

289. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1 to 224, which comprises a VH comprising CDRs of DH (SEQ ID NO:115), SPGNGD (SEQ ID NO:116), and SMANYFDY (SEQ ID NO:117); and a VL comprising CDRs of HSVLYSSNQKNY (SEQ ID NO:118), WAS (SEQ ID NO:119), and HQYLSSYT (SEQ ID NO:120).

290. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1 to 224, which comprises a VH comprising CDRs of DH (SEQ ID NO:121), SPGNDD (SEQ ID

NO:122), and SMANSFDF (SEQ ID NO:123); and a VL comprising CDRs of QSVLYSSDQKNY (SEQ ID NO:124), WAS (SEQ ID NO:125), and HQYLSSYT (SEQ ID NO:126).

291. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 1 to 290, which is a chimeric or humanized antibody or antigen-binding fragment of a chimeric or humanized antibody.

292. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1 to 291, which comprises a VH comprising an amino acid sequence having at least 95% sequence identity to QVQLQQSDAELVKPGASVRISCKAYGYTFTDHAIHWVKQKPEQGLEWLGYISPGNDDIQYNAK FKGKATLTADKSSSTAYMQLNSLTSDDSAVYFCKRSMANSFDYWGQGTTLTVSS (SEQ ID NO:1) and a VL comprising an amino acid sequence having at least 95% sequence identity to NIMLTQSPSSLAVSAGEKVTMSCKSSQSVLYSSDQKNYLAWYQQKPGQSPKLLIYWASTRES GVPDRFTGSGSGTDFTLTISNVQAEDLAVYYCHQYLSSYTFGGGTKLEIK (SEQ ID NO:2).

293. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1 to 291, which comprises a VH comprising an amino acid sequence having at least 97% sequence identity to QVQLQQSDAELVKPGASVRISCKAYGYTFTDHAIHWVKQKPEQGLEWLGYISPGNDDIQYNAK FKGKATLTADKSSSTAYMQLNSLTSDDSAVYFCKRSMANSFDYWGQGTTLTVSS (SEQ ID NO:1) and a VL comprising an amino acid sequence having at least 97% sequence identity to NIMLTQSPSSLAVSAGEKVTMSCKSSQSVLYSSDQKNYLAWYQQKPGQSPKLLIYWASTRES GVPDRFTGSGSGTDFTLTISNVQAEDLAVYYCHQYLSSYTFGGGTKLEIK (SEQ ID NO:2).

294. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1 to 291, which comprises a VH comprising an amino acid sequence having at least 99% sequence identity to QVQLQQSDAELVKPGASVRISCKAYGYTFTDHAIHWVKQKPEQGLEWLGYISPGNDDIQYNAK FKGKATLTADKSSSTAYMQLNSLTSDDSAVYFCKRSMANSFDYWGQGTTLTVSS (SEQ ID NO:1) and a VL comprising an amino acid sequence having at least 99% sequence identity to NIMLTQSPSSLAVSAGEKVTMSCKSSQSVLYSSDQKNYLAWYQQKPGQSPKLLIYWASTRES GVPDRFTGSGSGTDFTLTISNVQAEDLAVYYCHQYLSSYTFGGGTKLEIK (SEQ ID NO:2).

295. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1 to 291, which comprises a VH comprising the amino acid sequence of QVQLQQSDAELVKPGASVRISCKAYGYTFTDHAIHWVKQKPEQGLEWLGYISPGNDDIQYNAK FKGKATLTADKSSSTAYMQLNSLTSDDSAVYFCKRSMANSFDYWGQGTTLTVSS (SEQ ID NO:1) and a VL comprising the amino acid sequence of

NIMLTQSPSSLAVSAGEKVTMSCKSSQSVLYSSDQKNYLAWYQQKPGQSPKLLIYWASTRES GVPDRFTGSGSGTDFTLTISNVQAEDLAVYYCHQYLSSYTFGGGTKLEIK (SEQ ID NO:2).

296. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1 to 291, which comprises a VH comprising an amino acid sequence having at least 95% sequence identity to QVQLQQSDAELVKPGASVKISCKASGYTFTDHAIHWVKQKPEQGLEWIGYFSPGNGDIKYNEK FKGKATLTADRSSSTANMHLNSLTSEDSAVYFCKRSMANYFDYWGQGTTLTVSS (SEQ ID NO:23) and a VL comprising an amino acid sequence having at least 95% sequence identity to NIMMTQSPSSLVVSAGEKVTMSCKSSHSVLYSSNQKNYLAWYQQKPGQSPKLLIYWASTKNS GVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCHQYLSSYTFGGGTKLEIK (SEQ ID NO:24).

297. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1 to 291, which comprises a VH comprising an amino acid sequence having at least 97% sequence identity to QVQLQQSDAELVKPGASVKISCKASGYTFTDHAIHWVKQKPEQGLEWIGYFSPGNGDIKYNEK FKGKATLTADRSSSTANMHLNSLTSEDSAVYFCKRSMANYFDYWGQGTTLTVSS (SEQ ID NO:23) and a VL comprising an amino acid sequence having at least 97% sequence identity to NIMMTQSPSSLVVSAGEKVTMSCKSSHSVLYSSNQKNYLAWYQQKPGQSPKLLIYWASTKNS GVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCHQYLSSYTFGGGTKLEIK (SEQ ID NO:24).

298. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1 to 291, which comprises a VH comprising an amino acid sequence having at least 99% sequence identity to QVQLQQSDAELVKPGASVKISCKASGYTFTDHAIHWVKQKPEQGLEWIGYFSPGNGDIKYNEK FKGKATLTADRSSSTANMHLNSLTSEDSAVYFCKRSMANYFDYWGQGTTLTVSS (SEQ ID NO:23) and a VL comprising an amino acid sequence having at least 99% sequence identity to NIMMTQSPSSLVVSAGEKVTMSCKSSHSVLYSSNQKNYLAWYQQKPGQSPKLLIYWASTKNS GVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCHQYLSSYTFGGGTKLEIK (SEQ ID NO:24).

299. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1 to 291, which comprises a VH comprising the amino acid sequence of QVQLQQSDAELVKPGASVKISCKASGYTFTDHAIHWVKQKPEQGLEWIGYFSPGNGDIKYNEK FKGKATLTADRSSSTANMHLNSLTSEDSAVYFCKRSMANYFDYWGQGTTLTVSS (SEQ ID NO:23) and a VL comprising the amino acid sequence of NIMMTQSPSSLVVSAGEKVTMSCKSSHSVLYSSNQKNYLAWYQQKPGQSPKLLIYWASTKNS GVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCHQYLSSYTFGGGTKLEIK (SEQ ID NO:24).

300. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1 to 291, which comprises a VH comprising an amino acid sequence having at least 95% sequence identity to QVQLQQSDAELVEPGASVKISCKAYGYTFTDHAIHWVKQKPEQGLEWLGYISPGNDDIQYNAK FKGRATLTADKSSSTAYMQLNSLTSDDSAVYFCKRSMANSFDFWGQGTTLTVSS (SEQ ID NO:45) and a VL comprising an amino acid sequence having at least 95% sequence identity to NIMLTQSPSSLAVSAGEKVTMSCKSSQSVLYSSDQKNYLAWYQQKPGQSPKLLIYWASTRES GVPDRFTGSGSGTDFTLTISNVRAEDLAVYYCHQYLSSYTFGGGTKLEIK (SEQ ID NO:46).

301. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1 to 291, which comprises a VH comprising an amino acid sequence having at least 97% sequence identity to QVQLQQSDAELVEPGASVKISCKAYGYTFTDHAIHWVKQKPEQGLEWLGYISPGNDDIQYNAK FKGRATLTADKSSSTAYMQLNSLTSDDSAVYFCKRSMANSFDFWGQGTTLTVSS (SEQ ID NO:45) and a VL comprising an amino acid sequence having at least 97% sequence identity to NIMLTQSPSSLAVSAGEKVTMSCKSSQSVLYSSDQKNYLAWYQQKPGQSPKLLIYWASTRES GVPDRFTGSGSGTDFTLTISNVRAEDLAVYYCHQYLSSYTFGGGTKLEIK (SEQ ID NO:46).

302. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1 to 291, which comprises a VH comprising an amino acid sequence having at least 99% sequence identity to QVQLQQSDAELVEPGASVKISCKAYGYTFTDHAIHWVKQKPEQGLEWLGYISPGNDDIQYNAK FKGRATLTADKSSSTAYMQLNSLTSDDSAVYFCKRSMANSFDFWGQGTTLTVSS (SEQ ID NO:45) and a VL comprising an amino acid sequence having at least 99% sequence identity to NIMLTQSPSSLAVSAGEKVTMSCKSSQSVLYSSDQKNYLAWYQQKPGQSPKLLIYWASTRES GVPDRFTGSGSGTDFTLTISNVRAEDLAVYYCHQYLSSYTFGGGTKLEIK (SEQ ID NO:46).

303. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1 to 291, which comprises a VH comprising the amino acid sequence of QVQLQQSDAELVEPGASVKISCKAYGYTFTDHAIHWVKQKPEQGLEWLGYISPGNDDIQYNAK FKGRATLTADKSSSTAYMQLNSLTSDDSAVYFCKRSMANSFDFWGQGTTLTVSS (SEQ ID NO:45) and a VL comprising the amino acid sequence of NIMLTQSPSSLAVSAGEKVTMSCKSSQSVLYSSDQKNYLAWYQQKPGQSPKLLIYWASTRES GVPDRFTGSGSGTDFTLTISNVRAEDLAVYYCHQYLSSYTFGGGTKLEIK (SEQ ID NO:46).

304. An anti-glyco-MUC4 antibody or antigen-binding fragment that competes with a reference antibody or antigen binding fragment comprising:

(a) a heavy chain variable (VH) sequence of QVQLQQSDAELVKPGASVRISCKAYGYTFTDHAIHWVKQKPEQGLEWL GYISPGNDDIQYNAKFKGKATLTADKSSSTAYMQLNSLTSDDSAVYFCK RSMANSFDYWGQGTTLTVSS (SEQ ID NO:1) and a light chain variable (VL) sequence of NIMLTQSPSSLAVSAGEKVTMSCKSSQSVLYSSDQKNYLAWYQQKPG QSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISNVQAEDLAVYYCH QYLSSYTFGGGTKLEIK (SEQ ID NO:2);

(b) a heavy chain variable (VH) sequence of QVQLQQSDAELVKPGASVKISCKASGYTFTDHAIHWVKQKPEQGLEWI GYFSPGNGDIKYNEKFKGKATLTADRSSSTANMHLNSLTSEDSAVYFC KRSMANYFDYWGQGTTLTVSS (SEQ ID NO:23) and a light chain variable (VL) sequence of NIMMTQSPSSLVVSAGEKVTMSCKSSHSVLYSSNQKNYLAWYQQKPG QSPKLLIYWASTKNSGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCHQ YLSSYTFGGGTKLEIK (SEQ ID NO:24);

(c) a heavy chain variable (VH) sequence of QVQLQQSDAELVEPGASVKISCKAYGYTFTDHAIHWVKQKPEQGLEWL GYISPGNDDIQYNAKFKGRATLTADKSSSTAYMQLNSLTSDDSAVYFCK RSMANSFDFWGQGTTLTVSS (SEQ ID NO:45) and a light chain variable (VL) sequence of NIMLTQSPSSLAVSAGEKVTMSCKSSQSVLYSSDQKNYLAWYQQKPG QSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISNVRAEDLAVYYCHQ YLSSYTFGGGTKLEIK (SEQ ID NO:46); or

(d) a humanized heavy chain variable (VH) sequence of 2D5 (*e.g.*, SEQ ID NOS:133-144) and a humanized light chain variable (VL) sequence of 2D5 (*e.g.*, SEQ ID NOS:145-153),

for binding to a MUC4 peptide CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) that has been glycosylated with GalNAc on the serine and threonine residues shown with bold and underlined text ("the MUC4 glycopeptide"), the anti-glyco-MUC4 antibody or antigen-binding fragment comprising:

(a) a VH sequence with first, second and third CDR means within the VH sequence; and

(b) a VL sequence with fourth, fifth and sixth CDR means within the VL sequence,

wherein the first, second, third, fourth, fifth, and sixth CDR means cooperate to effect binding of the anti-glyco-MUC4 antibody or antigen-binding fragment to the MUC4 glycopeptide.

305. An anti-glyco-MUC4 antibody or antigen-binding fragment that competes with a reference antibody or antigen binding fragment comprising:

(a) a heavy chain variable (VH) sequence of QVQLQQSDAELVKPGASVRISCKAYGYTFTDHAIHWVKQKPEQGLEWL GYISPGNDDIQYNAKFKGKATLTADKSSSTAYMQLNSLTSDDSAVYFCK RSMANSFDYWGQGTTLTVSS (SEQ ID NO:1) and a light chain variable (VL) sequence of NIMLTQSPSSLAVSAGEKVTMSCKSSQSVLYSSDQKNYLAWYQQKPG QSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISNVQAEDLAVYYCH QYLSSYTFGGGTKLEIK (SEQ ID NO:2);

(b) a heavy chain variable (VH) sequence of QVQLQQSDAELVKPGASVKISCKASGYTFTDHAIHWVKQKPEQGLEWI GYFSPGNGDIKYNEKFKGKATLTADRSSSTANMHLNSLTSEDSAVYFC KRSMANYFDYWGQGTTLTVSS (SEQ ID NO:23) and a light chain variable (VL) sequence of NIMMTQSPSSLVVSAGEKVTMSCKSSHSVLYSSNQKNYLAWYQQKPG QSPKLLIYWASTKNSGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCHQ YLSSYTFGGGTKLEIK (SEQ ID NO:24);

(c) a heavy chain variable (VH) sequence of QVQLQQSDAELVEPGASVKISCKAYGYTFTDHAIHWVKQKPEQGLEWL GYISPGNDDIQYNAKFKGRATLTADKSSSTAYMQLNSLTSDDSAVYFCK RSMANSFDFWGQGTTLTVSS (SEQ ID NO:45) and a light chain variable (VL) sequence of NIMLTQSPSSLAVSAGEKVTMSCKSSQSVLYSSDQKNYLAWYQQKPG QSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISNVRAEDLAVYYCHQ YLSSYTFGGGTKLEIK (SEQ ID NO:46); or

(d) a humanized heavy chain variable (VH) sequence of 2D5 (*e.g.*, SEQ ID NOS:133-144) and a humanized light chain variable (VL) sequence of 2D5 (*e.g.*, SEQ ID NOS:145-153),

for binding to a MUC4 peptide CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) that has been glycosylated with GalNAc on the serine and threonine residues shown with bold and underlined text ("the MUC4 glycopeptide"), the anti-glyco-MUC4 antibody or antigen-binding fragment comprising a means for binding the MUC4 glycopeptide.

306. The anti-glyco-MUC4 antibody or antigen-binding fragment of embodiment 305, wherein the means for binding the MUC4 glycopeptide comprises a heavy chain variable (VH) domain and a light chain variable (VL) domain.

307. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 304 to 306, wherein the anti-glyco-MUC4 antibody or antigen-binding fragment competes with a reference antibody or antigen binding fragment comprising a VH sequence of QVQLQQSDAELVKPGASVRISCKAYGYTFTDHAIHWVKQKPEQGLEWLGYISPGNDDIQYNAK FKGKATLTADKSSSTAYMQLNSLTSDDSAVYFCKRSMANSFDYWGQGTTLTVSS (SEQ ID NO:1) and a VL sequence of NIMLTQSPSSLAVSAGEKVTMSCKSSQSVLYSSDQKNYLAWYQQKPGQSPKLLIYWASTRES GVPDRFTGSGSGTDFTLTISNVQAEDLAVYYCHQYLSSYTFGGGTKLEIK (SEQ ID NO:2).

308. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 304 to 306, wherein the anti-glyco-MUC4 antibody or antigen-binding fragment competes with a reference antibody or antigen binding fragment comprising a VH sequence of QVQLQQSDAELVKPGASVKISCKASGYTFTDHAIHWVKQKPEQGLEWIGYFSPGNGDIKYNEK FKGKATLTADRSSSTANMHLNSLTSEDSAVYFCKRSMANYFDYWGQGTTLTVSS (SEQ ID NO:23) and a VL sequence of QVQLQQSDAELVKPGASVKISCKASGYTFTDHAIHWVKQKPEQGLEWIGYFSPGNGDIKYNEK FKGKATLTADRSSSTANMHLNSLTSEDSAVYFCKRSMANYFDYWGQGTTLTVSS (SEQ ID NO:23).

309. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 304 to 306, wherein the anti-glyco-MUC4 antibody or antigen-binding fragment competes with a reference antibody or antigen binding fragment comprising a VH sequence of QVQLQQSDAELVEPGASVKISCKAYGYTFTDHAIHWVKQKPEQGLEWLGYISPGNDDIQYNAK FKGRATLTADKSSSTAYMQLNSLTSDDSAVYFCKRSMANSFDFWGQGTTLTVSS (SEQ ID NO:45) and a VL sequence of NIMLTQSPSSLAVSAGEKVTMSCKSSQSVLYSSDQKNYLAWYQQKPGQSPKLLIYWASTRES GVPDRFTGSGSGTDFTLTISNVRAEDLAVYYCHQYLSSYTFGGGTKLEIK (SEQ ID NO:46).

310. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 304 to 306, wherein the anti-glyco-MUC4 antibody or antigen-binding fragment competes with a reference antibody or antigen binding fragment comprising a humanized heavy chain variable (VH) sequence of 2D5 (*e.g.*, SEQ ID NOS:133-144) and a humanized light chain variable (VL) sequence of 2D5 (*e.g.*, SEQ ID NOS:145-153).

311. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 1 to 310, which preferentially binds to a glyco-MUC4 epitope that is overexpressed on cancer cells as compared to normal cells.

312. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 1 to 311, which specifically binds to a MUC4 peptide

CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) that has been glycosylated with STn on the serine and threonine residues shown with bold and underlined text.

313. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 1 to 311, which does not specifically bind to a MUC4 peptide CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) that has been glycosylated with STn on the serine and threonine residues shown with bold and underlined text.

314. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 313, which binds to the MUC4 glycopeptide with a binding affinity (KD) of 1 nM to 200 nM as measured by surface plasmon resonance or bio-layer interferometry.

315. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 313, which binds to the MUC4 glycopeptide with a binding affinity (KD) of 1 nM to 150 nM as measured by surface plasmon resonance or bio-layer interferometry.

316. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 313, which binds to the MUC4 glycopeptide with a binding affinity (KD) of 1 nM to 100 nM as measured by surface plasmon resonance or bio-layer interferometry.

317. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 313, which binds to the MUC4 glycopeptide with a binding affinity (KD) of 1 nM to 50 nM as measured by surface plasmon resonance or bio-layer interferometry.

318. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 313, which binds to the MUC4 glycopeptide with a binding affinity (KD) of 5 nM to 200 nM as measured by surface plasmon resonance or bio-layer interferometry.

319. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 313, which binds to the MUC4 glycopeptide with a binding affinity (KD) of 5 nM to 100 nM as measured by surface plasmon resonance or bio-layer interferometry.

320. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 313, which binds to the MUC4 glycopeptide with a binding affinity (KD) of 5 nM to 50 nM as measured by surface plasmon resonance or bio-layer interferometry.

321. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 313, which binds to the MUC4 glycopeptide with a binding affinity (KD) of 5 nM to 25 nM as measured by surface plasmon resonance or bio-layer interferometry.

322. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 313, which binds to the MUC4 glycopeptide with a binding affinity (KD) of 5 nM to 10 nM as measured by surface plasmon resonance or bio-layer interferometry.

323. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 313, which binds to the MUC4 glycopeptide with a binding affinity (KD) of 10 nM to 200 nM as measured by surface plasmon resonance or bio-layer interferometry.

324. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 313, which binds to the MUC4 glycopeptide with a binding affinity (KD) of 10 nM to 100 nM as measured by surface plasmon resonance or bio-layer interferometry.

325. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 313, which binds to the MUC4 glycopeptide with a binding affinity (KD) of 10 nM to 150 nM as measured by surface plasmon resonance or bio-layer interferometry.

326. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 313, which binds to the MUC4 glycopeptide with a binding affinity (KD) of 10 nM to 100 nM as measured by surface plasmon resonance or bio-layer interferometry.

327. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 313, which binds to the MUC4 glycopeptide with a binding affinity (KD) of 10 nM to 50 nM as measured by surface plasmon resonance or bio-layer interferometry.

328. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 313, which binds to the MUC4 glycopeptide with a binding affinity (KD) of 10 nM to 25 nM as measured by surface plasmon resonance or bio-layer interferometry.

329. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 313, which binds to the MUC4 glycopeptide with a binding affinity (KD) of 50 nM to 200 nM as measured by surface plasmon resonance or bio-layer interferometry.

330. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 313, which binds to the MUC4 glycopeptide with a binding affinity (KD) of 50 nM to 150 nM as measured by surface plasmon resonance or bio-layer interferometry.

331. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 313, which binds to the MUC4 glycopeptide with a binding affinity (KD) of 50 nM to 100 nM as measured by surface plasmon resonance or bio-layer interferometry.

332. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 313, which binds to the MUC4 glycopeptide with a binding affinity (KD) of 100 nM to 200 nM as measured by surface plasmon resonance or bio-layer interferometry.

333. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 313, which binds to the MUC4 glycopeptide with a binding affinity (KD) of 100 nM to 150 nM as measured by surface plasmon resonance or bio-layer interferometry.

334. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 314 to 333, in which the binding affinity to the MUC4 glycopeptide is as measured by surface plasmon resonance.

335. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 314 to 333, in which the binding affinity to the MUC4 glycopeptide is as measured by bio-layer interferometry.

336. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 335, which does not specifically bind to the unglycosylated MUC4 peptide CTIPSTAMHTRSTAAPIPILP (SEQ ID NO:155) (the "unglycosylated MUC4 peptide").

337. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 336, which has a binding affinity to the MUC4 glycopeptide which is at least 3 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the unglycosylated MUC4 peptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the unglycosylated MUC4 peptide (e.g., about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

338. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 337, which has a binding affinity to the MUC4 glycopeptide which is at least 5 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the unglycosylated MUC4 peptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the unglycosylated MUC4 peptide (e.g., about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

339. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 338, which has a binding affinity to the MUC4 glycopeptide which is at least 10 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the unglycosylated MUC4 peptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the unglycosylated MUC4 peptide (e.g., about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

340. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 339, which has a binding affinity to the MUC4 glycopeptide which is at least 20 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the unglycosylated MUC4 peptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the unglycosylated MUC4 peptide (e.g., about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

341. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 340, which has a binding affinity to the MUC4 glycopeptide which is at least 50 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the

unglycosylated MUC4 peptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the unglycosylated MUC4 peptide (e.g., about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

342. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 341, which has a binding affinity to the MUC4 glycopeptide which is at least 100 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the unglycosylated MUC4 peptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the unglycosylated MUC4 peptide (e.g., about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

343. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 342, which does not specifically bind to the MUC1 tandem repeat $(VTSAPDTRPAPGSTAPPAHG)_3$ (SEQ ID NO:201) that has been glycosylated in vitro using purified recombinant human glycosyltransferases GalNAc-T1, GalNAc-T2, and GalNAc-T4 ("the first MUC1 glycopeptide").

344. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 343, which has a binding affinity to the MUC4 glycopeptide which is at least 3 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the first MUC1 glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the first MUC1 peptide (e.g., about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

345. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 344, which has a binding affinity to the MUC4 glycopeptide which is at least 5 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the first MUC1 glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the first MUC1 peptide (e.g., about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

346. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 345, which has a binding affinity to the MUC4 glycopeptide which is at least 10 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the first MUC1 glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the

presence of saturating amounts of either the MUC4 glycopeptide or the first MUC1 peptide (e.g., about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

347. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 346, which has a binding affinity to the MUC4 glycopeptide which is at least 20 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the first MUC1 glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the first MUC1 peptide (e.g., about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

348. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 347, which has a binding affinity to the MUC4 glycopeptide which is at least 50 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the first MUC1 glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the first MUC1 peptide (e.g., about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

349. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 348, which has a binding affinity to the MUC4 glycopeptide which is at least 100 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the first MUC1 glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the first MUC1 peptide (e.g., about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

350. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 349, which does not specifically bind to the MUC1 peptide TAPPAHGV**TS**APD**T**RPAPG**ST**APPAHGVT (SEQ ID NO:202) that has been glycosylated in vitro with GalNAc on the serine and threonine residues shown with bold and underlined text (the “second MUC1 glycopeptide”).

351. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 350, which has a binding affinity to the MUC4 glycopeptide which is at least 3 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the second MUC1 glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the second MUC1 peptide (e.g., about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

352. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 351, which has a binding affinity to the MUC4 glycopeptide which is at least 5 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the

second MUC1 glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the second MUC1 peptide (e.g., about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

353. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 352, which has a binding affinity to the MUC4 glycopeptide which is at least 10 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the second MUC1 glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the second MUC1 peptide (e.g., about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

354. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 353, which has a binding affinity to the MUC4 glycopeptide which is at least 20 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the second MUC1 glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the second MUC1 peptide (e.g., about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

355. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 354, which has a binding affinity to the MUC4 glycopeptide which is at least 50 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the second MUC1 glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the second MUC1 peptide (e.g., about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

356. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 355, which has a binding affinity to the MUC4 glycopeptide which is at least 100 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the second MUC1 glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the second MUC1 peptide (e.g., about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

357. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 356, which does not specifically bind to the CD44v6 peptide GYRQ**T**PKEDSH**S**TTGTAAA (SEQ ID NO:218) that has been glycosylated in vitro with GalNAc on the threonine and serine residues shown with bold and underlined text (the “CD44v6 glycopeptide”).

358. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 357, which has a binding affinity to the MUC4 glycopeptide which is at least 3 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the CD44v6 glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the CD44v6 glycopeptide (*e.g.*, about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

359. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 358, which has a binding affinity to the MUC4 glycopeptide which is at least 5 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the CD44v6 glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the CD44v6 glycopeptide (*e.g.*, about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

360. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 359, which has a binding affinity to the MUC4 glycopeptide which is at least 10 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the CD44v6 glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the CD44v6 glycopeptide (*e.g.*, about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

361. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 360, which has a binding affinity to the MUC4 glycopeptide which is at least 20 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the CD44v6 glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the CD44v6 glycopeptide (*e.g.*, about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

362. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 361, which has a binding affinity to the MUC4 glycopeptide which is at least 50 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the CD44v6 glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the CD44v6 glycopeptide (*e.g.*, about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

363. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 362, which has a binding affinity to the MUC4 glycopeptide which is at least 100 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to

the CD44v6 glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the CD44v6 glycopeptide (*e.g.*, about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

364. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 363, which does not specifically bind to the LAMP1 peptide CEQDRP**S**P**TT**APPAPPSPSP (SEQ ID NO:219) that has been glycosylated in vitro with GalNAc on the serine and threonine residues shown with bold and underlined text (the "LAMP1 glycopeptide").

365. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 364, which has a binding affinity to the MUC4 glycopeptide which is at least 3 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the LAMP1 glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the LAMP1 glycopeptide (*e.g.*, about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

366. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 365, which has a binding affinity to the MUC4 glycopeptide which is at least 5 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the LAMP1 glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the LAMP1 glycopeptide (*e.g.*, about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

367. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 366, which has a binding affinity to the MUC4 glycopeptide which is at least 10 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the LAMP1 glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the LAMP1 glycopeptide (*e.g.*, about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

368. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 367, which has a binding affinity to the MUC4 glycopeptide which is at least 20 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the LAMP1 glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the LAMP1 glycopeptide (*e.g.*, about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

369. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 368, which has a binding affinity to the MUC4 glycopeptide which is at least 50 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the LAMP1 glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the LAMP1 glycopeptide (*e.g.*, about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

370. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 369, which has a binding affinity to the MUC4 glycopeptide which is at least 100 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the LAMP1 glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the LAMP1 glycopeptide (*e.g.*, about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

371. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 370, which does not specifically bind to the cMET peptide PTKSFISGG**ST**ITGVGKNLN (SEQ ID NO:220) that has been glycosylated in vitro with GalNAc on the serine and threonine residues shown with bold and underlined text (the "cMET glycopeptide").

372. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 371, which has a binding affinity to the MUC4 glycopeptide which is at least 3 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the cMET glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the cMET glycopeptide (*e.g.*, about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

373. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 372, which has a binding affinity to the MUC4 glycopeptide which is at least 5 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the cMET glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the cMET glycopeptide (*e.g.*, about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

374. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 373, which has a binding affinity to the MUC4 glycopeptide which is at least 10 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the cMET glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the

presence of saturating amounts of either the MUC4 glycopeptide or the cMET glycopeptide (*e.g.*, about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

375. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 374, which has a binding affinity to the MUC4 glycopeptide which is at least 20 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the cMET glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the cMET glycopeptide (*e.g.*, about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

376. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 375, which has a binding affinity to the MUC4 glycopeptide which is at least 50 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the cMET glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the cMET glycopeptide (*e.g.*, about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

377. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 376, which has a binding affinity to the MUC4 glycopeptide which is at least 100 times the binding affinity of the anti-glyco-MUC4 antibody or antigen-binding fragment to the cMET glycopeptide, optionally wherein the binding affinity is measured by surface plasmon resonance, and further optionally where in the surface plasmon resonance is measured in the presence of saturating amounts of either the MUC4 glycopeptide or the cMET glycopeptide (*e.g.*, about 1 μM, about 1.5 μM, or about 2 μM of either peptide).

378. An anti-glyco-MUC4 antibody or antigen-binding fragment comprising a means for binding a MUC4 epitope that is overexpressed on cancer cells as compared to normal cells.

379. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 378, wherein the means for binding the MUC4 epitope comprises a heavy chain variable (VH) domain and a light chain variable (VL) domain.

380. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 379, which is multivalent.

381. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 380, which is an antigen-binding fragment.

382. The anti-glyco-MUC4 antibody or antigen-binding fragment of embodiment 381, wherein the antigen-binding fragment is in the form of a single-chain variable fragment (scFv).

383. The anti-glyco-MUC4 antibody or antigen-binding fragment of embodiment 382, wherein the scFv comprises the heavy chain variable fragment N-terminal to the light chain variable fragment.

384. The anti-glyco-MUC4 antibody or antigen-binding fragment of embodiment 382, wherein the scFv comprises the heavy chain variable fragment C-terminal to the light chain variable fragment.

385. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 382 to 384, wherein the scFv heavy chain variable fragment and light chain variable fragment are covalently bound to a linker sequence, which is optionally 4-15 amino acids.

386. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 380, which is in the form of a multispecific antibody.

387. An anti-glyco-MUC4 antibody comprising a means for binding a MUC4 epitope that is overexpressed on cancer cells as compared to normal cells, which is in the form of a multispecific antibody.

388. The anti-glyco-MUC4 antibody of embodiment 387, wherein the means for binding the MUC4 epitope comprises a heavy chain variable (VH) domain and a light chain variable (VL) domain.

389. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 386 to 388, wherein the multispecific antibody is a bispecific antibody that binds to a second epitope that is different from the first epitope.

390. The anti-glyco-MUC4 antibody or antigen-binding fragment of embodiment 389, wherein the bispecific antibody is a bottle opener, mAb-Fv, mAb-scFv, central-scFv, one-armed central-scFv, or dual scFv format bispecific antibody.

391. The anti-glyco-MUC4 antibody or antigen-binding fragment of embodiment 390, wherein the bispecific antibody is a bottle opener format bispecific antibody.

392. The anti-glyco-MUC4 antibody or antigen-binding fragment of embodiment 390, wherein the bispecific antibody is a mAb-Fv format bispecific antibody.

393. The anti-glyco-MUC4 antibody or antigen-binding fragment of embodiment 390, wherein the bispecific antibody is a mAb-scFv format bispecific antibody.

394. The anti-glyco-MUC4 antibody or antigen-binding fragment of embodiment 390, wherein the bispecific antibody is a central-scFv format bispecific antibody.

395. The anti-glyco-MUC4 antibody or antigen-binding fragment of embodiment 390, wherein the bispecific antibody is a one-armed central-scFv format bispecific antibody.

396. The anti-glyco-MUC4 antibody or antigen-binding fragment of embodiment 390, wherein the bispecific antibody is a dual scFv format bispecific antibody.

397. The anti-glyco-MUC4 antibody or antigen-binding fragment of embodiment 389, wherein the bispecific antibody is a bispecific domain-exchanged antibody (*e.g.*, a CrossMab), a Fab-arm exchange antibody, a bispecific T-cell engager (BiTE), or a dual-affinity retargeting molecule (DART).

398. The anti-glyco-MUC4 antibody or antigen-binding fragment of embodiment 397, wherein the bispecific antibody is a bispecific domain-exchanged antibody (*e.g.*, a CrossMab).

399. The anti-glyco-MUC4 antibody or antigen-binding fragment of embodiment 398, wherein the bispecific antibody is a bispecific IgG comprising a Fab-arm having a domain crossover between heavy and light chains (*e.g.*, a CrossMabFAB).

400. The anti-glyco-MUC4 antibody or antigen-binding fragment of embodiment 398, wherein the bispecific antibody is a bispecific IgG comprising a Fab-arm having a domain crossover between variable heavy and variable light chains (*e.g.*, a CrossMabVH-VL).

401. The anti-glyco-MUC4 antibody or antigen-binding fragment of embodiment 398, wherein the bispecific antibody is a bispecific IgG comprising a Fab-arm having a domain crossover between constant heavy and constant light chains (*e.g.*, a CrossMabCH1-CL).

402. The anti-glyco-MUC4 antibody or antigen-binding fragment of embodiment 397, wherein the bispecific antibody is a Fab-arm exchange antibody.

403. The anti-glyco-MUC4 antibody or antigen-binding fragment of embodiment 390, wherein the bispecific antibody is a dual-affinity retargeting molecule (DART).

404. The anti-glyco-MUC4 antibody or antigen-binding fragment of embodiment 390, wherein the bispecific antibody is a bispecific T-cell engager (BiTE).

405. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 389 to 404, wherein the second epitope is a MUC4 epitope.

406. The anti-glyco-MUC4 antibody of antigen-binding fragment of any one of embodiments 389 to 404, wherein the second epitope is a MUC4 epitope that is overexpressed on cancer cells as compared to normal cells.

407. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 389 to 404, wherein the second epitope is a T-cell epitope.

408. The anti-glyco-MUC4 antibody or antigen-binding fragment of embodiment 407, wherein the T-cell epitope comprises a CD3 epitope, a CD8 epitope, a CD16 epitope, a CD25 epitope, a CD28 epitope, or an NKG2D epitope.

409. The anti-glyco-MUC4 antibody or antigen-binding fragment of embodiment 408, wherein the T-cell epitope comprises a CD3 epitope, which is optionally an epitope present in human CD3.

410. The anti-glyco-MUC4 antibody or antigen-binding fragment of embodiment 409, wherein the CD3 epitope comprises a CD3 gamma epitope, a CD3 delta epitope, a CD3 epsilon epitope, or a CD3 zeta epitope.

411. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of embodiments 1 to 410 which is conjugated to a detectable moiety.

412. The anti-glyco-MUC4 antibody or antigen binding fragment of embodiment 411 in which the detectable moiety is an enzyme, a radioisotope, or a fluorescent label.

413. The anti-glyco-MUC4 antibody or antigen binding fragment of any one of embodiments 1 to 412, wherein the anti-glyco-MUC4 antibody or antigen binding fragment is not a Tn-MUC4 binding polypeptide produced by the cell line 4D9 deposited with the European Collection of Authenticated Cell Cultures (ECACC) under accession number 09120102.

414. The anti-glyco-MUC4 antibody or antigen binding fragment of any one of embodiments 1 to 412, wherein the anti-glyco-MUC4 antibody or antigen binding fragment is not a Tn-MUC4 binding polypeptide produced by the cell line 6E3 deposited with the European Collection of Authenticated Cell Cultures (ECACC) under accession number 09120103.

415. A bispecific antibody comprising (a) a means for binding a MUC4 epitope that is overexpressed on cancer cells as compared to normal cells and (b) a means for binding a T-cell epitope, optionally wherein the bispecific antibody has the features described in any one of embodiments 389 to 414.

416. The bispecific antibody of embodiment 415, wherein the means for binding the MUC4 epitope comprises a heavy chain variable (VH) domain and a light chain variable (VL) domain.

417. The bispecific antibody of embodiment 415 or embodiment 416, wherein the means for binding the T-cell epitope comprises a heavy chain variable (VH) domain and a light chain variable (VL) domain.

418. The bispecific antibody of any one of embodiments 415 to 417, wherein the T-cell epitope comprises a CD3 epitope, a CD8 epitope, a CD16 epitope, a CD25 epitope, a CD28 epitope, or an NKG2D epitope.

419. The bispecific antibody of embodiment 418, wherein the T-cell epitope comprises a CD3 epitope, which is optionally an epitope present in human CD3.

420. The bispecific antibody of embodiment 419, wherein the CD3 epitope comprises a CD3 gamma epitope, a CD3 delta epitope, a CD3 epsilon epitope, or a CD3 zeta epitope.

421. A fusion protein comprising the amino acid sequence of the anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 414 or the bispecific antibody of any one of embodiments 415 to 420 operably linked to at least a second amino acid sequence.

422. The fusion protein of embodiment 421, wherein the second amino acid sequence is that of 4-1BB, CD2, CD3-zeta, or a fragment thereof.

423. The fusion protein of embodiment 421, wherein the second amino acid sequence is that of a fusion peptide.

424. The fusion protein of embodiment 423, wherein the fusion peptide is a CD28-CD3-zeta, a 4-1BB (CD137)-CD3-zeta fusion peptide, a CD2-CD3-zeta fusion peptide, a CD28-CD2-CD3-zeta fusion peptide, or a 4-1BB (CD137)-CD2-CD3-zeta fusion peptide.

425. The fusion protein of embodiment 421, wherein the second amino acid sequence is that of a modulator of T cell activation or a fragment thereof.

426. The fusion protein of embodiment 425, wherein the modulator of T cell activation is IL-15 or IL-15Rα.

427. The fusion protein of embodiment 421, wherein the second amino acid sequence is that of a MIC protein domain.

428. The fusion protein of embodiment 427, wherein the MIC protein domain is an α1-α2 domain.

429. The fusion protein of embodiment 428, wherein the α1-α2 domain is a MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, or OMCP α1-α2 domain.

430. The fusion protein of any one of embodiments 427 to 429, wherein the MIC protein domain is an engineered MIC protein domain.

431. The fusion protein of embodiment 421, wherein the second amino acid sequence is that of a neuraminidase (EC 3.2.1.18 or EC 3.2.1.129).

432. The fusion protein of embodiment 431, wherein the neuraminidase amino acid sequence is derived from *Micromonospora viridifaciens*.

433. The fusion protein of embodiment 431 or 432, wherein the neuraminidase comprises an amino acid sequence having at least 95% sequence identity to GGSPVPPGGEPLYTEQDLAVNGREGFPNYRIPALTVTPDGDLLASYDGRPTGIDAPGPNSILQ RRSTDGGRTWGEQQVVSAGQTTAPIKGFSDPSYLVDRETGTIFNFHVYSQRQGFAGSRPGTD PADPNVLHANVATSTDGGLTWSHRTITADITPDPGWRSRFAASGEGIQLRYGPHAGRLIQQYTI INAAGAFQAVSVYSDDHGRTWRAGEAVGVGMDENKTVELSDGRVLLNSRDSARSGYRKVAV STDGGHSYGPVTIDRDLPDPTNNASIIRAFPDAPAGSARAKVLLFSNAASQTSRSQGTIRMSCD DGQTWPVSKVFQPGSMSYSTLTALPDGTYGLLYEPGTGIRYANFNLAWLGG (SEQ ID NO:222).

434. The fusion protein of any one of embodiments 431 to 433, wherein the neuraminidase comprises an amino acid sequence having at least 97% sequence identity to GGSPVPPGGEPLYTEQDLAVNGREGFPNYRIPALTVTPDGDLLASYDGRPTGIDAPGPNSILQ RRSTDGGRTWGEQQVVSAGQTTAPIKGFSDPSYLVDRETGTIFNFHVYSQRQGFAGSRPGTD PADPNVLHANVATSTDGGLTWSHRTITADITPDPGWRSRFAASGEGIQLRYGPHAGRLIQQYTI INAAGAFQAVSVYSDDHGRTWRAGEAVGVGMDENKTVELSDGRVLLNSRDSARSGYRKVAV STDGGHSYGPVTIDRDLPDPTNNASIIRAFPDAPAGSARAKVLLFSNAASQTSRSQGTIRMSCD DGQTWPVSKVFQPGSMSYSTLTALPDGTYGLLYEPGTGIRYANFNLAWLGG (SEQ ID NO:222).

435. The fusion protein of any one of embodiments 431 to 434, wherein the neuraminidase comprises an amino acid sequence having at least 98% sequence identity to GGSPVPPGGEPLYTEQDLAVNGREGFPNYRIPALTVTPDGDLLASYDGRPTGIDAPGPNSILQ RRSTDGGRTWGEQQVVSAGQTTAPIKGFSDPSYLVDRETGTIFNFHVYSQRQGFAGSRPGTD PADPNVLHANVATSTDGGLTWSHRTITADITPDPGWRSRFAASGEGIQLRYGPHAGRLIQQYTI INAAGAFQAVSVYSDDHGRTWRAGEAVGVGMDENKTVELSDGRVLLNSRDSARSGYRKVAV

STDGGHSYGPVTIDRDLPDPTNNASIIRAFPDAPAGSARAKVLLFSNAASQTSRSQGTIRMSCD DGQTWPVSKVFQPGSMSYSTLTALPDGTYGLLYEPGTGIRYANFNLAWLGG (SEQ ID NO:222).

436. The fusion protein of any one of embodiments 431 to 435, wherein the neuraminidase comprises an amino acid sequence having at least 99% sequence identity to GGSPVPPGGEPLYTEQDLAVNGREGFPNYRIPALTVTPDGDLLASYDGRPTGIDAPGPNSILQ RRSTDGGRTWGEQQVVSAGQTTAPIKGFSDPSYLVDRETGTIFNFHVYSQRQGFAGSRPGTD PADPNVLHANVATSTDGGLTWSHRTITADITPDPGWRSRFAASGEGIQLRYGPHAGRLIQQYTI INAAGAFQAVSVYSDDHGRTWRAGEAVGVGMDENKTVELSDGRVLLNSRDSARSGYRKVAV STDGGHSYGPVTIDRDLPDPTNNASIIRAFPDAPAGSARAKVLLFSNAASQTSRSQGTIRMSCD DGQTWPVSKVFQPGSMSYSTLTALPDGTYGLLYEPGTGIRYANFNLAWLGG (SEQ ID NO:222).

437. The fusion protein of any one of embodiments 431 to 436, wherein the neuraminidase comprises the amino acid GGSPVPPGGEPLYTEQDLAVNGREGFPNYRIPALTVTPDGDLLASYDGRPTGIDAPGPNSILQ RRSTDGGRTWGEQQVVSAGQTTAPIKGFSDPSYLVDRETGTIFNFHVYSQRQGFAGSRPGTD PADPNVLHANVATSTDGGLTWSHRTITADITPDPGWRSRFAASGEGIQLRYGPHAGRLIQQYTI INAAGAFQAVSVYSDDHGRTWRAGEAVGVGMDENKTVELSDGRVLLNSRDSARSGYRKVAV STDGGHSYGPVTIDRDLPDPTNNASIIRAFPDAPAGSARAKVLLFSNAASQTSRSQGTIRMSCD DGQTWPVSKVFQPGSMSYSTLTALPDGTYGLLYEPGTGIRYANFNLAWLGG (SEQ ID NO:222).

438. The fusion protein of any one of embodiments 431 to 437, which comprises a signal sequence.

439. The fusion protein of embodiment 438, wherein the signal sequence is a granulysin signal sequence.

440. The fusion protein of embodiment 438, wherein the signal sequence is a granzymeK signal sequence.

441. The fusion protein of embodiment 438, wherein the signal sequence is an NPY signal sequence.

442. The fusion protein of embodiment 438, wherein the signal sequence is an IFN signal sequence.

443. The fusion protein of any one of embodiments 431 to 442, which comprises a self-cleaving peptide sequence.

444. The fusion protein of embodiment 443, wherein the self-cleaving peptide sequence is a 2A peptide.

445. The fusion protein of embodiment 444, wherein the 2A peptide is T2A.

446. A chimeric antigen receptor (CAR) comprising one or more antigen-binding fragments according to any one of embodiments 381 to 385.

447. The CAR of embodiment 446, which comprises one or more scFvs according to any one of embodiments 382 to 385.

448. The CAR of embodiment 447, which comprises one scFv according to any one of embodiments 382 to 385.

449. The CAR of embodiment 448, which comprises two scFvs according to any one of embodiments 382 to 385.

450. The CAR of embodiment 449, wherein the two scFvs have the same amino acid sequence.

451. The CAR of embodiment 449 or 450, wherein the two scFvs are covalently bound by a linker sequence, which is optionally 4-15 amino acids.

452. The CAR of any one of embodiments 446 to 451, comprising in amino- to carboxy-terminal order: (i) the one or more antigen-binding fragments, (ii) a transmembrane domain, and (iii) an intracellular signaling domain.

453. A chimeric antigen receptor (CAR) comprising in amino- to carboxy-terminal order: (i) one or more means for binding a MUC4 epitope that is overexpressed on cancer cells as compared to normal cells, (ii) a transmembrane domain, and (iii) an intracellular signaling domain.

454. The CAR of embodiment 453, wherein the means for binding the MUC4 epitope comprises a heavy chain variable (VH) domain and a light chain variable (VL) domain.

455. The CAR of any one of embodiments 452 to 454, wherein the transmembrane domain comprises a CD28 transmembrane domain.

456. The CAR of embodiment 455, wherein the CD28 transmembrane domain comprises the amino acid sequence FWVLVVVGGVLACYSLLVTVAFIIFWV (SEQ ID NO:163).

457. The CAR of any one of embodiments 452 to 456, wherein the intracellular signaling domain comprises a co-stimulatory signaling region.

458. The CAR of embodiment 457, wherein the co-stimulatory signaling region comprises a signaling portion of, or the entire, cytoplasmic domain of CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, DAP10, GITR, or a combination thereof.

459. The CAR of embodiment 458, wherein the CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, DAP10, or GITR a human CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, DAP10, or GITR.

460. The CAR of embodiment 458 or embodiment 459, wherein a signaling portion of, or the entire co-stimulatory signaling domain comprises the cytoplasmic domain of CD2.

461. The CAR of embodiment 460, wherein the cytoplasmic domain of CD2 comprises the amino acid sequence TKRKKQRSRRNDEELETRAHRVATEERGRKPHQIPASTPQNPATSQHPPPPPGHRSQAPSHR PPPPGHRVQHQPQKRPPAPSGTQVHQQKGPPLPRPRVQPKPPHGAAENSLSPSSN (SEQ ID NO:217).

462. The CAR of any one of embodiments 458 to 461, wherein the co-stimulatory signaling domain comprises a signaling portion of, or the entire, cytoplasmic domain of CD28.

463. The CAR of embodiment 462, wherein the cytoplasmic domain of CD28 comprises the amino acid sequence RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS (SEQ ID NO:169).

464. The CAR of any one of embodiments 452 to 463, wherein the intracellular signaling domain comprises a T cell signaling domain.

465. The CAR of embodiment 464, wherein the T cell signaling domain is C-terminal to the co-stimulatory signaling region.

466. The CAR of embodiment 464 or embodiment 465, wherein the T cell signaling domain comprises a CD3-zeta signaling domain.

467. The CAR of embodiment 466, wherein the CD3-zeta signaling domain comprises the amino acid sequence RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYN ELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO:168).

468. The CAR of any one of embodiments 452 to 467, which further comprises a signal peptide N-terminal to the one or more antibody fragments, one or more scFvs or one or more means for binding a MUC4 epitope.

469. The CAR of embodiment 468, wherein the signal peptide is a human CD8 signal peptide.

470. The CAR of embodiment 469, wherein the human CD8 signal peptide comprises the amino acid sequence MALPVTALLLPLALLLHAARP (SEQ ID NO:161).

471. The CAR of any one of embodiments 452 to 470, which further comprises a hinge between (a) the one or more antigen-binding fragments or the one or more means for binding a MUC4 epitope and (b) the transmembrane domain.

472. The CAR of embodiment 471, wherein the hinge comprises a human CD8a hinge.

473. The CAR of embodiment 472, wherein the human CD8a hinge comprises the amino acid sequence TTTPAPRPPTPAPTIASPLSLRPEACRPAAGGAVHTRGLDFAC (SEQ ID NO:164).

474. The CAR of embodiment 472, wherein the human CD8a hinge comprises the amino acid sequence TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO:223).

475. The CAR of embodiment 471, wherein the hinge comprises a human IgG4-short hinge comprising the amino acid sequence ESKYGPPCPSCP (SEQ ID NO:166).

476. The CAR of embodiment 471, wherein the hinge comprises a human IgG4-long hinge comprising the amino acid sequence ESKYGPPCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDG VEVHNAKTKPREEQFQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPR EPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGKM (SEQ ID NO:167).

477. A chimeric antigen receptor (CAR), whose amino acid sequence comprises the amino acid sequence of 2D5-CART of Table 14 (SEQ ID NO:206).

478. A chimeric antigen receptor (CAR), whose amino acid sequence comprises the amino acid sequence of 15F3-CART of Table 14 (SEQ ID NO:207).

479. A chimeric antigen receptor (CAR), whose amino acid sequence comprises the amino acid sequence of 5B8-CART of Table 14 (SEQ ID NO:208).

480. An antibody-drug conjugate comprising the anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 414, the bispecific antibody of any one of embodiments 415 to 420, or the fusion protein of any one of embodiments 421 to 445 conjugated to a cytotoxic agent.

481. The antibody-drug conjugate of embodiment 480, wherein the cytotoxic agent is an auristatin, a DNA minor groove binding agent, an alkylating agent, an enediyne, a lexitropsin, a duocarmycin, a taxane, a dolastatin, a maytansinoid, a vinca alkaloid, or an amanitin toxin.

482. The antibody-drug conjugate of embodiment 481, wherein the anti-glyco-MUC4 antibody or antigen-binding fragment or bispecific antibody is conjugated to the cytotoxic agent via a linker.

483. The antibody-drug conjugate of embodiment 482, wherein the linker is cleavable under intracellular conditions.

484. The antibody-drug conjugate of embodiment 483, wherein the cleavable linker is cleavable by an intracellular protease.

485. The antibody-drug conjugate of embodiment 484, wherein the linker comprises a dipeptide.

486. The antibody-drug conjugate of embodiment 485, wherein the dipeptide is val-cit or phe-lys.

487. The antibody-drug conjugate of embodiment 483, wherein the cleavable linker is hydrolyzable at a pH of less than 5.5.

488. The antibody-drug conjugate of embodiment 487, wherein the hydrolyzable linker is a hydrazone linker.

489. The antibody-drug conjugate of embodiment 483, wherein the cleavable linker is a disulfide linker.

490. A chimeric T cell receptor (TCR) comprising:

(a) an antigen-binding fragment according to any one of embodiments 381 to 385;

(b) a first polypeptide chain comprising a first TCR domain comprising a first TCR transmembrane domain from a first TCR subunit; and

(c) a second polypeptide chain comprising a second TCR domain comprising a second TCR transmembrane domain from a second TCR subunit.

491. The chimeric TCR of embodiment 490, which comprises one or more scFvs according to any one of embodiments 382 to 385.

492. The chimeric TCR of embodiment 490 or 491, which comprises one scFv according to any one of embodiments 382 to 385.

493. A chimeric T cell receptor (TCR) comprising:

(a) a means for binding a MUC4 epitope that is overexpressed on cancer cells as compared to normal cells;

(b) a first polypeptide chain comprising a first TCR domain comprising a first TCR transmembrane domain from a first TCR subunit; and

(c) a second polypeptide chain comprising a second TCR domain comprising a second TCR transmembrane domain from a second TCR subunit.

494. The chimeric TCR of embodiment 493, wherein the means for binding a MUC4 epitope that is overexpressed on cancer cells as compared to normal cells comprises an scFv.

495. The chimeric TCR of embodiment 492 or embodiment 494, wherein the first polypeptide chain further comprises the scFv, and optionally further comprises a linker between the first TCR domain and the scFv.

496. The chimeric TCR of embodiment 492 or embodiment 494, wherein the second polypeptide chain further comprises the scFv, and optionally further comprises a linker between the second TCR domain and the scFv.

497. The chimeric TCR of embodiment 490 or 491, which comprises two scFvs according to any one of embodiments 382 to 385.

498. The chimeric TCR of embodiment 493, wherein the means for binding a MUC4 epitope that is overexpressed on cancer cells as compared to normal cells comprises two scFvs.

499. The chimeric TCR of embodiment 497 or embodiment 498, wherein the two scFvs have the same amino acid sequence.

500. The chimeric TCR of embodiment 497 or embodiment 498, wherein the two scFvs have different amino acid sequences.

501. The chimeric TCR of any one of embodiments 497 to 500, wherein the two scFvs are covalently bound by a linker sequence, which is optionally 4-15 amino acids in length.

502. The chimeric TCR of any one of embodiments 497 to 501, wherein the first polypeptide chain further comprises the two scFvs, and optionally further comprises a linker between the first TCR domain and a first scFv of the two scFvs.

503. The chimeric TCR of any one of embodiments 497 to 501, wherein the second polypeptide chain further comprises the two scFvs, and optionally further comprises a linker between the second TCR domain and a first scFv of the two scFvs.

504. The chimeric TCR of any one of embodiments 497 to 501, wherein the first polypeptide chain comprises a first scFv of the two scFvs, and the second polypeptide chain comprises a second scFv of the two scFvs, and optionally wherein (i) the first polypeptide chain comprises a first linker between the first TCR domain and the first scFv, and (ii) the second polypeptide chain comprises a second linker between the second TCR domain and the second scFv.

505. The chimeric TCR of embodiment 490, wherein the antigen-binding fragment is an anti-glyco-MUC4 Fv fragment.

506. The chimeric TCR of embodiment 493, wherein the means for binding a MUC4 epitope that is overexpressed on cancer cells as compared to normal cells is an anti-glyco-MUC4 Fv fragment.

507. The chimeric TCR of embodiment 505 or embodiment 506, wherein the Fv fragment comprises an anti-glyco-MUC4 variable heavy chain (VH) and an anti-glyco-MUC4 variable light chain (VL), optionally wherein the VH and VL are a VH and a VL of an anti-glyco-MUC4 antibody or binding fragment according to any one of embodiments 1 to 414.

508. The chimeric TCR of embodiment 507, wherein the first polypeptide chain further comprises the anti-glyco-MUC4 VH and the second polypeptide chain further comprises the anti-glyco-MUC4 VL, optionally wherein (i) the first polypeptide chain further comprises a linker between the first TCR domain and the anti-glyco-MUC4 VH, and (ii) the second polypeptide chain further comprises a linker between the second TCR domain and the anti-glyco-MUC4 VL.

509. The chimeric TCR of embodiment 507, wherein the first polypeptide chain further comprises the anti-glyco-MUC4 VL and the second polypeptide chain further comprises the anti-glyco-MUC4 VH, optionally wherein (i) the first polypeptide chain further comprises a linker between the first TCR domain and the anti-glyco-MUC4 VL, and (ii) the second polypeptide chain further comprises a linker between the second TCR domain and the anti-glyco-MUC4 VH.

510. The chimeric TCR of any one of embodiments 490 and 505 to 509, wherein the first polypeptide chain further comprises a common heavy chain 1 (CH1) domain.

511. The chimeric TCR of any one of embodiments 490 and 505 to 510, wherein the second polypeptide chain further comprises a common light chain (CL) domain.

512. The chimeric TCR of embodiment 490, wherein the antigen-binding fragment is an anti-glyco-MUC4 Fab domain.

513. The chimeric TCR of embodiment 493, wherein the means for binding a MUC4 epitope that is overexpressed on cancer cells as compared to normal cells is an anti-glyco-MUC4 Fab domain.

514. The chimeric TCR of embodiment 512 or embodiment 513, which comprises one anti-glyco-MUC4 Fab domain.

515. The chimeric TCR of embodiment 512 or embodiment 513, which comprises two anti-glyco-MUC4 Fab domains.

516. The chimeric TCR of embodiment 515, wherein the two Fab domains have the same amino acid sequence.

517. The chimeric TCR of embodiment 515, wherein the two Fab domains have different amino acid sequences.

518. The chimeric TCR of any one of embodiments 512 to 517, wherein the Fab domain or each Fab domain comprises an anti-glyco-MUC4 variable heavy chain (VH) and an anti-glyco-MUC4 variable light chain (VL), optionally wherein the VH and VL are a VH and a VL of an anti-glyco-MUC4 antibody or binding fragment according to any one of embodiments 1 to 414.

519. The chimeric TCR of embodiment 518, wherein the first polypeptide chain comprises the anti-glyco-MUC4 VH and a CH1 domain or a CL domain, optionally wherein the first polypeptide chain comprises a linker between the first TCR domain and the CH1 domain or the CL domain.

520. The chimeric TCR of embodiment 519, wherein the second polypeptide chain comprises the anti-glyco-MUC4 VL and a CL domain or a CH1 domain, optionally wherein the second polypeptide chain comprises a linker between the second TCR domain and the CL domain or the CH1 domain.

521. The chimeric TCR of embodiment 519, comprising a third polypeptide chain comprising the anti-glyco-MUC4 VL and a CL domain or a CH1 domain, the third polypeptide chain being capable of associating with the anti-glyco-MUC4 VH and the CH1 domain or the CL domain of the first polypeptide chain.

522. The chimeric TCR of embodiment 518, wherein the second polypeptide chain comprises the anti-glyco-MUC4 VH and a CH1 domain or a CL domain, optionally wherein the second polypeptide chain comprises a linker between the second TCR domain and the CH1 domain or the CL domain.

523. The chimeric TCR of embodiment 522, wherein the first polypeptide chain comprises the anti-glyco-MUC4 VL and a CL or a CH1 domain, optionally wherein the first polypeptide chain comprises a linker between the second TCR domain and the CL domain or the CH1.

524. The chimeric TCR of embodiment 522, comprising a third polypeptide chain comprising the anti-glyco-MUC4 VL and a CL domain or a CH1 domain, the third polypeptide chain being capable of associating with the anti-glyco-MUC4 VH and the CH1 domain or the CL domain of the second polypeptide chain.

525. The chimeric TCR of embodiment 518, wherein the first polypeptide chain comprises a first anti-glyco-MUC4 VH and a first chain CH1 domain or a first chain CL domain and the second polypeptide chain comprises a second anti-glyco-MUC4 VH and a second chain CH1 domain or a second chain CL domain, optionally wherein the first polypeptide chain comprises a linker between the first TCR domain and the first chain CH1 domain or the first chain CL domain, and optionally wherein the second polypeptide chain comprises a linker between the second TCR domain and the second chain CH1 domain or the second chain CL domain.

526. The chimeric TCR of embodiment 525, comprising:

(a) a third polypeptide chain comprising a first anti-glyco-MUC4 VL and a third chain CL domain or a third chain CH1 domain, capable of associating with the first anti-glyco-MUC4 VH and the first chain CH1 domain or the first chain CL domain of the first polypeptide; and

(b) a fourth polypeptide chain comprising a second anti-glyco-MUC4 VL and a fourth chain CL domain or a fourth chain CH1 domain, capable of associating with the second anti-glyco-MUC4 VH and the second chain CH1 domain or the second chain CL domain of the second polypeptide.

527. The chimeric TCR of any one of embodiments 490 to 526, when depending directly or indirectly from embodiment 490, wherein the antigen-binding fragment comprises an anti-glyco-MUC4 variable heavy chain comprising the amino acid sequence of QVQLQQSDAELVKPGASVRISCKAYGYTFTDHAIHWVKQKPEQGLEWLGYISPGNDDIQYNAK FKGKATLTADKSSSTAYMQLNSLTSDDSAVYFCKRSMANSFDYWGQGTTLTVSS (SEQ ID NO:1).

528. The chimeric TCR of any one of embodiments 490 to 526, when depending directly or indirectly from embodiment 490, wherein the antigen-binding fragment comprises an anti-glyco-MUC4 variable heavy chain comprising the amino acid sequence of QVQLQQSDAELVKPGASVKISCKASGYTFTDHAIHWVKQKPEQGLEWIGYFSPGNGDIKYNEK FKGKATLTADRSSSTANMHLNSLTSEDSAVYFCKRSMANYFDYWGQGTTLTVSS (SEQ ID NO:23).

529. The chimeric TCR of any one of embodiments 490 to 526, when depending directly or indirectly from embodiment 490, wherein the antigen-binding fragment comprises an anti-glyco-MUC4 variable heavy chain comprising the amino acid sequence of QVQLQQSDAELVEPGASVKISCKAYGYTFTDHAIHWVKQKPEQGLEWLGYISPGNDDIQYNAK FKGRATLTADKSSSTAYMQLNSLTSDDSAVYFCKRSMANSFDFWGQGTTLTVSS (SEQ ID NO:45).

530. The chimeric TCR of any one of embodiments 490 to 526, when depending directly or indirectly from embodiment 490, wherein the antigen-binding fragment comprises an anti-glyco-MUC4 variable heavy chain comprising the amino acid sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRAVLSADKSVSTAYLQISSLKAEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:133).

531. The chimeric TCR of any one of embodiments 490 to 526, when depending directly or indirectly from embodiment 490, wherein the antigen-binding fragment comprises an anti-glyco-MUC4 variable heavy chain comprising the amino acid sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNDDIQYNQ KFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:134).

532. The chimeric TCR of any one of embodiments 490 to 526, when depending directly or indirectly from embodiment 490, wherein the antigen-binding fragment comprises an anti-glyco-MUC4 variable heavy chain comprising the amino acid sequence of QVQLVQSGSELKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISTGNANITYAQ GFTGRAVLSLDKSVSTAYLQISSLKAEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:135).

533. The chimeric TCR of any one of embodiments 490 to 526, when depending directly or indirectly from embodiment 490, wherein the antigen-binding fragment comprises an anti-glyco-MUC4 variable heavy chain comprising the amino acid sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIQYNAK FKGHATLSADKSSSTAYLQWSSLKASDAAMYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:136).

534. The chimeric TCR of any one of embodiments 490 to 526, when depending directly or indirectly from embodiment 490, wherein the antigen-binding fragment comprises an anti-glyco-MUC4 variable heavy chain comprising the amino acid sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNDDIRYNAK FKGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:137).

535. The chimeric TCR of any one of embodiments 490 to 526, when depending directly or indirectly from embodiment 490, wherein the antigen-binding fragment comprises an

anti-glyco-MUC4 variable heavy chain comprising the amino acid sequence of EVQLLQSAAEVKRPGESLRISCKASGYTFTDHAIHWVRQMPGKELEWLGYISPGNADTRYSAS FQGHVTISADKSSSTAYLQWSSLKASDAAMYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:138).

536. The chimeric TCR of any one of embodiments 490 to 526, when depending directly or indirectly from embodiment 490, wherein the antigen-binding fragment comprises an anti-glyco-MUC4 variable heavy chain comprising the amino acid sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNA KFKGRATLTADKSTSTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:139).

537. The chimeric TCR of any one of embodiments 490 to 526, when depending directly or indirectly from embodiment 490, wherein the antigen-binding fragment comprises an anti-glyco-MUC4 variable heavy chain comprising the amino acid sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDHAIHWVRQAPGQGLEWLGYISPGNDDIQYNQ KFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:140).

538. The chimeric TCR of any one of embodiments 490 to 526, when depending directly or indirectly from embodiment 490, wherein the antigen-binding fragment comprises an anti-glyco-MUC4 variable heavy chain comprising the amino acid sequence of QVQLVQSGAEVKKPGSSVKVSCKASGYTFSDHAIHWVRQAPGQGLEWLGYISPGNADINYAQ KFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:141).

539. The chimeric TCR of any one of embodiments 490 to 526, when depending directly or indirectly from embodiment 490, wherein the antigen-binding fragment comprises an anti-glyco-MUC4 variable heavy chain comprising the amino acid sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYNA KFKGRATLTADKSASTAYMELSSLRSEDTAVYFCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:142).

540. The chimeric TCR of any one of embodiments 490 to 526, when depending directly or indirectly from embodiment 490, wherein the antigen-binding fragment comprises an anti-glyco-MUC4 variable heavy chain comprising the amino acid sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNDDIQYSQ KFKGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:143).

541. The chimeric TCR of any one of embodiments 490 to 526, when depending directly or indirectly from embodiment 490, wherein the antigen-binding fragment comprises an anti-glyco-MUC4 variable heavy chain comprising the amino acid sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAIHWVRQAPGQRLEWLGYISPGNADTQYS

QKFQGRVTITADKSASTAYMELSSLRSEDTAVYYCKRSMANSFDYWGQGTLVTVSS (SEQ ID NO:144).

542. The chimeric TCR of any one of embodiments 490 to 526, when depending directly or indirectly from embodiment 490, wherein the anti-glyco-MUC4 variable heavy chain comprises:

(a) a complementarity determining region (CDR) H1 comprising the amino acid sequence of GYTFTDHA (SEQ ID NO:67), DHAIH (SEQ ID NO:73), GYTFTDH (SEQ ID NO:79), GYTFTDHAIH (SEQ ID NO:103), or DH (SEQ ID NO:127);

(b) a CDR-H2 comprising the amino acid sequence of X_1SPGNX_2DI (SEQ ID NO:68), YX_1SPGNX_2DIX_3YNX_4KFKG (SEQ ID NO:74), SPGNX_2D (SEQ ID NO:80), YX_1SPGNX_2DIX_3YNX_4KFKG (SEQ ID NO:104), or SPGNX_2 (SEQ ID NO:128); and

(c) a CDR-H3 comprising the amino acid sequence of KRSMANX_5FDX_6 (SEQ ID NO:69), SMANX_5FDX_6 (SEQ ID NO:75), SMANX_5FDX_6 (SEQ ID NO:81), KRSMANX_5FDX_6 (SEQ ID NO:105), or SMANX_5FDX_6 (SEQ ID NO:129).

543. The chimeric TCR of any one of embodiments 490 to 542 when depending directly or indirectly from embodiment 490, wherein the antigen-binding fragment comprises an anti-glyco-MUC4 variable light chain comprising the amino acid sequence of NIMLTQSPSSLAVSAGEKVTMSCKSSQSVLYSSDQKNYLAWYQQKPGQSPKLLIYWASTRES GVPDRFTGSGSGTDFTLTISNVQAEDLAVYYCHQYLSSYTFGGGTKLEIK (SEQ ID NO:2).

544. The chimeric TCR of any one of embodiments 490 to 542 when depending directly or indirectly from embodiment 490, wherein the antigen-binding fragment comprises an anti-glyco-MUC4 variable light chain comprising the amino acid sequence of NIMMTQSPSSLVVSAGEKVTMSCKSSHSVLYSSNQKNYLAWYQQKPGQSPKLLIYWASTKNS GVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCHQYLSSYTFGGGTKLEIK (SEQ ID NO:24).

545. The chimeric TCR of any one of embodiments 490 to 542 when depending directly or indirectly from embodiment 490, wherein the antigen-binding fragment comprises an anti-glyco-MUC4 variable light chain comprising the amino acid sequence of NIMLTQSPSSLAVSAGEKVTMSCKSSQSVLYSSDQKNYLAWYQQKPGQSPKLLIYWASTRES GVPDRFTGSGSGTDFTLTISNVRAEDLAVYYCHQYLSSYTFGGGTKLEIK (SEQ ID NO:46).

546. The chimeric TCR of any one of embodiments 490 to 542 when depending directly or indirectly from embodiment 490, wherein the antigen-binding fragment comprises an anti-glyco-MUC4 variable light chain comprising the amino acid sequence of DIVLTQSPDSLAVSLGERATINCKSSQSVLYSSDQKNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:145).

547. The chimeric TCR of any one of embodiments 490 to 542 when depending directly or indirectly from embodiment 490, wherein the antigen-binding fragment comprises an anti-glyco-MUC4 variable light chain comprising the amino acid sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNLRNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:146).

548. The chimeric TCR of any one of embodiments 490 to 542 when depending directly or indirectly from embodiment 490, wherein the antigen-binding fragment comprises an anti-glyco-MUC4 variable light chain comprising the amino acid sequence of DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNERNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:147).

549. The chimeric TCR of any one of embodiments 490 to 542 when depending directly or indirectly from embodiment 490, wherein the antigen-binding fragment comprises an anti-glyco-MUC4 variable light chain comprising the amino acid sequence of EIVLTQSPGTLSLSPGERATLSCKSSQSVLYSSDQKNYLAWYQQKPGQAPRLLIYWASTRESG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:148).

550. The chimeric TCR of any one of embodiments 490 to 542 when depending directly or indirectly from embodiment 490, wherein the antigen-binding fragment comprises an anti-glyco-MUC4 variable light chain comprising the amino acid sequence of EIVLTQSPGTLSLSPGERATLSCRSSQSVLYSSDQKSYLAWYQQKPGQAPRLLIYWASTRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:149).

551. The chimeric TCR of any one of embodiments 490 to 542 when depending directly or indirectly from embodiment 490, wherein the antigen-binding fragment comprises an anti-glyco-MUC4 variable light chain comprising the amino acid sequence of EIVLTQSPGTLSLSPGERATLSCRASQSVSYSSDQKSYLAWYQQKPGQAPRLLIYWASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:150).

552. The chimeric TCR of any one of embodiments 490 to 542 when depending directly or indirectly from embodiment 490, wherein the antigen-binding fragment comprises an anti-glyco-MUC4 variable light chain comprising the amino acid sequence of DIVLTQTPLSLPVTPGEPASISCKSSQSVLYSSDQKNYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:151).

553. The chimeric TCR of any one of embodiments 490 to 542 when depending directly or indirectly from embodiment 490, wherein the antigen-binding fragment comprises an anti-glyco-MUC4 variable light chain comprising the amino acid sequence of DIVMTQTPLSLPVTPGEPASISCRSSQSVLYSSDEKTYLAWYLQKPGQSPQLLIYWASTRESGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:152).

554. The chimeric TCR of any one of embodiments 490 to 542 when depending directly or indirectly from embodiment 490, wherein the antigen-binding fragment comprises an anti-glyco-MUC4 variable light chain comprising the amino acid sequence of

DIVMTQTPLSLPVTPGEPASISCRSSQSLLYSSDERTYLAWYLQKPGQSPQLLIYWASTRASGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCHQYLSSYTFGQGTKLEIK (SEQ ID NO:153).

555. The chimeric TCR of any one of embodiments 490 to 542 when depending directly or indirectly from embodiment 490, wherein the anti-glyco-MUC4 variable light chain comprises:

(a) a CDR-L1 comprising the amino acid sequence of X_7SVLYSSX_8QKNY (SEQ ID NO:70), KSSX_7SVLYSSX_8QKNYLA (SEQ ID NO:76), KSSX_7SVLYSSX_8QKNYLA (SEQ ID NO:82), KSSX_7SVLYSSX_8QKNYLA (SEQ ID NO:106), or X_7SVLYSSX_8QKNY (SEQ ID NO:130);

(b) a CDR-L2 comprising the amino acid sequence of WAS (SEQ ID NO:71), WASTX_9X_{10}S (SEQ ID NO:77), WASTX_9X_{10}S (SEQ ID NO:83), WASTX_9X_{10}S (SEQ ID NO:107), or WAS (SEQ ID NO:131); and

(c) a CDR-L3 comprising the amino acid sequence of HQYLSSYT (SEQ ID NO:72), HQYLSSYT (SEQ ID NO:78), HQYLSSYT (SEQ ID NO:84), HQYLSSYT (SEQ ID NO:108), or HQYLSSYT (SEQ ID NO:132).

556. The chimeric TCR of any one of embodiments 495, 496, 502 to 504, 508, and 509, when comprising a first and/or a second linker, the first and/or second linkers comprise, individually, a constant domain or fragment thereof from an immunoglobulin or from a T cell receptor subunit.

557. The chimeric TCR of embodiment 556, wherein the first and/or second linkers comprise, individually, a CH1, CH2, CH3, CH4, or CL antibody domain, or a fragment of any one thereof.

558. The chimeric TCR of embodiment 556, wherein the first and/or second linkers comprise, individually, a Cα, Cβ, Cγ, or Cδ TCR domain, or a fragment of any one thereof.

559. The chimeric TCR of embodiment 558, wherein the first polypeptide chain comprises a Cα TCR domain or a fragment thereof, and the second polypeptide chain comprises a Cβ TCR domain or a fragment thereof.

560. The chimeric TCR of embodiment 558, wherein the first polypeptide chain comprises a Cβ TCR domain or a fragment thereof, and the second polypeptide chain comprises a Cα TCR domain or a fragment thereof.

561. The chimeric TCR of embodiment 558, wherein the first polypeptide chain comprises a Cγ TCR domain or a fragment thereof, and the second polypeptide chain comprises a Cδ TCR domain or a fragment thereof.

562. The chimeric TCR of embodiment 558, wherein the first polypeptide chain comprises a Cδ TCR domain or a fragment thereof, and the second polypeptide chain comprises a Cγ TCR domain or a fragment thereof.

563. The chimeric TCR of any one of embodiments 559 to 562, wherein the first TCR constant region domain and the second TCR constant region domain each comprise at least one mutation relative to the wildtype TCR constant region domain.

564. The chimeric TCR of embodiment 563, wherein the TCRα constant region domain comprises a substitution at amino acid position 48 of wildtype TCRα constant region and the TCRβ constant region domain comprises a substitution at amino acid position 57 of wildtype TCRβ constant region.

565. The chimeric TCR of embodiment 563 or 564 wherein the Cα TCR domain comprises a substitution at an amino acid corresponding to amino acid position 85 of wildtype human Cα TCR and the Cβ TCR domain comprises a substitution at an amino acid corresponding to amino acid position 88 of wildtype human Cβ TCR.

566. The chimeric TCR of any one of embodiments 490 to 565, wherein the first TCR constant region domain is a TCRγ constant region domain and the second TCR constant region domain is a TCRδ constant region domain.

567. The chimeric TCR of any one of embodiments 490 to 566, wherein the first TCR constant further comprises a first connecting peptide of a TCR subunit, or a fragment thereof, N-terminal to the first TCR transmembrane domain.

568. The chimeric TCR of any one of embodiments 490 to 567, wherein the second TCR domain further comprises a second connecting peptide of a TCR subunit, or a fragment thereof, N-terminal to the second TCR transmembrane domain.

569. The chimeric TCR of embodiment 568, comprising a disulfide bond between a residue in the first connecting peptide and a residue in the second connecting peptide.

570. The chimeric TCR of any one of embodiments 490 to 569, wherein the first TCR domain further comprises a first TCR intracellular domain comprising a TCR intracellular sequence C-terminal to the first transmembrane domain.

571. The chimeric TCR of any one of embodiments 490 to 570, wherein the second TCR domain further comprises a second TCR intracellular domain comprising a TCR intracellular sequence C-terminal to the second transmembrane domain.

572. The chimeric TCR of any one of embodiments 490 to 571, wherein the first polypeptide chain further comprises a first accessory intracellular domain comprising a co-stimulatory intracellular signaling sequence C-terminal to the first transmembrane domain.

573. The chimeric TCR of any one of embodiments 490 to 572, wherein the second polypeptide chain further comprises a second accessory intracellular domain comprising a co-stimulatory intracellular signaling sequence C-terminal to the second transmembrane domain.

574. The chimeric TCR of any one of embodiments 490 to 573, further comprising a cleavable peptide linker, configured to temporarily associate the first polypeptide chain with the second polypeptide chain during and/or shortly after protein translation.

575. The chimeric TCR of embodiment 574, wherein the cleavable peptide linker is a protease cleavable peptide linker.

576. The chimeric TCR of embodiment 574 or 575, wherein the peptide linker comprises the sequence ATNFSLLKQAGDVEENPGP (SEQ ID NO:200).

577. The chimeric TCR of any one of embodiments 490 to 576, wherein the first TCR domain is a TCR α chain or a fragment thereof and the second TCR domain is a TCR β chain or a fragment thereof.

578. The chimeric TCR of any one of embodiments 490 to 576, wherein the first TCR domain is a TCR β chain or a fragment thereof and the second TCR domain is a TCR α chain or a fragment thereof.

579. The chimeric TCR of any one of embodiments 490 to 576, wherein the first TCR domain is a TCR δ chain or a fragment thereof and the second TCR domain is a TCR γ chain or a fragment thereof.

580. The chimeric TCR of any one of embodiments 490 to 576, wherein the first TCR domain is a TCR γ chain or a fragment thereof and the second TCR domain is a TCR δ chain or a fragment thereof.

581. The chimeric TCR of any one of embodiments 490 to 580, comprising, from N- to C-terminus, (i) an anti-glyco-MUC4 variable heavy chain (VH), (ii) the first TCR domain, (iii) a cleavable peptide linker, (iv) an anti-glyco-MUC4 variable light chain (VL), and (v) the second TCR domain.

582. The chimeric TCR of any one of embodiments 490 to 580, comprising, from N- to C-terminus, (i) an anti-glyco-MUC4 variable heavy chain (VH), (ii) the second TCR domain, (iii) a cleavable peptide linker, (iv) an anti-glyco-MUC4 common light chain (CL), and (v) the first second TCR domain.

583. The chimeric TCR of any one of embodiments 490 to 580, comprising, from N- to C-terminus, (i) an anti-glyco-MUC4 variable light chain (VL), (ii) the first TCR domain, (iii) a cleavable peptide linker, (iv) an anti-glyco-MUC4 variable heavy chain (VH), and (v) the second TCR domain.

584. The chimeric TCR of any one of embodiments 490 to 580, comprising, from N- to C-terminus, (i) an anti-glyco-MUC4 variable light chain (VL), (ii) the second TCR domain, (iii) a cleavable peptide linker, (iv) an anti-glyco-MUC4 variable heavy chain (VH), and (v) the first TCR domain.

585. A nucleic acid comprising a coding region for an anti-glyco-MUC4 antibody or antigen-binding fragment of any of embodiments 1 to 414, the bispecific antibody of any one of embodiments 415 to 420, the fusion protein of any one of embodiments 421 to 445, the CAR of any one of embodiments 446 to 479, or the chimeric TCR of any one of embodiments 490 to 584.

586. The nucleic acid of embodiment 585 in which the coding region is codon-optimized for expression in a human cell.

587. A vector comprising the nucleic acid of embodiment 585 or embodiment 586.

588. The vector of embodiment 587 which is a viral vector.

589. The vector of embodiment 588 wherein the viral vector is a lentiviral vector.

590. A host cell engineered to express the nucleic acid of embodiment 585 or embodiment 586.

591. The host cell of embodiment 590, which is a human T-cell engineered to express the CAR of any one of embodiments 446 to 479.

592. The host cell of embodiment 590, which is a human NK cell engineered to express the CAR of any one of embodiments 446 to 479.

593. The host cell of embodiment 590, which is a human T-cell engineered to express the chimeric TCR of any one of embodiments 490 to 584.

594. A host cell comprising the vector of any one of embodiments 587 to 589.

595. The host cell of embodiment 594 which is a T-cell and wherein the vector encodes the CAR of any one of embodiments 446 to 479.

596. The host cell of embodiment 594 which is a T-cell and wherein the vector encodes the chimeric TCR of any one of embodiments 490 to 584.

597. A pharmaceutical composition comprising (a) the anti-glyco-MUC4 antibody or antigen binding fragment of any of embodiments 1 to 414, the bispecific antibody of any one of embodiments 415 to 420, the fusion protein of any one of embodiments 421 to 445, the CAR of any one of embodiments 446 to 479, the antibody-drug conjugate of any one of embodiments 480 to 489, the chimeric TCR of any one of embodiments 490 to 584, the nucleic acid of embodiment 585 or embodiment 586, the vector of any one of embodiments 587 to 589, or the host cell of any one of embodiments 590 to 596, and (b) a physiologically suitable buffer, adjuvant, diluent, or combination thereof.

598. A method of treating cancer comprising administering to a subject in need thereof an effective amount of the anti-glyco-MUC4 antibody or antigen binding fragment of any of embodiments 1 to 414, the bispecific antibody of any one of embodiments 415 to 420, the fusion protein of any one of embodiments 421 to 445, the CAR of any one of embodiments 446 to 479, the antibody-drug conjugate of any one of embodiments 480 to 489, the chimeric TCR of any one of embodiments 490 to 584, the nucleic acid of embodiment 585 or embodiment 586, the vector of any one of embodiments 587 to 589, the host cell of any one of embodiments 590 to 596, or the pharmaceutical composition of embodiment 597.

599. The method of embodiment 598, wherein the subject is suffering from pancreatic cancer, lung cancer, breast cancer, cancer of the gall bladder, salivary gland cancer, prostate cancer, biliary tract cancer, esophageal cancer, papillary thyroid carcinoma, low-grade fibromyxoid sarcoma, and ovarian cancer.

600. The method of embodiment 599, wherein the subject is suffering from breast cancer.

601. The method of embodiment 599, wherein the subject is suffering from lung cancer.

602. The method of embodiment 599, wherein the subject is suffering from prostate cancer.

603. The method of embodiment 599, wherein the subject is suffering from a urogenital cancer.

604. The method of embodiment 599, wherein the subject is suffering from esophageal cancer.

605. The method of embodiment 599, wherein the subject is suffering from ovarian cancer.

606. The method of embodiment 599, wherein the subject is suffering from pancreatic cancer.

607. The method of embodiment 599, wherein the subject is suffering from cancer of the gall bladder.

608. The method of embodiment 599, wherein the subject is suffering from salivary gland cancer.

609. The method of embodiment 599, wherein the subject is suffering from biliary tract cancer.

610. The method of embodiment 599, wherein the subject is suffering from papillary thyroid carcinoma.

611. The method of embodiment 599, wherein the subject is suffering from low-grade fibromyxoid sarcoma.

612. A method of detecting cancer in a biological sample, comprising contacting a sample (*e.g.*, a sample comprising or suspected of comprising cancer cells and/or cancer-derived extracellular vesicles) with an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of embodiments 1 to 414 and detecting binding of the anti-glyco-MUC4 antibody or antigen-binding fragment.

613. The method of embodiment 612, further comprising quantitating the binding of the anti-glyco-MUC4 antibody or antigen-binding fragment.

614. The method of embodiment 612 or embodiment 613, wherein the binding is compared to a normal tissue control as a negative/baseline control and/or to a cancerous tissue control as a positive control.

615. The method of any one of embodiments 612 to 614, wherein the cancer is pancreatic cancer, lung cancer, breast cancer, cancer of the gall bladder, salivary gland cancer, prostate cancer, biliary tract cancer, esophageal cancer, papillary thyroid carcinoma, low-grade fibromyxoid sarcoma, and ovarian cancer.

616. The method of embodiment 615, wherein the cancer is pancreatic cancer.

617. The method of embodiment 615, wherein the cancer is lung cancer.

618. The method of embodiment 615, wherein the cancer is breast cancer

619. The method of embodiment 615, wherein the cancer is cancer of the gall bladder.

620. The method of embodiment 615, wherein the cancer is salivary gland cancer.

621. The method of embodiment 615, wherein the cancer is prostate cancer.

622. The method of embodiment 615, wherein the cancer is biliary tract cancer.

623. The method of embodiment 615, wherein the cancer is esophageal cancer.

624. The method of embodiment 615, wherein the cancer is papillary thyroid carcinoma.

625. The method of embodiment 615, wherein the cancer is low-grade fibromyxoid sarcoma.

626. The method of embodiment 615, wherein the cancer is ovarian cancer.

627. The method of any one of embodiments 598 to 626, when depending from any one of embodiments 427 to 430, which further comprises administering to the subject genetically modified T-cells engineered to express a CAR comprising a NKG2D receptor capable of specifically binding the MIC protein domain.

628. The anti-glyco-MUC4 antibody or antigen binding fragment of any of embodiments 1 to 414, the bispecific antibody of any one of embodiments 415 to 420, the fusion protein of any one of embodiments 421 to 445, the CAR of any one of embodiments 446 to 479, the antibody-drug conjugate of any one of embodiments 480 to 489, the chimeric TCR of any one of embodiments 490 to 584, the nucleic acid of embodiment 585 or embodiment 586, the vector of any one of embodiments 587 to 589, the host cell of any one of embodiments 590 to 596, or the pharmaceutical composition of embodiment 597 for use as a medicament.

629. The anti-glyco-MUC4 antibody or antigen binding fragment of any of embodiments 1 to 414, the bispecific antibody of any one of embodiments 415 to 420, the fusion protein of any one of embodiments 421 to 445, the CAR of any one of embodiments 446 to 479, the antibody-drug conjugate of any one of embodiments 480 to 489, the chimeric TCR of any one of embodiments 490 to 584, the nucleic acid of embodiment 585 or embodiment 586, the vector of any one of embodiments 587 to 589, the host cell of any one of embodiments 590 to 596, or the pharmaceutical composition of embodiment 597 for use in the treatment of cancer, optionally wherein the cancer is pancreatic cancer, lung cancer, breast cancer, cancer of the gall bladder, salivary gland cancer, prostate cancer, biliary tract cancer, esophageal cancer, papillary thyroid carcinoma, low-grade fibromyxoid sarcoma, and ovarian cancer.

630. The anti-glyco-MUC4 antibody or antigen binding fragment of any of embodiments 1 to 414, the bispecific antibody of any one of embodiments 415 to 420, the fusion protein of any one of embodiments 421 to 445, the CAR of any one of embodiments 446

to 479, the antibody-drug conjugate of any one of embodiments 480 to 489, the chimeric TCR of any one of embodiments 490 to 584, the nucleic acid of embodiment 585 or embodiment 586, the vector of any one of embodiments 587 to 589, the host cell of any one of embodiments 590 to 596, or the pharmaceutical composition of embodiment 597 for use in the treatment of pancreatic cancer.

631. The anti-glyco-MUC4 antibody or antigen binding fragment of any of embodiments 1 to 414, the bispecific antibody of any one of embodiments 415 to 420, the fusion protein of any one of embodiments 421 to 445, the CAR of any one of embodiments 446 to 479, the antibody-drug conjugate of any one of embodiments 480 to 489, the chimeric TCR of any one of embodiments 490 to 584, the nucleic acid of embodiment 585 or embodiment 586, the vector of any one of embodiments 587 to 589, the host cell of any one of embodiments 590 to 596, or the pharmaceutical composition of embodiment 597 for use in the treatment of lung cancer.

632. The anti-glyco-MUC4 antibody or antigen binding fragment of any of embodiments 1 to 414, the bispecific antibody of any one of embodiments 415 to 420, the fusion protein of any one of embodiments 421 to 445, the CAR of any one of embodiments 446 to 479, the antibody-drug conjugate of any one of embodiments 480 to 489, the chimeric TCR of any one of embodiments 490 to 584, the nucleic acid of embodiment 585 or embodiment 586, the vector of any one of embodiments 587 to 589, the host cell of any one of embodiments 590 to 596, or the pharmaceutical composition of embodiment 597 for use in the treatment of breast cancer.

633. The anti-glyco-MUC4 antibody or antigen binding fragment of any of embodiments 1 to 414, the bispecific antibody of any one of embodiments 415 to 420, the fusion protein of any one of embodiments 421 to 445, the CAR of any one of embodiments 446 to 479, the antibody-drug conjugate of any one of embodiments 480 to 489, the chimeric TCR of any one of embodiments 490 to 584, the nucleic acid of embodiment 585 or embodiment 586, the vector of any one of embodiments 587 to 589, the host cell of any one of embodiments 590 to 596, or the pharmaceutical composition of embodiment 597 for use in the treatment of cancer of the gall bladder.

634. The anti-glyco-MUC4 antibody or antigen binding fragment of any of embodiments 1 to 414, the bispecific antibody of any one of embodiments 415 to 420, the fusion protein of any one of embodiments 421 to 445, the CAR of any one of embodiments 446 to 479, the antibody-drug conjugate of any one of embodiments 480 to 489, the chimeric TCR of any one of embodiments 490 to 584, the nucleic acid of embodiment 585 or embodiment 586, the vector of any one of embodiments 587 to 589, the host cell of any one of embodiments 590 to 596, or the pharmaceutical composition of embodiment 597 for use in the treatment of salivary gland cancer.

635. The anti-glyco-MUC4 antibody or antigen binding fragment of any of embodiments 1 to 414, the bispecific antibody of any one of embodiments 415 to 420, the fusion protein of any one of embodiments 421 to 445, the CAR of any one of embodiments 446 to 479, the antibody-drug conjugate of any one of embodiments 480 to 489, the chimeric TCR of any one of embodiments 490 to 584, the nucleic acid of embodiment 585 or embodiment 586, the vector of any one of embodiments 587 to 589, the host cell of any one of embodiments 590 to 596, or the pharmaceutical composition of embodiment 597 for use in the treatment of prostate cancer.

636. The anti-glyco-MUC4 antibody or antigen binding fragment of any of embodiments 1 to 414, the bispecific antibody of any one of embodiments 415 to 420, the fusion protein of any one of embodiments 421 to 445, the CAR of any one of embodiments 446 to 479, the antibody-drug conjugate of any one of embodiments 480 to 489, the chimeric TCR of any one of embodiments 490 to 584, the nucleic acid of embodiment 585 or embodiment 586, the vector of any one of embodiments 587 to 589, the host cell of any one of embodiments 590 to 596, or the pharmaceutical composition of embodiment 597 for use in the treatment of biliary tract cancer.

637. The anti-glyco-MUC4 antibody or antigen binding fragment of any of embodiments 1 to 414, the bispecific antibody of any one of embodiments 415 to 420, the fusion protein of any one of embodiments 421 to 445, the CAR of any one of embodiments 446 to 479, the antibody-drug conjugate of any one of embodiments 480 to 489, the chimeric TCR of any one of embodiments 490 to 584, the nucleic acid of embodiment 585 or embodiment 586, the vector of any one of embodiments 587 to 589, the host cell of any one of embodiments 590 to 596, or the pharmaceutical composition of embodiment 597 for use in the treatment of esophageal cancer.

638. The anti-glyco-MUC4 antibody or antigen binding fragment of any of embodiments 1 to 414, the bispecific antibody of any one of embodiments 415 to 420, the fusion protein of any one of embodiments 421 to 445, the CAR of any one of embodiments 446 to 479, the antibody-drug conjugate of any one of embodiments 480 to 489, the chimeric TCR of any one of embodiments 490 to 584, the nucleic acid of embodiment 585 or embodiment 586, the vector of any one of embodiments 587 to 589, the host cell of any one of embodiments 590 to 596, or the pharmaceutical composition of embodiment 597 for use in the treatment of papillary thyroid carcinoma.

639. The anti-glyco-MUC4 antibody or antigen binding fragment of any of embodiments 1 to 414, the bispecific antibody of any one of embodiments 415 to 420, the fusion protein of any one of embodiments 421 to 445, the CAR of any one of embodiments 446 to 479, the antibody-drug conjugate of any one of embodiments 480 to 489, the chimeric TCR of any one of embodiments 490 to 584, the nucleic acid of embodiment 585 or embodiment 586, the vector of any one of embodiments 587 to 589, the host cell of any one of embodiments

590 to 596, or the pharmaceutical composition of embodiment 597 for use in the treatment of low-grade fibromyxoid sarcoma.

640. The anti-glyco-MUC4 antibody or antigen binding fragment of any of embodiments 1 to 414, the bispecific antibody of any one of embodiments 415 to 420, the fusion protein of any one of embodiments 421 to 445, the CAR of any one of embodiments 446 to 479, the antibody-drug conjugate of any one of embodiments 480 to 489, the chimeric TCR of any one of embodiments 490 to 584, the nucleic acid of embodiment 585 or embodiment 586, the vector of any one of embodiments 587 to 589, the host cell of any one of embodiments 590 to 596, or the pharmaceutical composition of embodiment 597 for use in the treatment of ovarian cancer.

641. Use of the anti-glyco-MUC4 antibody or antigen binding fragment of any of embodiments 1 to 414, the bispecific antibody of any one of embodiments 415 to 420, the fusion protein of any one of embodiments 421 to 445, the CAR of any one of embodiments 446 to 479, the antibody-drug conjugate of any one of embodiments 480 to 489, the chimeric TCR of any one of embodiments 490 to 584, the nucleic acid of embodiment 585 or embodiment 586, the vector of any one of embodiments 587 to 589, the host cell of any one of embodiments 590 to 596, or the pharmaceutical composition of embodiment 597 for the manufacture of a medicament for the treatment of cancer, optionally wherein the cancer is pancreatic cancer, lung cancer, breast cancer, cancer of the gall bladder, salivary gland cancer, prostate cancer, biliary tract cancer, esophageal cancer, papillary thyroid carcinoma, low-grade fibromyxoid sarcoma, and ovarian cancer.

642. The use according to embodiment 641, wherein the cancer is pancreatic cancer.

643. The use according to embodiment 641, wherein the cancer is lung cancer.

644. The use according to embodiment 641, wherein the cancer is breast cancer.

645. The use according to embodiment 641, wherein the cancer is a cancer of the gall bladder.

646. The use according to embodiment 641, wherein the cancer is salivary gland cancer.

647. The use according to embodiment 641, wherein the cancer is prostate cancer.

648. The use according to embodiment 641, wherein the cancer is biliary tract cancer.

649. The use according to embodiment 641, wherein the cancer is esophageal cancer.

650. The use according to embodiment 641, wherein the cancer is papillary thyroid carcinoma.

651. The use according to embodiment 641, wherein the cancer is low-grade fibromyxoid sarcoma.

652. The use according to embodiment 641, wherein the cancer is ovarian cancer.

653. A peptide of 13-30 amino acids in length comprising (a) amino acids 4-16 of a MUC4 peptide CTIPSTAMHTRSTAAPIPILP (SEQ ID NO:155) or (b) an amino acid sequence corresponding to amino acids 4-16 of the MUC4 peptide CTIPSTAMHTRSTAAPIPILP (SEQ ID NO:155) with one or two amino acid substitutions at positions other than the serine corresponding to position 12 of CTIPSTAMHTRSTAAPIPILP (SEQ ID NO:155) and/or the threonine corresponding to position 13 of CTIPSTAMHTRSTAAPIPILP (SEQ ID NO:155).

654. The peptide of embodiment 653 which is 15-25 amino acids in length.

655. The peptide of embodiment 653 which is 18-25 amino acids in length.

656. The peptide of embodiment 653 which comprises CTIPSTAMHTRSTAAPIPILP (SEQ ID NO:155).

657. The peptide of embodiment 653 which consists of CTIPSTAMHTRSTAAPIPILP (SEQ ID NO:155).

658. The peptide of any one of embodiments 653 to 657 which is O-glycosylated at the serine corresponding to position 12 of CTIPSTAMHTRSTAAPIPILP (SEQ ID NO:155) and/or the threonine corresponding to position 13 of CTIPSTAMHTRSTAAPIPILP (SEQ ID NO:155).

659. The peptide of embodiment 658, wherein the O-glycosylation comprises or consists of GalNAc.

660. A peptide of 13-30 amino acids in length comprising (a) amino acids 4-16 of a MUC4 peptide CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) that has been O-glycosylated on the serine and threonine residues shown with bold and underlined text or (b) an amino acid sequence corresponding to amino acids 4-16 of the MUC4 peptide CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) that has been O-glycosylated on the serine and threonine residues shown with bold and underlined text with one or two amino acid substitutions at positions other than the serine corresponding to position 12 of CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) and/or the threonine corresponding to position 13 of CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154).

661. The peptide of embodiment 660 which is 15-25 amino acids in length.

662. The peptide of embodiment 660 which is 18-25 amino acids in length.

663. The peptide of embodiment 660 which comprises CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154).

664. The peptide of embodiment 660 which consists of CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154).

665. The peptide of any one of embodiments 660 to 664, wherein the O-glycosylation comprises or consists of GalNAc.

666. A composition comprising the peptide of any one of embodiments 653 to 665 and adjuvant.

667. The composition of embodiment 666, wherein the adjuvant comprises a Freund's adjuvant and/or an aluminum salt (*e.g.*, aluminum hydroxide).

668. A method of generating antibodies against a tumor-associated form of MUC4, comprising administering to an animal:

(a) the peptide of any one of embodiments 660 to 665; or

(b) the composition of embodiment 666 or 667 wherein the composition comprises the peptide of any one of embodiments 660 to 665.

669. The method of embodiment 668, further comprising collecting antibodies from the animal following the administering step.

670. A method of eliciting an immune response against a tumor-associated form of MUC4, comprising administering to a subject:

(a) the peptide of any one of embodiments 660 to 665; or

(b) The composition of embodiment 666 or 667 wherein the composition comprises the peptide of any one of embodiments 660 to 665.

671. The method of any one of embodiments 668 to 670, wherein the animal is a mouse or a rabbit.

672. The anti-glyco-MUC4 antibody or antigen binding fragment, bispecific antibody, fusion protein, CAR, antibody-drug conjugate, the chimeric TCR, pharmaceutical composition method or use as described in any one of the preceding embodiments, wherein the determination of competing is made using an antibody competition assay, optionally wherein the assay is an assay described in Section 5.1.

673. The anti-glyco-MUC4 antibody or antigen binding fragment, bispecific antibody, fusion protein, CAR, antibody-drug conjugate, the chimeric TCR, pharmaceutical composition method or use of embodiment 672, wherein competing is present if the anti-glyco-MUC4 antibody or anti-glyco-MUC4 antibody fragment decreases binding of a reference antibody by at least about 20% 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% when tested at a reference antibody concentration that is 80% of maximal binding under the specific assay conditions used and a test antibody concentration that is 10-fold higher than the reference antibody concentration.

[0448] All publications, patents, patent applications and other documents cited in this application are hereby incorporated by reference in their entireties for all purposes to the same extent as if each individual publication, patent, patent application or other document were individually indicated to be incorporated by reference for all purposes. In the event that there is an inconsistency between the teachings of one or more of the references incorporated herein and the present disclosure, the teachings of the present specification are intended.

CLAIMS:

What is claimed is:

1. An anti-glyco-MUC4 antibody or antigen binding fragment that specifically binds to a MUC4 peptide CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) that has been glycosylated with GalNAc on the serine and threonine residues shown with bold and underlined text ("the MUC4 glycopeptide").

2. The anti-glyco-MUC4 antibody or antigen binding fragment of claim 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence and a light chain variable (VL) sequence of:

(a) SEQ ID NO:1 and SEQ ID NO:2, respectively;

(b) SEQ ID NO:23 and SEQ ID NO:24, respectively; or

(c) SEQ ID NO:45 and SEQ ID NO:46, respectively,

for binding to the MUC4 glycopeptide.

3. The anti-glyco-MUC4 antibody or antigen binding fragment of claim 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of any one of SEQ ID NOs:133-144 and a light chain variable (VL) sequence of any one of SEQ ID NOs:145-153 for binding to the MUC4 glycopeptide.

4. The anti-glyco-MUC4 antibody or antigen binding fragment of any one of claims 1 to 3, which specifically binds to COSMC knock-out T3M4 cells.

5. The anti-glyco-MUC4 antibody or antigen binding fragment of claim 4, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence and a light chain variable (VL) sequence of:

(a) SEQ ID NO:1 and SEQ ID NO:2, respectively;

(b) SEQ ID NO:23 and SEQ ID NO:24, respectively; or

(c) SEQ ID NO:45 and SEQ ID NO:46, respectively,

for binding to COSMC knock-out T3M4 cells.

6. The anti-glyco-MUC4 antibody or antigen binding fragment of claim 4, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of any one of SEQ

ID NOs:133-144 and a light chain variable (VL) sequence of any one of SEQ ID NOs:145-153 for binding to COSMC knock-out T3M4 cells.

7. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of claims 1 to 6, comprising:

(a) a complementarity determining region (CDR) H1 comprising the amino acid sequence of SEQ ID NO:67, SEQ ID NO:73, SEQ ID NO:79, SEQ ID NO:103, or SEQ ID NO:127;

(b) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:68, SEQ ID NO:74, SEQ ID NO:80, SEQ ID NO:104, or SEQ ID NO:128;

(c) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:69, SEQ ID NO:75, SEQ ID NO:81, SEQ ID NO:105, or SEQ ID NO:129;

(d) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:70, SEQ ID NO:76, SEQ ID NO:82, SEQ ID NO:106, or SEQ ID NO:130;

(e) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:71, SEQ ID NO:77, SEQ ID NO:83, SEQ ID NO:107, or SEQ ID NO:131; and

(f) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:72, SEQ ID NO:78, SEQ ID NO:84, SEQ ID NO:108, or SEQ ID NO:132.

8. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of claims 1 to 7, which comprises:

(a) a VH comprising CDR-H1, CDR-H2, and CDR-H3 having the amino sequences of SEQ ID NOs:3-5, respectively, and a VL comprising CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NOs:6-8, respectively;

(b) a VH comprising CDR-H1, CDR-H2, and CDR-H3 having the amino sequences of SEQ ID NOs:9-11, respectively, and a VL comprising CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NOs:12-14, respectively; or

(c) a VH comprising CDR-H1, CDR-H2, and CDR-H3 having the amino sequences of SEQ ID NOs:15-17, respectively, and a VL comprising CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NOs:18-20, respectively.

9. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of claims 1 to 7, which comprises:

(a) a VH comprising CDR-H1, CDR-H2, and CDR-H3 having the amino sequences of SEQ ID NOs:25-27, respectively, and a VL comprising CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NOs:28-30, respectively;

(b) a VH comprising CDR-H1, CDR-H2, and CDR-H3 having the amino sequences of SEQ ID NOs:31-33, respectively, and a VL comprising CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NOs:34-36, respectively; or

(c) a VH comprising CDR-H1, CDR-H2, and CDR-H3 having the amino sequences of SEQ ID NOs:37-39, respectively, and a VL comprising CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NOs:40-42, respectively.

10. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of claims 1 to 7, which comprises:

(a) a VH comprising CDR-H1, CDR-H2, and CDR-H3 having the amino sequences of SEQ ID NOs:47-49, respectively, and a VL comprising CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NOs:50-52, respectively;

(b) a VH comprising CDR-H1, CDR-H2, and CDR-H3 having the amino sequences of SEQ ID NOs:53-55, respectively, and a VL comprising CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NOs:56-58, respectively; or

(c) a VH comprising CDR-H1, CDR-H2, and CDR-H3 having the amino sequences of SEQ ID NOs:59-61, respectively, and a VL comprising CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NOs:62-64, respectively.

11. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of claims 1 to 10, which is a chimeric or humanized antibody or antigen-binding fragment of a chimeric or humanized antibody.

12. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of claims 1 to 11, which comprises:

(a) a VH comprising an amino acid sequence having at least 95% sequence identity to SEQ ID NO:1 and a VL comprising an amino acid sequence having at least 95% sequence identity to SEQ ID NO:2;

(b) a VH comprising an amino acid sequence having at least 95% sequence identity to SEQ ID NO:23 and a VL comprising an amino acid sequence having at least 95% sequence identity to SEQ ID NO:24.

(c) a VH comprising an amino acid sequence having at least 95% sequence identity to SEQ ID NO:45 and a VL comprising an amino acid sequence having at least 95% sequence identity to SEQ ID NO:46.

13. An anti-glyco-MUC4 antibody or antigen-binding fragment that competes with a reference antibody or antigen binding fragment comprising:

(a) a heavy chain variable (VH) sequence of SEQ ID NO:1 and a light chain variable (VL) sequence of SEQ ID NO:2;
(b) a heavy chain variable (VH) sequence of SEQ ID NO:23 and a light chain variable (VL) sequence of SEQ ID NO:24;
(c) a heavy chain variable (VH) sequence of SEQ ID NO:45 and a light chain variable (VL) sequence of SEQ ID NO:46; or
(d) a heavy chain variable (VH) sequence of any one of SEQ ID NOs:133-144 and a light chain variable (VL) sequence of any one of SEQ ID NOs:145-153,

for binding to a MUC4 peptide CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) that has been glycosylated with GalNAc on the serine and threonine residues shown with bold and underlined text ("the MUC4 glycopeptide"), the anti-glyco-MUC4 antibody or antigen-binding fragment comprising:

(a) a VH sequence with first, second and third CDR means within the VH sequence; and
(b) a VL sequence with fourth, fifth and sixth CDR means within the VL sequence,

wherein the first, second, third, fourth, fifth, and sixth CDR means cooperate to effect binding of the anti-glyco-MUC4 antibody or antigen-binding fragment to the MUC4 glycopeptide.

14. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of claims 1 to 13, which preferentially binds to a glyco-MUC4 epitope that is overexpressed on cancer cells as compared to normal cells.

15. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of claims 1 to 14, which specifically binds to a MUC4 peptide CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) that has been glycosylated with STn on the serine and threonine residues shown with bold and underlined text.

16. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of claims 1 to 14, which does not specifically bind to a MUC4 peptide CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) that has been glycosylated with STn on the serine and threonine residues shown with bold and underlined text.

17. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of claims 1 to 16, which binds to the MUC4 glycopeptide with a binding affinity (KD) of:

(a) 1 nM to 200 nM as measured by surface plasmon resonance or bio-layer interferometry;

(b) 1 nM to 150 nM as measured by surface plasmon resonance or bio-layer interferometry;

(c) 1 nM to 100 nM as measured by surface plasmon resonance or bio-layer interferometry;

(d) 1 nM to 50 nM as measured by surface plasmon resonance or bio-layer interferometry;

(e) 5 nM to 200 nM as measured by surface plasmon resonance or bio-layer interferometry;

(f) 5 nM to 100 nM as measured by surface plasmon resonance or bio-layer interferometry;

(g) 5 nM to 50 nM as measured by surface plasmon resonance or bio-layer interferometry;

(h) 5 nM to 25 nM as measured by surface plasmon resonance or bio-layer interferometry;

(i) 5 nM to 10 nM as measured by surface plasmon resonance or bio-layer interferometry;

(j) 10 nM to 200 nM as measured by surface plasmon resonance or bio-layer interferometry;

(k) 10 nM to 100 nM as measured by surface plasmon resonance or bio-layer interferometry;

(l) 10 nM to 150 nM as measured by surface plasmon resonance or bio-layer interferometry;

(m) 10 nM to 100 nM as measured by surface plasmon resonance or bio-layer interferometry;

(n) 10 nM to 50 nM as measured by surface plasmon resonance or bio-layer interferometry;

(o) 10 nM to 25 nM as measured by surface plasmon resonance or bio-layer interferometry;

(p) 50 nM to 200 nM as measured by surface plasmon resonance or bio-layer interferometry;

(q) 50 nM to 150 nM as measured by surface plasmon resonance or bio-layer interferometry;

(r) 50 nM to 100 nM as measured by surface plasmon resonance or bio-layer interferometry;

(s) 100 nM to 200 nM as measured by surface plasmon resonance or bio-layer interferometry; or

(t) 100 nM to 150 nM as measured by surface plasmon resonance or bio-layer interferometry.

18. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of claims 1 to 17, which does not specifically bind to the unglycosylated MUC4 peptide CTIPSTAMHTRSTAAPIPILP (SEQ ID NO:155) (the "unglycosylated MUC4 peptide").

19. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of claims 1 to 18, which does not specifically bind to the MUC1 tandem repeat $(VTSAPDTRPAPGSTAPPAHG)_3$ (SEQ ID NO:201) that has been glycosylated in vitro using purified recombinant human glycosyltransferases GalNAc-T1, GalNAc-T2, and GalNAc-T4 ("the first MUC1 glycopeptide").

20. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of claims 1 to 19, which does not specifically bind to the MUC1 peptide TAPPAHGV**TS**APD**T**RPAPG**ST**APPAHGVT (SEQ ID NO:202) that has been glycosylated in vitro with GalNAc on the serine and threonine residues shown with bold and underlined text (the "second MUC1 glycopeptide").

21. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of claims 1 to 20, which does not specifically bind to the CD44v6 peptide GYRQ**T**PKEDSH**S**TTGTAAA (SEQ ID NO:218) that has been glycosylated in vitro with GalNAc on the threonine and serine residues shown with bold and underlined text (the "CD44v6 glycopeptide").

22. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of claims 1 to 21, which does not specifically bind to the LAMP1 peptide CEQDRP**S**P**TT**APPAPPSPSP (SEQ ID NO:219) that has been glycosylated in vitro with GalNAc on the serine and threonine residues shown with bold and underlined text (the "LAMP1 glycopeptide").

23. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of claims 1 to 22, which does not specifically bind to the cMET peptide PTKSFISGG**ST**ITGVGKNLN (SEQ ID NO:220) that has been glycosylated in vitro with GalNAc on the serine and threonine residues shown with bold and underlined text (the "cMET glycopeptide").

24. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of claims 1 to 23, which is multivalent.

25. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of claims 1 to 24, which is an antigen-binding fragment.

26. The anti-glyco-MUC4 antibody or antigen-binding fragment of claim 25, wherein the antigen-binding fragment is in the form of a single-chain variable fragment (scFv).

27. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of claims 1 to 24, which is in the form of a multispecific antibody.

28. The anti-glyco-MUC4 antibody or antigen-binding fragment of claim 27, wherein the multispecific antibody is a bispecific antibody that binds to a second epitope that is different from the first epitope.

29. The anti-glyco-MUC4 antibody or antigen-binding fragment of claim 28, wherein the bispecific antibody is a bottle opener, mAb-Fv, mAb-scFv, central-scFv, one-armed central-scFv, or dual scFv format bispecific antibody.

30. The anti-glyco-MUC4 antibody or antigen-binding fragment of claim 28, wherein the bispecific antibody is a bispecific domain-exchanged antibody (*e.g.*, a CrossMab), a Fab-arm exchange antibody, a bispecific T-cell engager (BiTE), or a dual-affinity retargeting molecule (DART).

31. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of claims 28 to 30, wherein the second epitope is a MUC4 epitope.

32. The anti-glyco-MUC4 antibody of antigen-binding fragment of any one of claims 28 to 30, wherein the second epitope is a MUC4 epitope that is overexpressed on cancer cells as compared to normal cells.

33. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of claims 28 to 30, wherein the second epitope is a T-cell epitope.

34. The anti-glyco-MUC4 antibody or antigen-binding fragment of claim 33, wherein the T-cell epitope comprises a CD3 epitope, a CD8 epitope, a CD16 epitope, a CD25 epitope, a CD28 epitope, or an NKG2D epitope.

35. A fusion protein comprising the amino acid sequence of the anti-glyco-MUC4 antibody or antigen-binding fragment of any of claims 1 to 34 operably linked to at least a second amino acid sequence.

36. A chimeric antigen receptor (CAR) comprising one or more antigen-binding fragments according to claim 25 or claim 26.

37. An antibody-drug conjugate comprising the anti-glyco-MUC4 antibody or antigen-binding fragment of any of claims 1 to 34 or the fusion protein of claim 35 conjugated to a cytotoxic agent.

38. A chimeric T cell receptor (TCR) comprising:

(a) an antigen-binding fragment according to claim 25 or claim 26;

(b) a first polypeptide chain comprising a first TCR domain comprising a first TCR transmembrane domain from a first TCR subunit; and

(c) a second polypeptide chain comprising a second TCR domain comprising a second TCR transmembrane domain from a second TCR subunit.

39. A nucleic acid comprising a coding region for an anti-glyco-MUC4 antibody or antigen-binding fragment of any of claims 1 to 34, the fusion protein of claim 35, the CAR of claim 36, or the chimeric TCR of claim 38.

40. A vector comprising the nucleic acid of claim 39.

41. A host cell engineered to express the nucleic acid of claim 39 or comprising the vector of claim 40.

42. A pharmaceutical composition comprising (a) the anti-glyco-MUC4 antibody or antigen binding fragment of any of claims 1 to 34, the fusion protein of claim 35, the CAR of claim 36, the antibody-drug conjugate of claim 37, the chimeric TCR of claim 38, the nucleic acid of claim 39, the vector of claim 40, or the host cell of claim 41, and (b) a physiologically suitable buffer, adjuvant, diluent, or combination thereof.

43. A method of treating cancer comprising administering to a subject in need thereof an effective amount of the anti-glyco-MUC4 antibody or antigen binding fragment of any of claims 1 to 34, the fusion protein of claim 35, the CAR of claim 36, the antibody-drug conjugate of claim 37, the chimeric TCR of claim 38, the nucleic acid of claim 39, the vector of claim 40, the host cell of claim 41, or the pharmaceutical composition of claim 42.

44. The method of claim 43, wherein the subject is suffering from pancreatic cancer, lung cancer, breast cancer, cancer of the gall bladder, salivary gland cancer, prostate cancer, biliary tract cancer, esophageal cancer, papillary thyroid carcinoma, low-grade fibromyxoid sarcoma, and ovarian cancer.

45. A method of detecting cancer in a biological sample, comprising contacting a sample (*e.g.*, a sample comprising or suspected of comprising cancer cells and/or cancer-derived extracellular vesicles) with an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of claims 1 to 34 and detecting binding of the anti-glyco-MUC4 antibody or antigen-binding fragment.

46. The method of any one of claims 45, wherein the cancer is pancreatic cancer, lung cancer, breast cancer, cancer of the gall bladder, salivary gland cancer, prostate cancer, biliary tract cancer, esophageal cancer, papillary thyroid carcinoma, low-grade fibromyxoid sarcoma, and ovarian cancer.

47. A peptide of 13-30 amino acids in length comprising (a) amino acids 4-16 of a MUC4 peptide CTIPSTAMHTRSTAAPIPILP (SEQ ID NO:155) or (b) an amino acid sequence corresponding to amino acids 4-16 of the MUC4 peptide CTIPSTAMHTRSTAAPIPILP (SEQ ID NO:155) with one or two amino acid substitutions at positions other than the serine corresponding to position 12 of CTIPSTAMHTRSTAAPIPILP (SEQ ID NO:155) and/or the threonine corresponding to position 13 of CTIPSTAMHTRSTAAPIPILP (SEQ ID NO:155).

48. A peptide of 13-30 amino acids in length comprising (a) amino acids 4-16 of a MUC4 peptide CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) that has been O-glycosylated on the serine and threonine residues shown with bold and underlined text or (b) an amino acid sequence corresponding to amino acids 4-16 of the MUC4 peptide CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) that has been O-glycosylated on the serine and threonine residues shown with bold and underlined text with one or two amino acid substitutions at positions other than the serine corresponding to position 12 of CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) and/or the threonine corresponding to position 13 of CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154).

49. A composition comprising the peptide of claim 47 or claim 48 and an adjuvant.

50. A method of generating antibodies against a tumor-associated form of MUC4, comprising administering to an animal:

(a) the peptide of claim 48; or

(b) the composition of claim 49, wherein the composition comprises the peptide of claim 48.

51. A method of eliciting an immune response against a tumor-associated form of MUC4, comprising administering to a subject:

(a) the peptide of claim 48; or

(b) The composition of claim 49, wherein the composition comprises the peptide of claim 48.

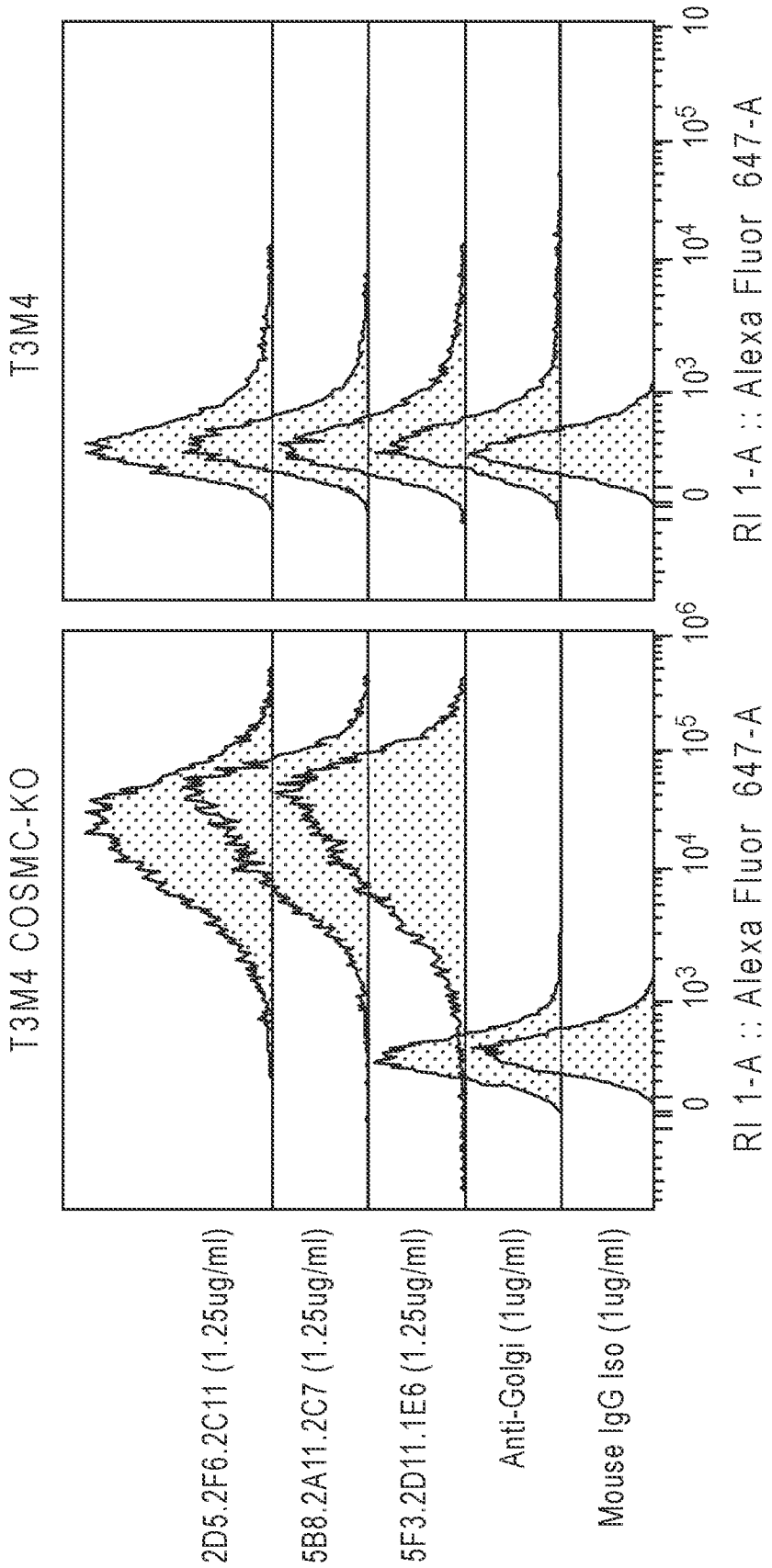

T3M4 COSMC-KO
T3M4
2D5.2F6.2C11 (1.25ug/ml)
5B8.2A11.2C7 (1.25ug/ml)
15F3.2D11.1E6 (1.25ug/ml)
Anti-Golgi (1ug/ml)
Mouse IgG Iso (1ug/ml)
RI 1-A :: Alexa Fluor 647-A
RI 1-A :: Alexa Fluor 647-A
0
10^3
10^4
10^5
10^6

FIG. 1A

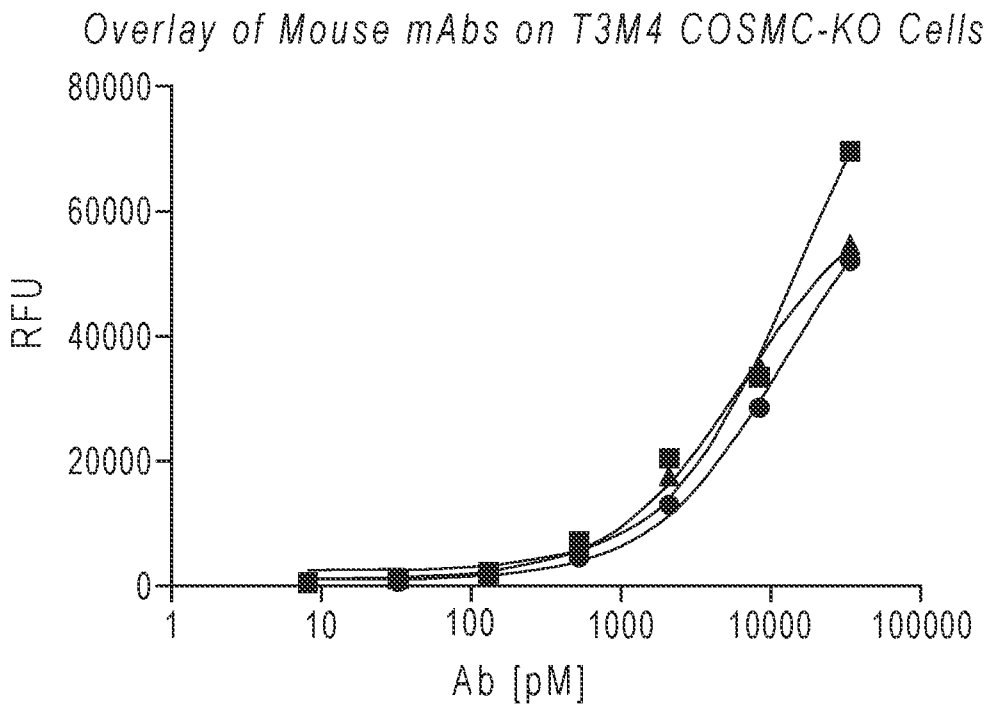

Overlay of Mouse mAbs on T3M4 COSMC-KO Cells
80000
60000
40000
20000
0
RFU
1
10
100
1000
10000
100000
Ab [pM]

FIG. 1B

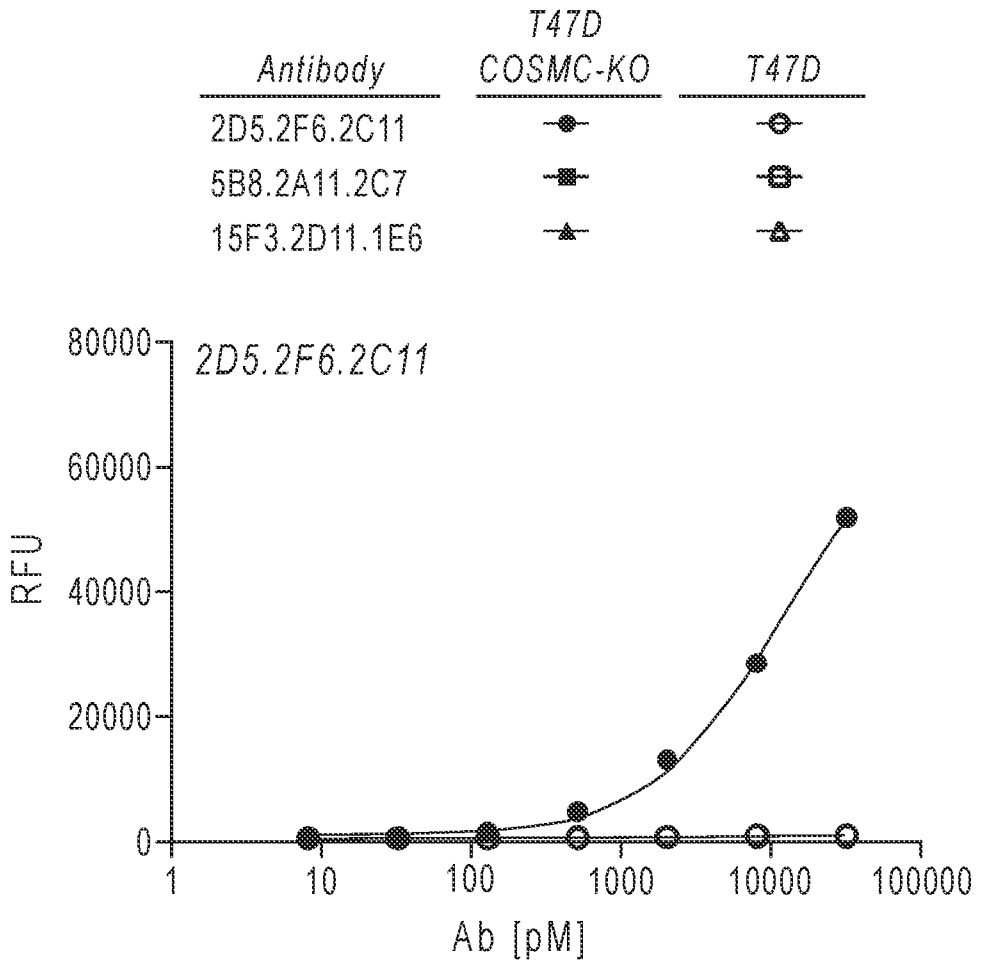

T47D
Antibody
COSMC-KO
T47D
2D5.2F6.2C11
5B8.2A11.2C7
15F3.2D11.1E6
2D5.2F6.2C11
80000
60000
40000
20000
0
RFU
1
10
100
1000
10000
100000
Ab [pM]

FIG. 1C

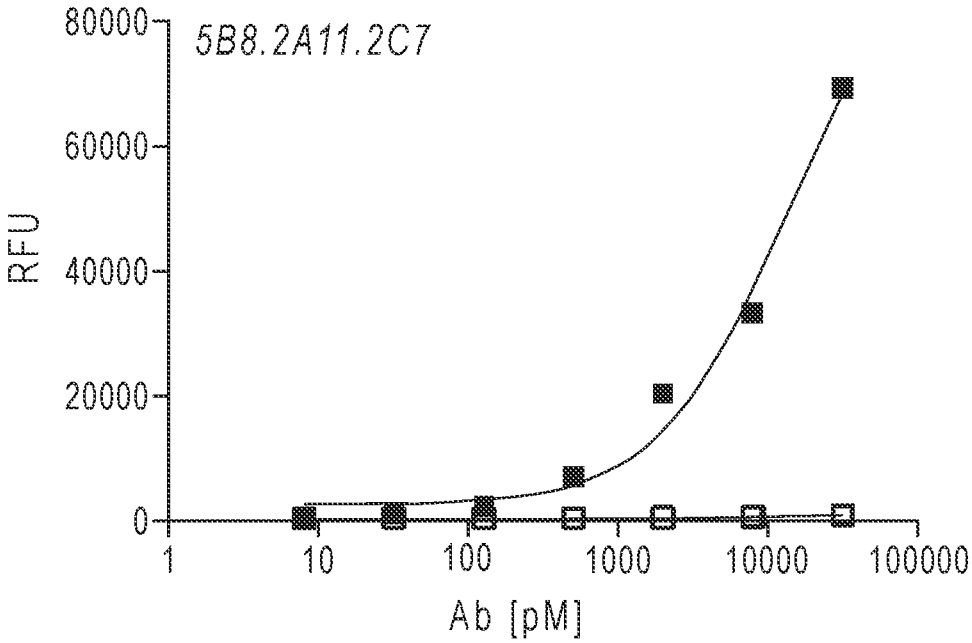


FIG. 1D

Antibody	T47D COSMC-KO	T47D
2D5.2F6.2C11	●	○
5B8.2A11.2C7	■	□
15F3.2D11.1E6	▲	△

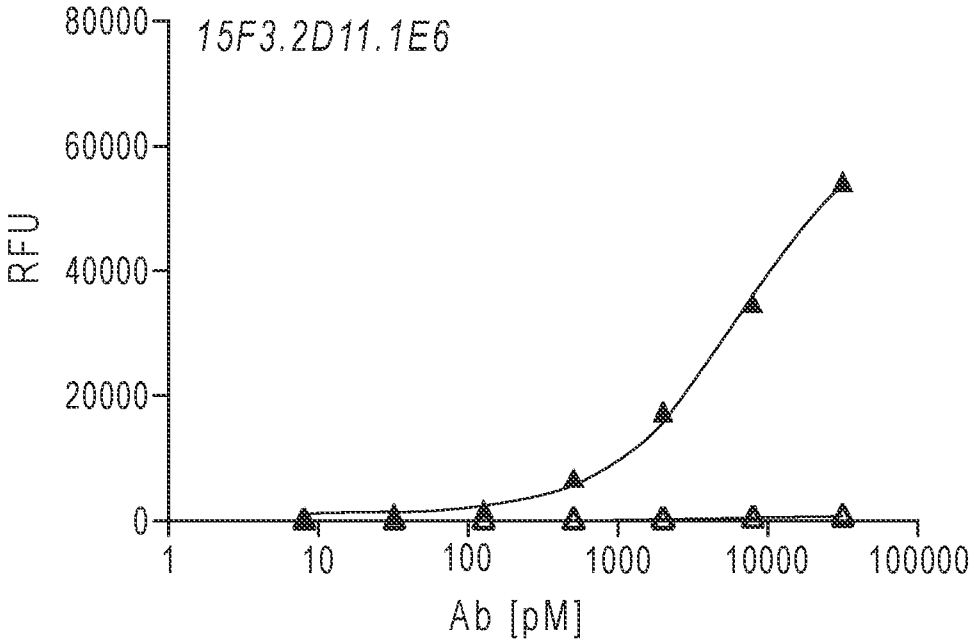


FIG. 1E

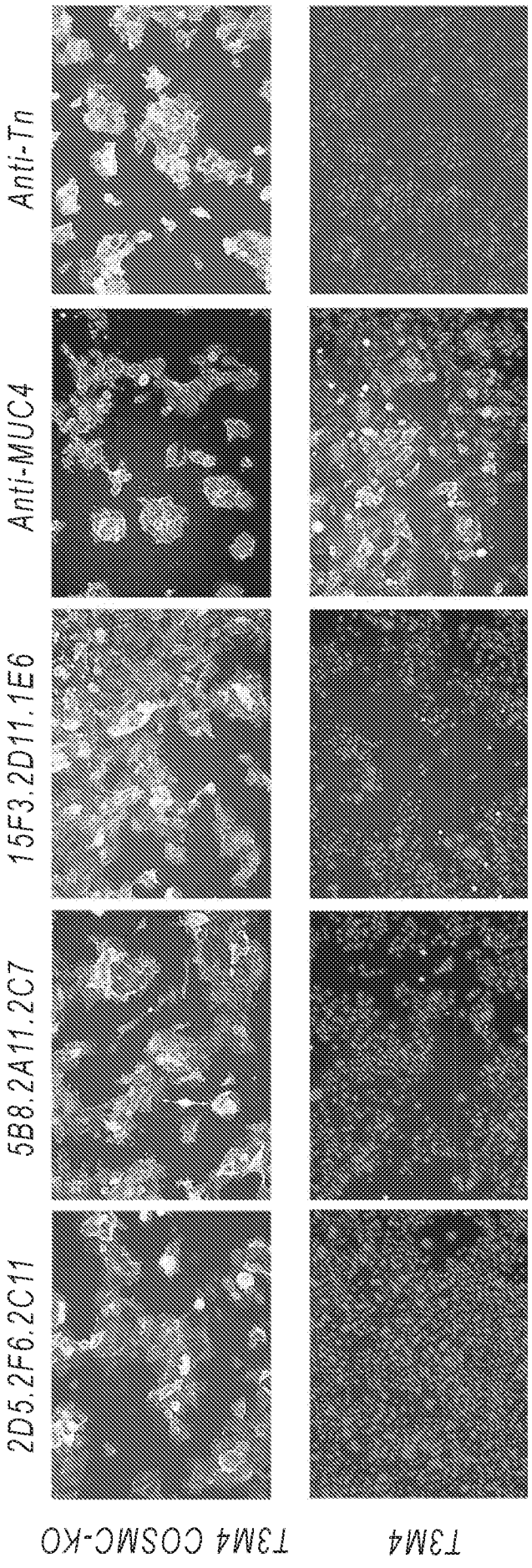

Anti-Tn
Anti-MUC4
15F3.2D11.1E6
5B8.2A11.2C7
2D5.2F6.2C11
T3M4 COSMC-KO
T3M4

FIG. 2

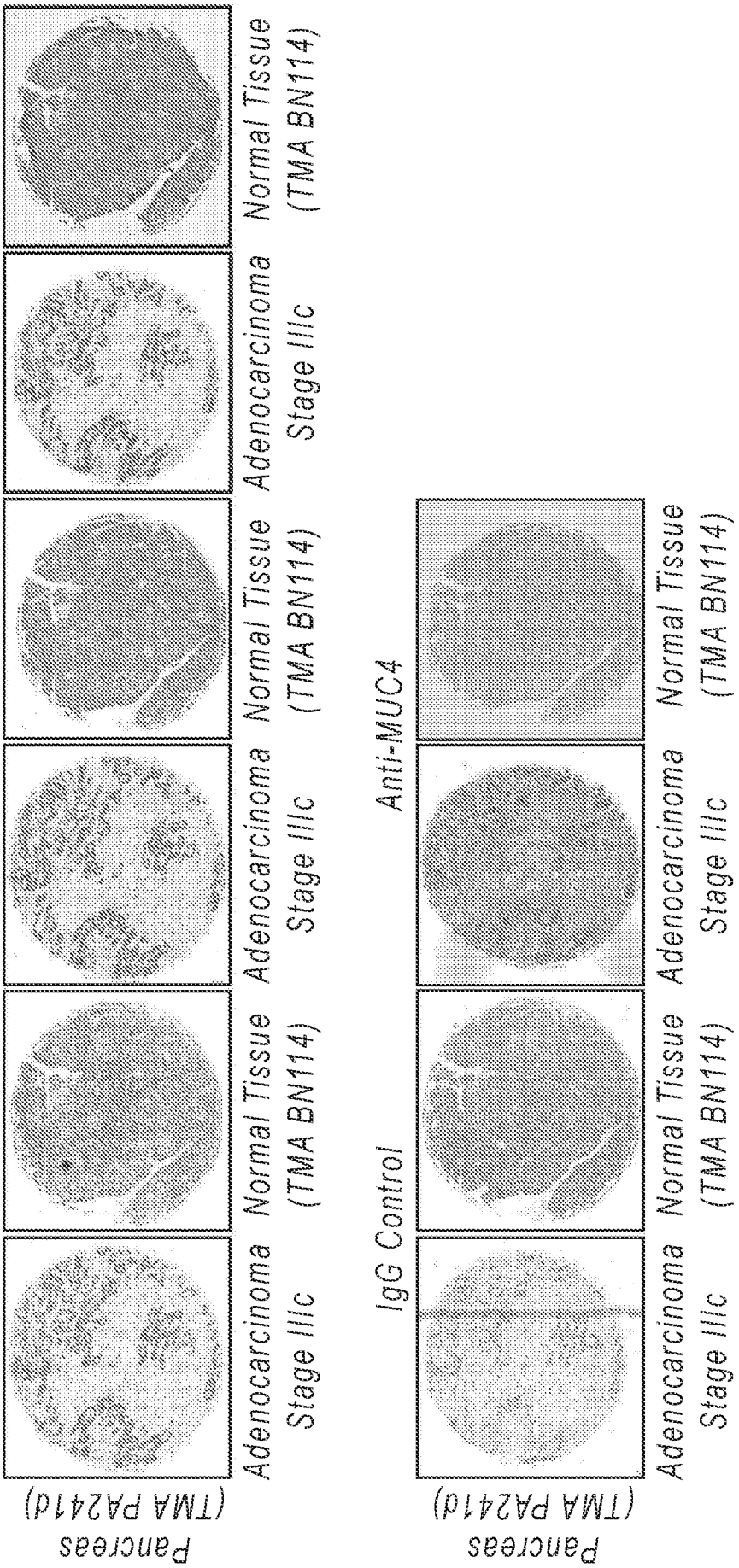

Pancreas (TMA PA241d)
Adenocarcinoma Stage IIIc
Normal Tissue (TMA BN114)
Adenocarcinoma Stage IIIc
Normal Tissue (TMA BN114)
IgG Control
2D5.2F6.2C11
5B8.2A11.2C7
15F3.2D11.1E6
Anti-MUC4

FIG. 3A

Pancreatic Cancer (TMA PA241d)

Antibody	Cases	Positive	Negative
2D5.2F6.2C11	6	3 (50%)	3 (50%)
5B8.2A11.2C7	6	3 (50%)	3 (50%)
15F3.2D11.1E6	6	3 (50%)	3 (50%)
Anti-MUC4	6	3 (50%)	3 (50%)

FIG. 3B

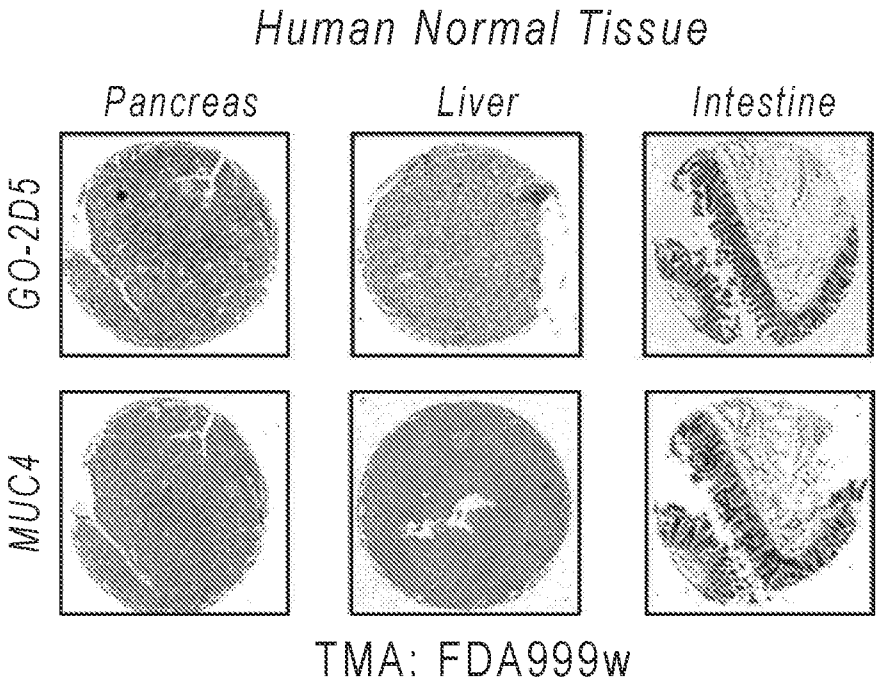


FIG. 3C

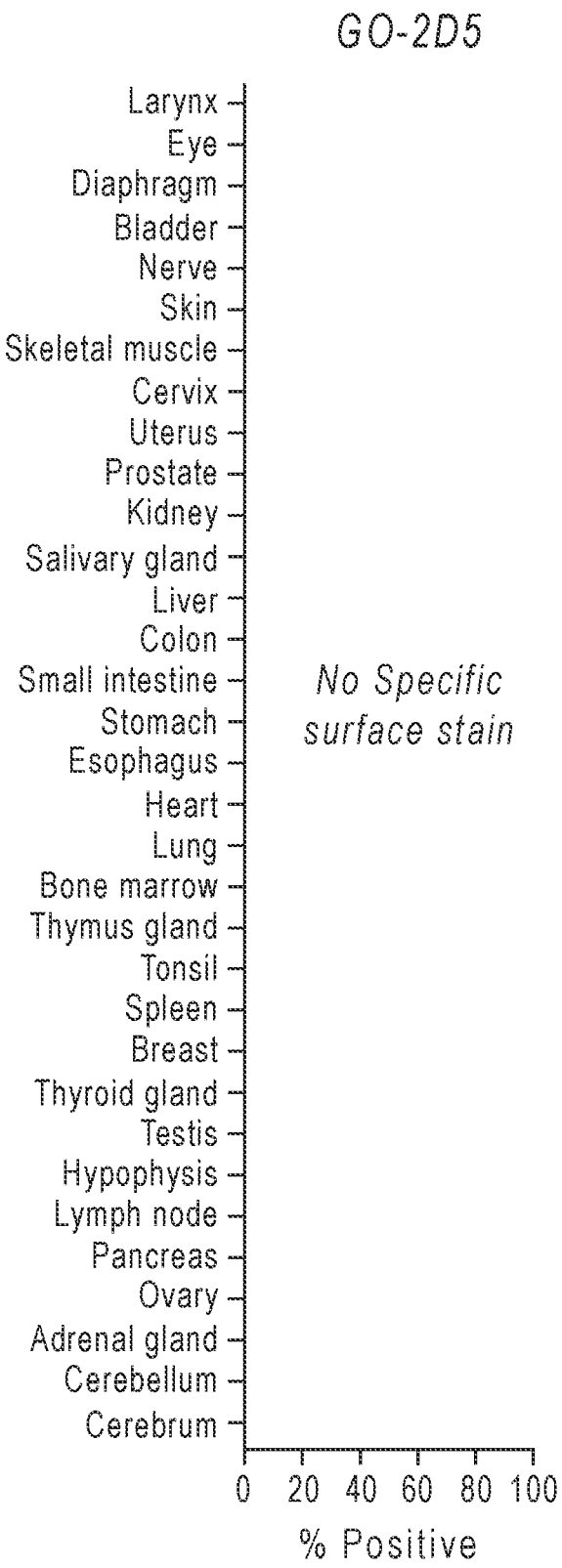

GO-2D5
Larynx
Eye
Diaphragm
Bladder
Nerve
Skin
Skeletal muscle
Cervix
Uterus
Prostate
Kidney
Salivary gland
Liver
Colon
Small intestine
Stomach
Esophagus
Heart
Lung
Bone marrow
Thymus gland
Tonsil
Spleen
Breast
Thyroid gland
Testis
Hypophysis
Lymph node
Pancreas
Ovary
Adrenal gland
Cerebellum
Cerebrum
No Specific surface stain
0 20 40 60 80 100
% Positive

FIG. 3D

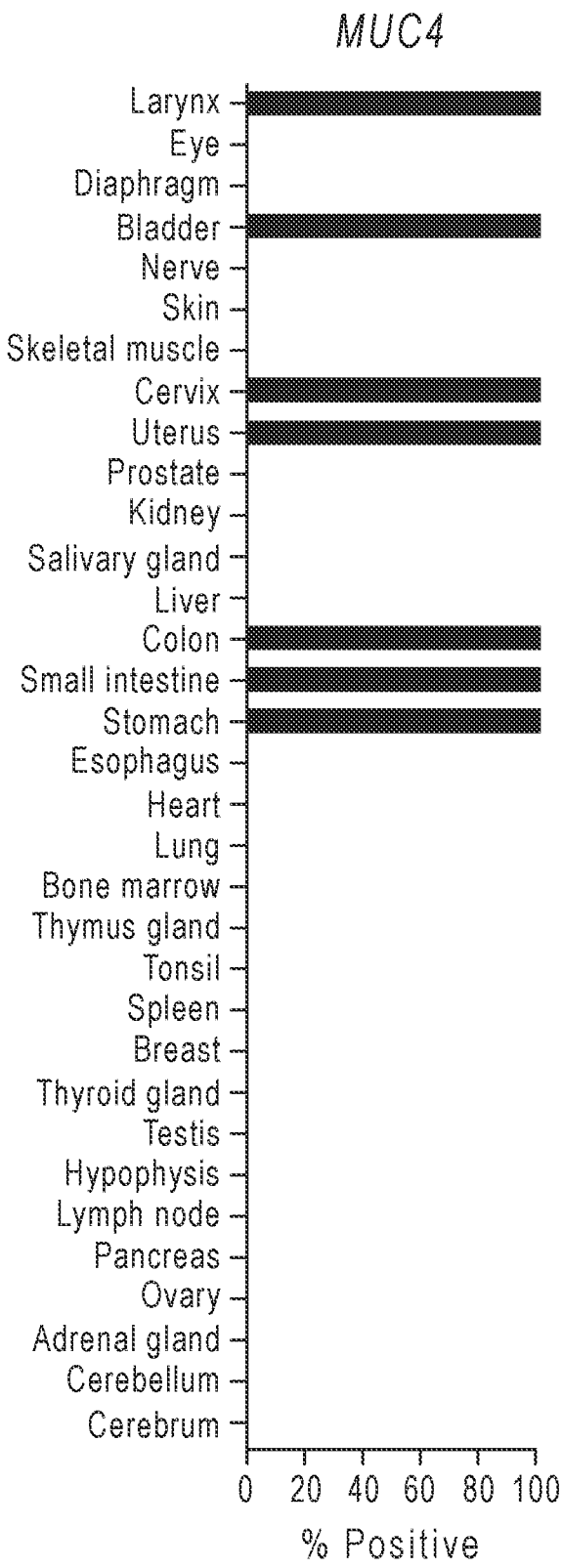

MUC4
Larynx
Eye
Diaphragm
Bladder
Nerve
Skin
Skeletal muscle
Cervix
Uterus
Prostate
Kidney
Salivary gland
Liver
Colon
Small intestine
Stomach
Esophagus
Heart
Lung
Bone marrow
Thymus gland
Tonsil
Spleen
Breast
Thyroid gland
Testis
Hypophysis
Lymph node
Pancreas
Ovary
Adrenal gland
Cerebellum
Cerebrum
0 20 40 60 80 100
% Positive

FIG. 3E

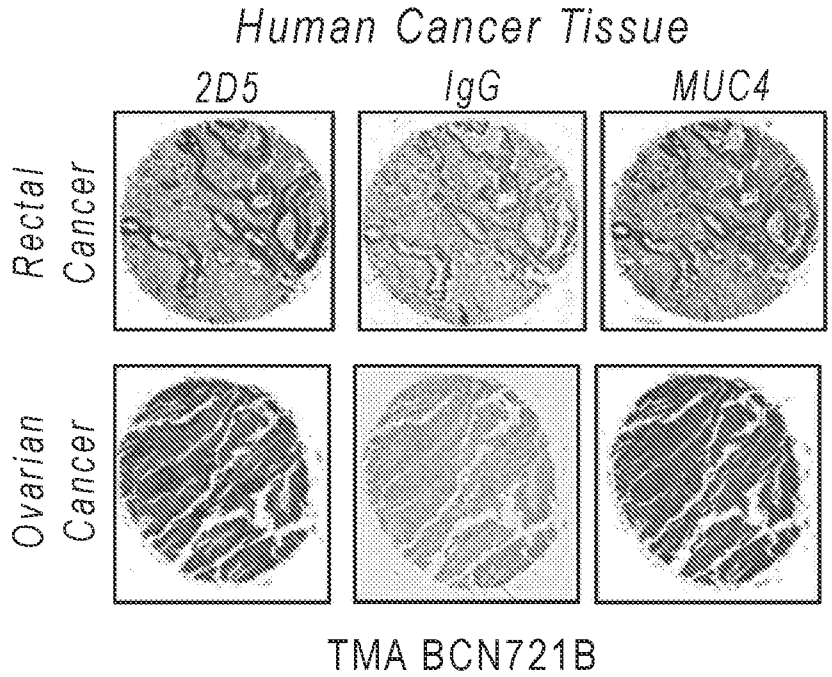
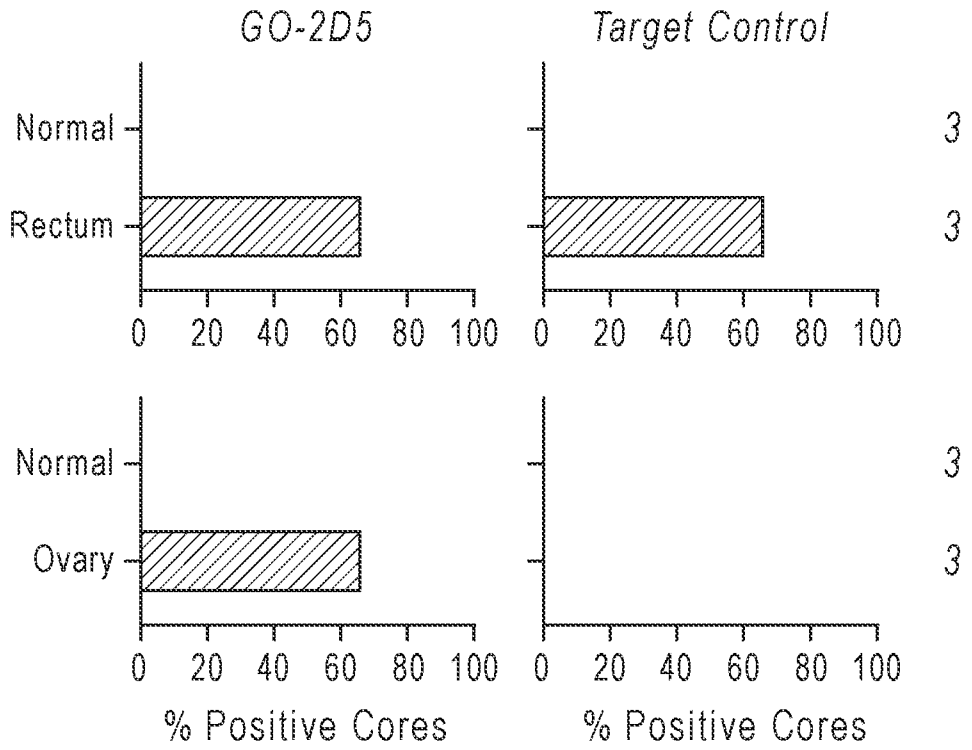


FIG. 3F



Positive Core = >70% positive surface stain on cancer cells

FIG. 3G

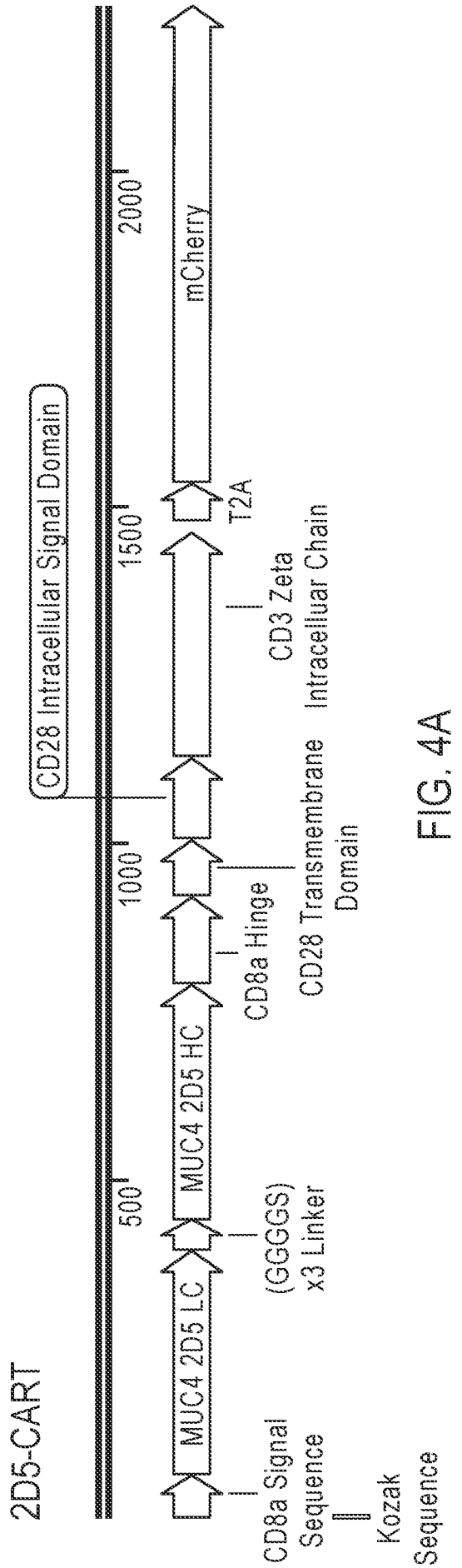

2D5-CART
500
1000
1500
2000
CD28 Intracellular Signal Domain
MUC4 2D5 LC
MUC4 2D5 HC
mCherry
CD8a Signal Sequence
Kozak Sequence
(GGGGS) x3 Linker
CD8a Hinge
CD28 Transmembrane Domain
CD3 Zeta Intracelluar Chain
T2A

FIG. 4A

15F3-CART
500
1000
1500
2000
CD28 Intracellular Signal Domain
MUC4 15F3 LC
MUC4 15F3 HC
mCherry
CD8a Signal Sequence
Kozak Sequence
(GGGGS) x3 Linker
CD8a Hinge
CD28 Transmembrane Domain
CD3 Zeta Intracelluar Chain
T2A

FIG. 4B

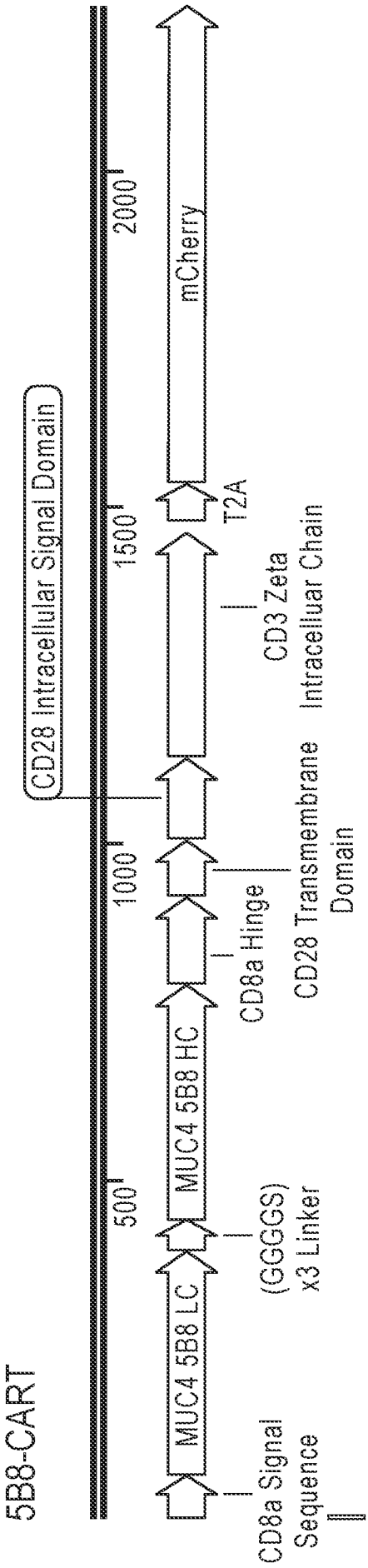

5B8-CART
CD8a Signal Sequence
Kozak Sequence
MUC4 5B8 LC
(GGGGS) x3 Linker
MUC4 5B8 HC
CD8a Hinge
CD28 Transmembrane Domain
CD28 Intracellular Signal Domain
CD3 Zeta Intracelluar Chain
T2A
mCherry
500
1000
1500
2000

FIG. 4C

FIG. 5A

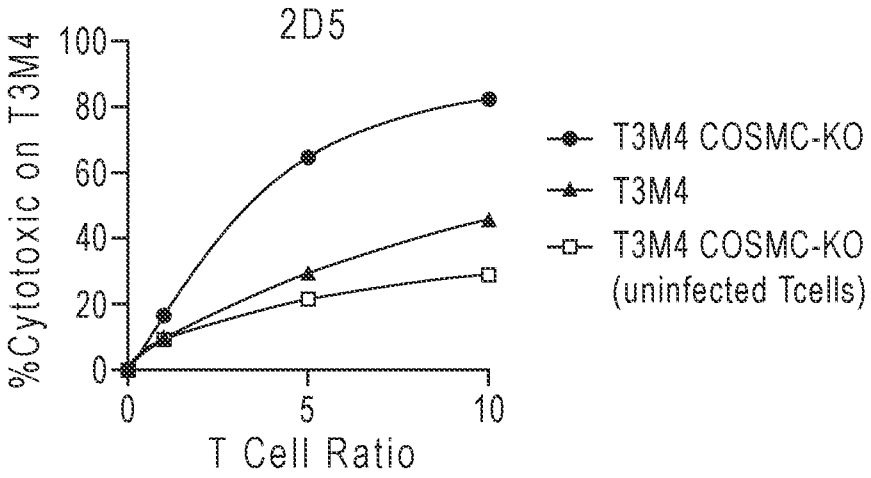


FIG. 5B

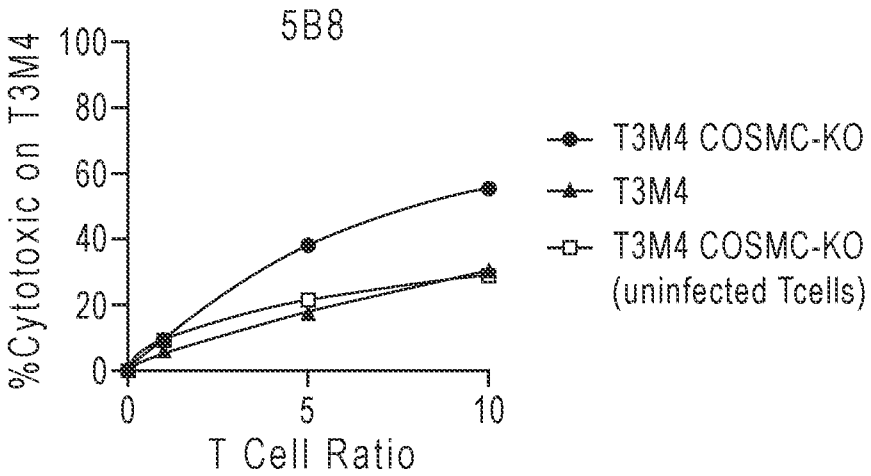
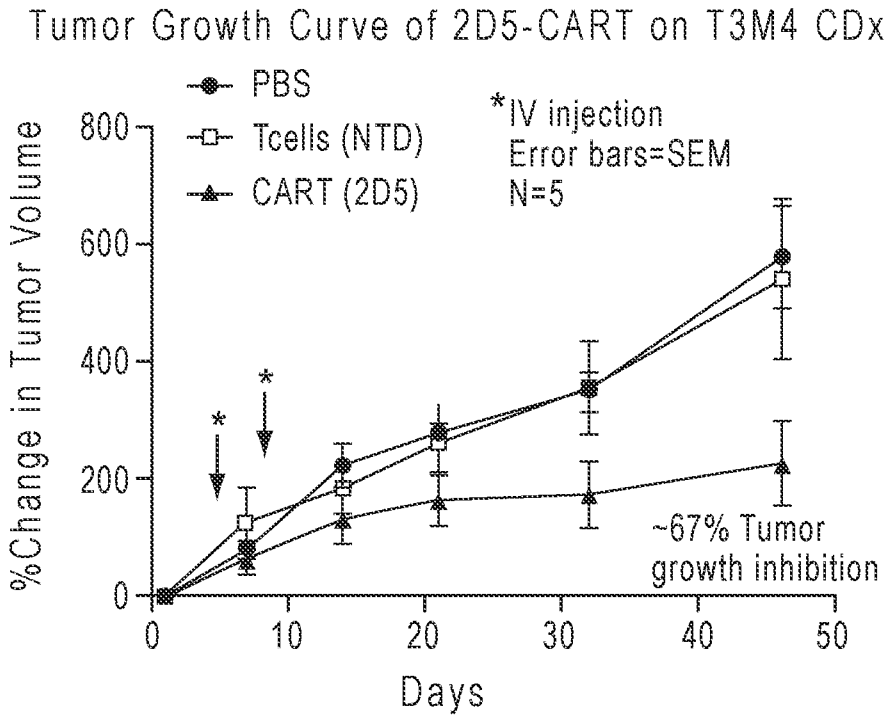


FIG. 6



Long HC MUC4(2D5)-GP/CD3	mVH-MUC4-GP	hCH1	Linker 1	hVH-CD3 Roche	Linker 2	hCL-kappa (mut)	Linker 3	hCH2 mut	hCH3 (long)	CHS
Short HC MUC4(2D5)-GP	mVH-MUC4-GP	hCH1	Hinge 1	hCH2 mut	hCH3 (short)	CHS				
Cross VL CD3	hVL-lambda-CD3	Linker 4	hCH1	Hinge 2						
VL kappa MUC4(2D5)-GP	mVL-kappa-MUC4-GP	hCL-kappa								

FIG. 7

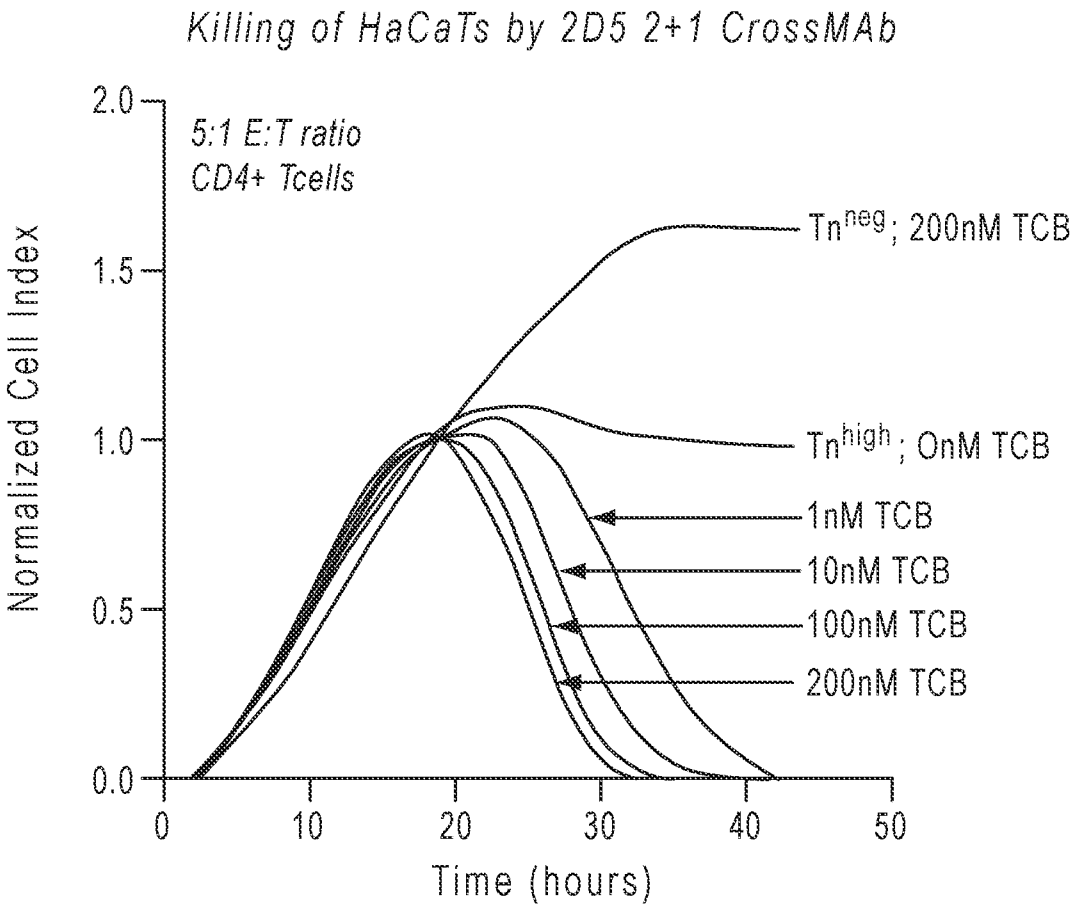

Killing of HaCaTs by 2D5 2+1 CrossMAb
5:1 E:T ratio
CD4+ Tcells
Normalized Cell Index
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
Time (hours)
Tn^neg; 200nM TCB
Tn^high; OnM TCB
1nM TCB
10nM TCB
100nM TCB
200nM TCB

FIG. 8

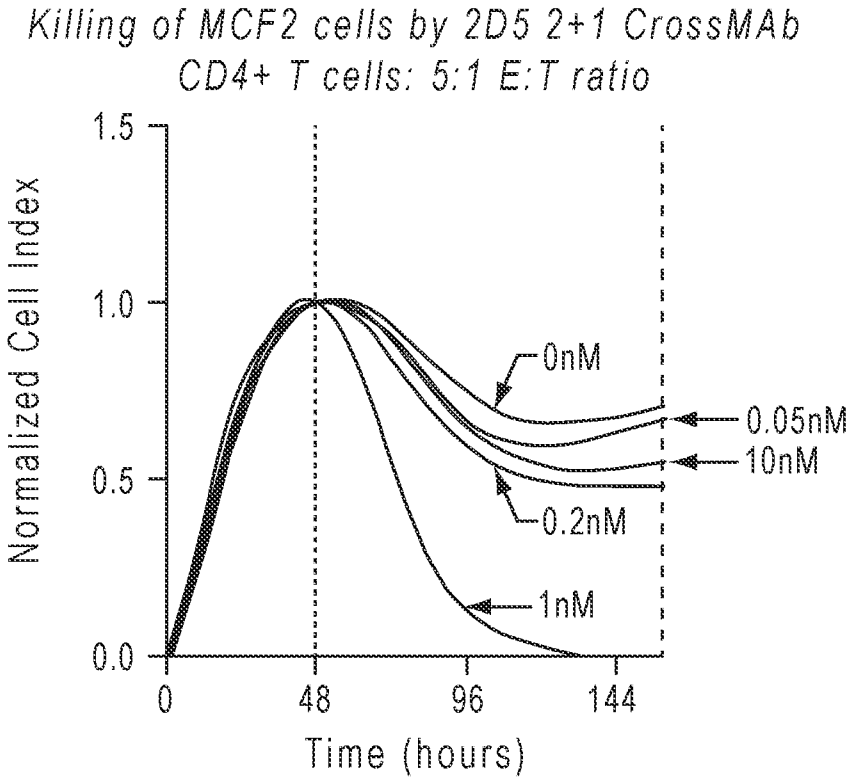

Killing of MCF2 cells by 2D5 2+1 CrossMAb
CD4+ T cells: 5:1 E:T ratio
Normalized Cell Index
1.5
1.0
0.5
0.0
0
48
96
144
Time (hours)
0nM
0.05nM
10nM
0.2nM
1nM

FIG. 9A

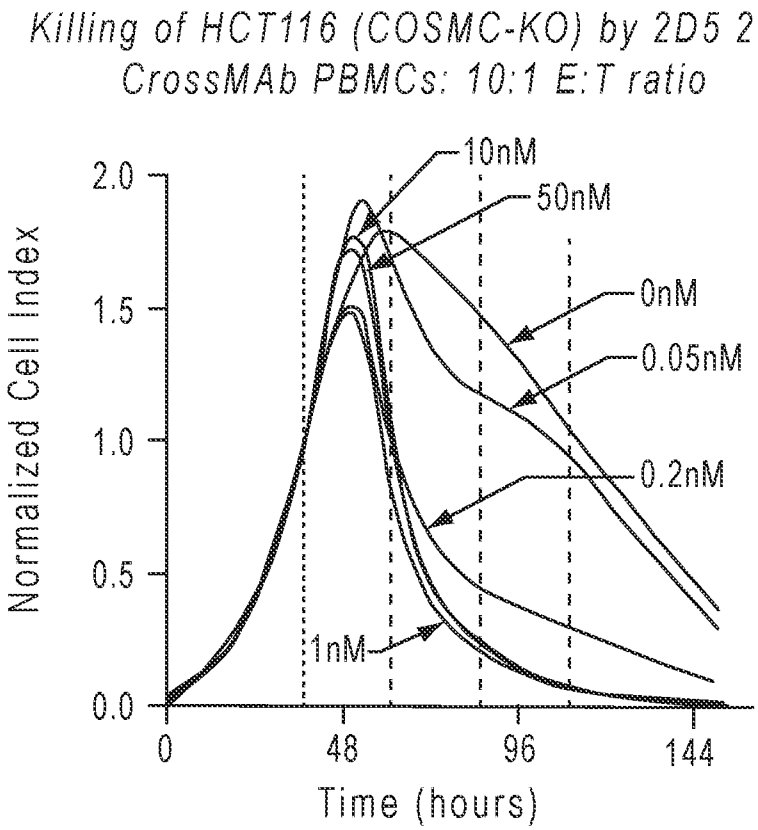

Killing of HCT116 (COSMC-KO) by 2D5 2+1
CrossMAb PBMCs: 10:1 E:T ratio
Normalized Cell Index
2.0
1.5
1.0
0.5
0.0
0
48
96
144
Time (hours)
10nM
50nM
0nM
0.05nM
0.2nM
1nM

FIG. 9B

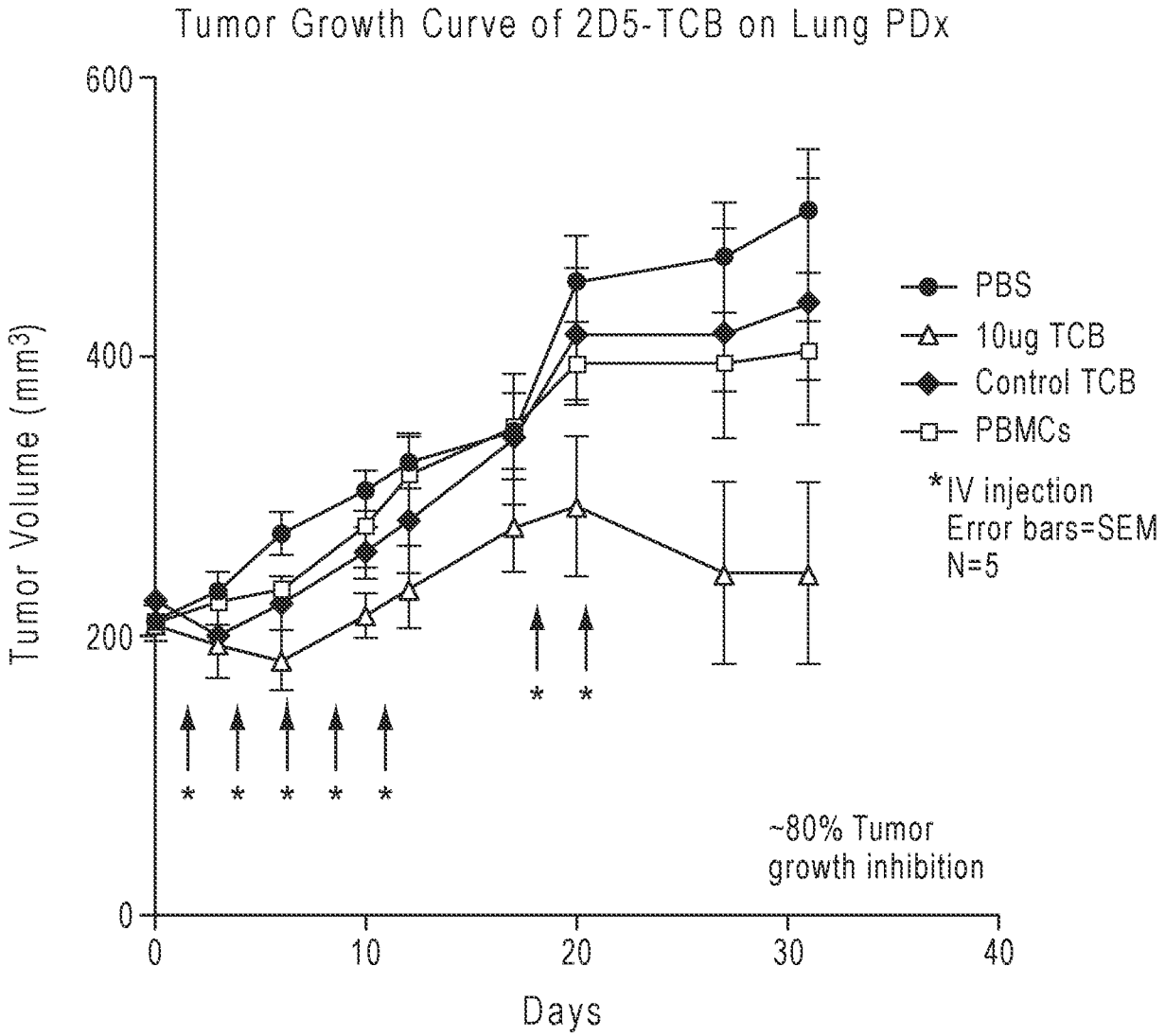

Tumor Growth Curve of 2D5-TCB on Lung PDx
Tumor Volume (mm3)
600
400
200
0
0
10
20
30
40
Days
PBS
10ug TCB
Control TCB
PBMCs
*IV injection
Error bars=SEM
N=5
~80% Tumor
growth inhibition

FIG. 10

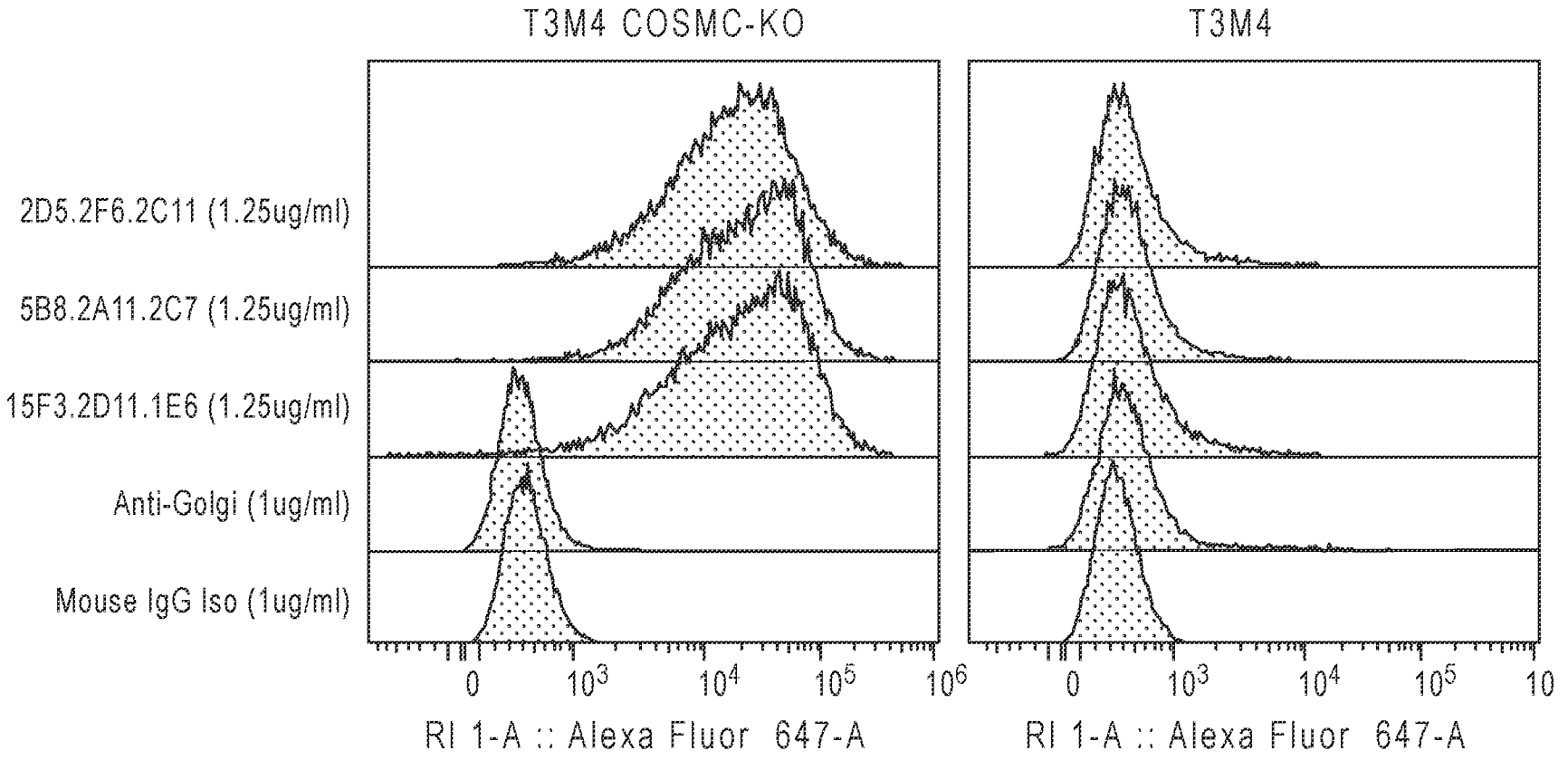

T3M4 COSMC-KO
T3M4
2D5.2F6.2C11 (1.25ug/ml)
5B8.2A11.2C7 (1.25ug/ml)
15F3.2D11.1E6 (1.25ug/ml)
Anti-Golgi (1ug/ml)
Mouse IgG Iso (1ug/ml)
0
10^3
10^4
10^5
10^6
RI 1-A :: Alexa Fluor 647-A
0
10^3
10^4
10^5
10
RI 1-A :: Alexa Fluor 647-A

FIG. 1A