example of antibody-induced oncosis is anti-Porimin[63]. Increase in membrane permeability of anti-Porimin treated Jurkat cells was detected by PI uptake. Moreover, formation of pores on the cells membrane was also visualized under Scanning Electron Microscope (SEM). Another hallmark of anti-Porimin induced oncosis is the re-arrangement of cytoskeletal proteins. A more relevant example is mAb84-induced hESC death via oncosis. Studies have also shown some oncotic features of mAb84-induced hESC death, such as rapid cell death, formation of cell aggregation, loss of membrane integrity and degradation of actin cytoskeleton-associated proteins[82]. However, for all mentioned studies, though the mode of cell death was identified, the detailed mechanism of antibody-induced cell death is still unknown.

WO2012/011876 describes a method of selecting an antibody as a candidate for having cytotoxic activity against a cell which expresses podocalyxin-like protein (PODXL) where the antibody binds PODXL, the method comprising a step of comparing the binding of a PODXL-binding antibody molecule to a glycan comprising Fucα1-2Galß1-3GlcNAc with the binding of a non-cytotoxic PODXL-binding antibody molecule to a glycan comprising Fucα1-2Galß1-3GlcNAc.

Summary of the Invention

In one aspect, the present invention refers to an antibody that binds to the glycan motif Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1 or Fucα1-2Galβ1-3GlcNAc, wherein the antibody is cytotoxic against undifferentiated pluripotent cells, and wherein the antibody comprises: at least one light chain variable region incorporating the following CDRs: CDR1: SASSSVSYMF (SEQ ID NO: 1); CDR2: LTSNLAS (SEQ ID NO: 2); CDR3: QQWSSNPYT (SEQ ID NO: 3); and at least one heavy chain variable region incorporating the following CDRs: CDR1: GFTFSNYYMN (SEQ ID NO: 4); CDR2: EIRLKSNNYATHYAESVKG (SEQ ID NO: 5); CDR3: FGY (SEQ ID NO: 6). In another aspect, the present invention refers to a monoclonal antibody produced from hybridoma cell line TAG-A1, deposited with American Type Culture Collection under Accession Number PTA-121134. In still another aspect, the present invention refers to a composition comprising the antibody or polypeptide of the present invention and at least one pharmaceutically-acceptable carrier. In another aspect, the present invention refers to a isolated nucleic acid encoding the antibody or polypeptide of the present invention. In another aspect, the present invention refers to a vector comprising the nucleic acid of the present invention. In another aspect, the present invention refers to a host cell comprising the vector of the present invention. In another aspect, the present invention refers to a

method for making an antibody or polypeptide of the present invention comprising culturing the host cell of the present invention under conditions suitable for the expression of a vector encoding the antibody or polypeptide, and recovering the antibody or polypeptide. In another aspect, the present invention refers to a method of destroying an undifferentiated pluripotent cell or cells in a sample containing such cells, the method comprising contacting a sample of cells containing an undifferentiated pluripotent cell or cells with an antibody according to the present invention. In another aspect, the present invention refers to a method of removing an undifferentiated pluripotent cell or cells from a sample containing such cells, the method comprising contacting a sample of cells containing an undifferentiated pluripotent cell or cells with an antibody of the present invention. In another aspect, the present invention refers to a method of enriching cells that have undergone or are undergoing differentiation from a mixture comprising undifferentiated pluripotent cells and such cells that have undergone or are undergoing differentiation, the method comprising contacting the mixture with an antibody of the present invention for a period of time sufficient for the antibody to kill undifferentiated pluripotent cells. In another aspect, the present invention refers to a method of preparing a composition containing cells differentiated from undifferentiated pluripotent cells which composition contains substantially no undifferentiated pluripotent cells, the method comprising: providing a population of cells comprising undifferentiated pluripotent cells and cells differentiated from undifferentiated pluripotent stem cells; contacting the population with an antibody of the present invention under conditions permitting the antibody to kill undifferentiated pluripotent cells; and separating live cells remaining after step (ii) from dead cells. In another aspect, the present invention refers to an *in vitro* complex, optionally isolated, comprising an undifferentiated pluripotent cell bound to an antibody of the present invention. In another aspect, the present invention refers to a hybridoma cell line TAG-A1, deposited with American Type Culture Collection under Accession Number PTA-121134.

Also described herein is an antibody, the amino acid sequence of the antibody may comprise the amino acid sequences i) to iii), or the amino acid sequences iv) to vi), or preferably the amino acid sequences i) to vi):

i)	SASSSVSYMF	(SEQ ID NO: 1)
ii)	LTSNLAS	(SEQ ID NO: 2)
iii)	QQWSSNPYT	(SEQ ID NO: 3)
iv)	GFTFSNYYMN	(SEQ ID NO: 4)
v)	EIRLKSNNYATHYAESVKG	(SEQ ID NO: 5)
vi)	FGY	(SEQ ID NO: 6)

or a variant thereof in which one or two or three amino acids in one or more of the sequences (i) to (vi) are replaced with another amino acid.

The antibody may comprise at least one light chain variable region incorporating the following CDRs:

CDR1: SASSSVSYMF	(SEQ ID NO: 1)
CDR2: LTSNLAS	(SEQ ID NO: 2)

Also described herein is an antibody that binds a glycosylated protein, wherein the glycosylation comprises the glycan motif Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1 or Fucα1-2Galβ1-3GlcNAc. The antibody may optionally have amino acid sequence components as described above.

In any example described herein, the antibody is preferably cytotoxic against undifferentiated pluripotent cells. Upon contact with an undifferentiated pluripotent cell it may kill the cell in one of less than 30 minutes, less than 20 minutes, less than 10 minutes, less than 5 minutes, less than 2 minutes or less than 1 minute.

In any example described herein, the antibody preferably specifically binds an undifferentiated pluripotent cell over a cell differentiated from an undifferentiated pluripotent cell. For example, the antibody exhibits specific binding against undifferentiated pluripotent cell(s) over embryoid bodies formed from such cells.

In one example an isolated heavy chain variable region polypeptide is provided, the heavy chain variable region polypeptide comprising the following CDRs:

CDR1: GFTFSNYYMN	(SEQ ID NO: 4)
CDR2: EIRLKSNNYATHYAESVKG	(SEQ ID NO: 5)
CDR3: FGY	(SEQ ID NO: 6)

In one example an antibody is provided, the antibody comprising a heavy chain and a light chain variable region sequence, wherein:

the heavy chain comprises a CDR1, CDR2, CDR3, having at least 85% overall sequence identity to CDR1: GFTFSNYYMN (SEQ ID NO: 4), CDR2: EIRLKSNNYATHYAESVKG (SEQ ID NO: 5), CDR3: FGY (SEQ ID NO: 6), and the light chain comprises a CDR1, CDR2, CDR3, having at least 85% overall sequence identity to CDR1: SASSSVSYMF (SEQ ID NO: 1), CDR2: LTSNLAS (SEQ ID NO: 2), CDR3: QQWSSNPYT (SEQ ID NO: 3), respectively.

In some examples the degree of sequence identity may be one of 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%.

In another example an antibody, optionally isolated, is provided comprising a heavy chain and a light chain variable region sequence, wherein:

the heavy chain sequence has at least 85% sequence identity to the A1H heavy chain sequence shown in Figure 15A, and

the light chain sequence has at least 85% sequence identity to the A1L light chain sequence shown in Figure 15B.
In some examples the degree of sequence identity may be one of 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%.

In some examples the antibody or polypeptide further comprises variable region heavy chain framework sequences between the CDRs. The framework sequences may be derived from human consensus framework sequences.

In one example an isolated light chain variable region polypeptide, optionally in combination with a heavy chain variable region polypeptide as described herein, is provided, the light chain variable region polypeptide comprising the following CDRs:

CDR1: SASSSVSYMF	(SEQ ID NO: 1)
CDR2: LTSNLAS	(SEQ ID NO: 2)
CDR3: QQWSSNPYT	(SEQ ID NO: 3)

In some examples the antibody or polypeptide further comprises variable region light chain framework sequences between the CDRs. The framework sequences may be derived from human consensus framework sequences.

In another example, a composition, e.g. a pharmaceutical composition or medicament, is provided. The composition may comprise an antibody or polypeptide as described herein and at least one pharmaceutically-acceptable carrier, excipient, adjuvant or diluent.

The antibody may be an IgG. It may have a molecular weight of about 140 to 160kDa, preferably about 150kDa.

In some examples, preferred antibodies are bivalent.

In some examples the antibody may be mAb A1.

Antibody mAb A1 was produced from a hybridoma cell, TAG-A1, which has been deposited with American Type Culture Collection (ATCC), Manassas, VA, USA by Agency for Science, Technology and Research, 1 Fusionoplois Way, #20-10 Connexis, Singapore 138632 on 20 March 2014 under accession number PTA-121134 in accordance with the provisions of the Budapest Treaty.

Accordingly, a hybridoma cell line TAG-A1, deposited with American Type Culture Collection under Accession Number PTA-121134 is provided. A monoclonal antibody produced from hybridoma cell line TAG-A1, deposited with American Type Culture Collection under Accession Number PTA-121134 is provided.

The antibody preferably exhibits specific binding towards more than one glycosylated protein antigen, particularly glycosylated protein antigens present in, or on, undifferentiated pluripotent cells, wherein the glycosylation on the protein antigens comprises the glycan motif Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1 or Fucα1-2Galβ1-3GlcNAc. This motif may optionally be present as part of a larger glycan or may represent the complete glycan. The glycan may preferably be O-linked to the protein.

The pluripotent cells may express one or more glycosylated proteins having the glycan motif Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1 or Fucα1-2Galβ1-3GlcNAc either in the cell and/or on the cell surface. In this specification undifferentiated pluripotent cells may be undifferentiated pluripotent stem cells. Preferably, they are human or mammalian.

In another example an isolated nucleic acid encoding an antibody or polypeptide as described herein is provided.

In one example there is provided a vector comprising a nucleic acid described herein. In another example, there is provided a host cell comprising the vector. For example, the host cell may be eukaryotic, or mammalian, e.g. Chinese Hamster Ovary (CHO), or human or may be a prokaryotic cell, e.g. *E. coli.*

In one example a method for making an antibody or polypeptide as described herein is provided, the method comprising culturing a host cell as described herein under conditions suitable for the expression of a vector encoding the antibody or polypeptide, and recovering the antibody or polypeptide.

In another example there is provided a method of destroying an undifferentiated pluripotent cell or cells in a sample containing such cells, the method

comprising contacting a sample of cells containing an undifferentiated pluripotent cell or cells with an antibody as described herein.

In another example there is provided a method of removing an undifferentiated pluripotent cell or cells from a sample containing such cells, the method comprising contacting a sample of cells containing an undifferentiated pluripotent cell or cells with an antibody as described herein.

In another example there is provided a method of enriching pluripotent cells that have undergone or are undergoing differentiation from a mixture comprising undifferentiated pluripotent cells and such cells that have undergone or are undergoing differentiation, the method comprising contacting the mixture with an antibody as described herein for a period of time sufficient for the antibody to kill undifferentiated pluripotent cells. The method may further comprise the step of separating live cells from dead cells.

In another example there is provided a method of preparing a composition containing cells differentiated from undifferentiated pluripotent cells which composition contains substantially no undifferentiated pluripotent cells, the method comprising:

(i) providing a population of cells comprising undifferentiated pluripotent cells and cells differentiated from undifferentiated pluripotent cells;

(ii) contacting the population with an antibody as described herein under conditions permitting the antibody to kill undifferentiated pluripotent cells; and

(iii) separating live cells remaining after step (ii) from dead cells.

The method may further comprise mixing the separated cells from (iii) with a pharmaceutically acceptable carrier, adjuvant or diluent.

In another example there is provided an *in vitro* complex comprising an undifferentiated pluripotent cell bound to an antibody as described herein. The complex may be isolated.

Methods as described herein may preferably be performed *in vitro*. The methods may comprise incubating cells and antibody together for a predetermined period of time sufficient for the antibody to exert a cytotoxic effect. Such period of time may be

one of at least 1 minute, at least 2 minutes, at least 5 minutes, at least 10 minutes, at least 20 minutes, at least 30 minutes, at least 40 minutes, at least 50 minutes, or at least 60 minutes.

In another example a method of treating a patient in need of cell therapy is provided, the method comprising administering to the patient a composition containing cells differentiated from undifferentiated pluripotent cells which composition contains substantially no undifferentiated pluripotent cells, wherein the composition is obtained by a method described herein.

The use of cells differentiated from undifferentiated pluripotent cells obtained by a method described herein in the manufacture of a medicament for treating a patient in need of cell therapy is also provided.

A cell or cells differentiated from undifferentiated pluripotent cells and obtained by a method described herein is also provided for use in treating a patient in need of cell therapy.

Description

Antibodies

Antibodies according to the present invention preferably to a glycan comprising the glycan motif Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1 or Fucα1-2Galβ1-3GlcNAc, or to a glycosylated protein, wherein the glycosylation comprises the glycan motif Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1 or Fucα1-2Galβ1-3GlcNAc.

In some embodiments, antibodies according to the present invention bind to a glycan or glycosylated protein comprising the glycan motif Fucα1-2Galβ1-3(Fucα1-4)GlcNAc.

In some embodiments, antibodies according to the present invention bind to a glycan or glycosylated protein comprising the glycan motif (Fucα1-2)Galβ1-3GlcNAcβ1-3Galβ1-4Glc.

In some embodiments, antibodies according to the present invention bind to a glycan or glycosylated protein comprising the glycan motif Fucα1-2Galβ1-3GlcNAc.

Claims:

1. An antibody that binds to the glycan motif Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1 or Fucα1-2Galβ1-3GlcNAc, wherein the antibody is cytotoxic against undifferentiated pluripotent cells, and wherein the antibody comprises:

at least one light chain variable region incorporating the following CDRs:

CDR1: SASSSVSYMF	(SEQ ID NO: 1)
CDR2: LTSNLAS	(SEQ ID NO: 2)
CDR3: QQWSSNPYT	(SEQ ID NO: 3); and

at least one heavy chain variable region incorporating the following CDRs:

CDR1: GFTFSNYYMN	(SEQ ID NO: 4)
CDR2: EIRLKSNNYATHYAESVKG	(SEQ ID NO: 5)
CDR3: FGY	(SEQ ID NO: 6).

2. A monoclonal antibody produced from hybridoma cell line TAG-A1, deposited with American Type Culture Collection under Accession Number PTA-121134.

3. The antibody according to claim 1 comprising a heavy chain and a light chain variable region sequence, wherein:

the heavy chain sequence has at least 85% sequence identity to the A1H heavy chain sequence shown in Figure 15A, and

the light chain sequence has at least 85% sequence identity to the A1L light chain sequence shown in Figure 15B.

4. A composition comprising the antibody or polypeptide of any one of claims 1 to 3 and at least one pharmaceutically-acceptable carrier.

5. An isolated nucleic acid encoding the antibody or polypeptide of any of one of claims 1 to 3.

6. A vector comprising the nucleic acid of claim 5.

7. A host cell comprising the vector of claim 6.

8. A method for making an antibody or polypeptide of any of one of claims 1 to 3 comprising culturing the host cell of claim 8 under conditions suitable for the expression

of a vector encoding the antibody or polypeptide, and recovering the antibody or polypeptide.

9. The antibody of any one of claims 1 to 3, wherein the antibody is cytotoxic against undifferentiated pluripotent cells.

10. A method of destroying an undifferentiated pluripotent cell or cells in a sample containing such cells, the method comprising contacting a sample of cells containing an undifferentiated pluripotent cell or cells with an antibody according to any one of claims 1 to 3.

11. A method of removing an undifferentiated pluripotent cell or cells from a sample containing such cells, the method comprising contacting a sample of cells containing an undifferentiated pluripotent cell or cells with an antibody according to any one of claims 1 to 3.

12. A method of enriching cells that have undergone or are undergoing differentiation from a mixture comprising undifferentiated pluripotent cells and such cells that have undergone or are undergoing differentiation, the method comprising contacting the mixture with an antibody according to any one of claims 1 to 3 for a period of time sufficient for the antibody to kill undifferentiated pluripotent cells.

13. The method of claim 12, further comprising the step of separating live cells from dead cells.

14. A method of preparing a composition containing cells differentiated from undifferentiated pluripotent cells which composition contains substantially no undifferentiated pluripotent cells, the method comprising:

(i) providing a population of cells comprising undifferentiated pluripotent cells and cells differentiated from undifferentiated pluripotent stem cells;

(ii) contacting the population with an antibody according to any one of claims 1 to 3 under conditions permitting the antibody to kill undifferentiated pluripotent cells; and

(iii) separating live cells remaining after step (ii) from dead cells.

15. The method of claim 14 further comprising mixing the separated cells with a pharmaceutically acceptable carrier, adjuvant or diluent.

16. An *in vitro* complex, optionally isolated, comprising an undifferentiated pluripotent cell bound to an antibody according to any one of claims 1 to 3.

17. A hybridoma cell line TAG-A1, deposited with American Type Culture Collection under Accession Number PTA-121134.