It is an object of the present invention to go some way towards overcoming this problem and/or to provide the public with a useful choice.

In this specification where reference has been made to patent specifications, other external documents, or other sources of information, this is generally for the purpose of providing a context for discussing the features of the invention. Unless specifically stated otherwise, reference to such external documents or such sources of information is not to be construed as an admission that such documents or such sources of information, in any jurisdiction, are prior art or form part of the common general knowledge in the art.

SUMMARY ~~OF THE INVENTION~~

The present ~~invention~~disclosure provides a use of complete genome sequencing (CGS) as a tool to select PERV-C and PERV-A/C negative pigs, i.e. pigs that have no or inactive, non-replicating PERV C and PERV-A/C, for xenotransplantation of tissues and/or cells into human recipients; and/or as foundation pigs for further manipulation, for example by gene editing xenoantigens, to provide pigs that are not only PERV-A and PERV-A/C negative but are also free of xenoantigens that are responsible for hyperacute organ rejection so that this novel pig strain is suitable as donors for whole organ, tissue and/or cell xenotransplantation into human recipients.

In a first aspect, the present ~~invention~~disclosure provides a method of selecting pigs suitable as donors for xenotransplantation of tissues and/or cells, or as foundation pigs for further manipulation, said method comprising the step:

a) providing a designated pathogen-free (DPF) pig herd having a low PERV copy number of between 4 and 40;

b) testing the PERV status of individual pigs of the herd using complete genome sequencing (CGS);

c) identifying individual pigs that have PERV-C negative and PERV-A/C negative status;

d) selecting said PERV-C negative and PERV-A/C negative pigs as donor pigs for xenotransplantation, or as foundation pigs for further manipulation.

The method preferably further comprises identifying the PERV-A and PERV-B status of the PERV-C negative and PERV-A/C negative pigs of step d) and further selecting pigs that have a very low number of full-length and potentially functional PERV-A and

PERV-B sequences of between 1-10, preferably between 1-5, and most preferably between 1-2.

Preferably the pigs are Auckland Island (AI) pigs, a swine breed that has designated pathogen-free (DPF) status and has a surprising low PERV copy number. More preferably, the AI pigs have been selectively bred for very low PERV copy number in a biosecure facility. More preferably, the AI pigs are PERV-null, i.e. any PERV sequences present in their genome are not able to produce infectious PERV particles (Garkavenko et al., 2008).

Other pig breeds/strains that are designated pathogen-free and have similar low PERV copy number and PERV-null status can also be used in the present ~~invention~~disclosure.

In a second aspect the ~~invention~~disclosure provides pigs selected by the method of the first aspect.

It was surprisingly found that full-length and potentially functional PERV-A/B sequences, in the PERV-C and PERV-A/C negative pigs selected by the method of the first aspect, were located on the Y-chromosome only so could be used to breed out functioning PERV. In a third aspect the present ~~invention~~disclosure therefore provides a method of breeding a herd of pigs that have no functional PERV genomic sequences suitable as donors for xenotransplantation, or as foundation pigs for further manipulation, said method comprising the steps:

a) selecting PERV-C and PERV-A/C negative male and female pigs using the CGS selection method of the first aspect;

b) analysing the chromosomal location of any full-length, potentially functional PERV-A and PERV-B gene sequences in the male pigs of step a);

c) selecting male pigs that have full-length, potentially functional PERV-A and/or PERV-B present in the Y-chromosome only;

d) breeding the male pigs of step c) with the female pigs of step a) to produce progeny; and

e) selecting female progeny that will lack the paternal full length and potentially functional PERV-A and/or PERV-B as future breeding stock to produce a herd of pigs that have no functional PERV genomic sequences suitable as donors of tissues and/or cells for xenotransplantation, or as foundation pigs for further manipulation.

The present ~~invention~~disclosure also contemplates selecting the female progeny of step e) as suitable donors for xenotransplantation of tissues and/or cells, or as foundation pigs for further manipulation.

In a fourth aspect the ~~invention~~disclosure provides a pig herd bred by the method of the third aspect.

The foundation pigs selected by the CGS selection method of the first aspect of the ~~invention~~disclosure, or bred by the method of the third aspect of the ~~invention~~disclosure can be further manipulated using gene editing technology to eliminate and/or deactivate one or more xenoantigens selected from GGTA1, CAMH, B4GalNT2, Neu5Gc, ASGR1 and SLA, for example, to minimise the risk of hyperacute rejection of tissues and/or cells, as well as whole organs transplanted into human recipients. In a fifth aspect the ~~invention~~disclosure therefore provides a method of providing donor pigs suitable for xenotransplantation of whole organs, tissues and/or cells into a human recipient comprising the steps:

(a) selecting PERV-C and PERV-A/C negative donor pigs using the CGS method of the first aspect;

(b) establishing a PERV-C and PERV-A/C negative cell line from the pigs of step (a);

(c) gene editing selected cells of step (b) to eliminate or deactivate one or more xenoantigen genes;

(d) optionally gene editing selected isolated cells of step (c) to express one or more human genes selected from A20, CD39, CD46, CD47, CD55, CD59, hemoxygenase-1 (HO-1), HLA-E, HLA-G, thrombomodulin (TM), CTLA4-Ig, and LEA29Y;

(e) establishing a gene-edited cell line from the pig cells of step (c) or step (d);

(f) carrying out somatic cell nuclear transfer from one or more of said gene edited cells of step (e) into an oocyte from a PERV-C and PERV-A/C negative pig *in vitro*;

(g) culturing to blastocyst stage;

(h) transferring said blastocyst(s) to a female PERV-C and PERV-A/C negative pig and growing to full term; and

(i) providing donor pigs that are PERV-C and PERV-A/C negative and gene edited for one or more xenoantigens.

The one or more xenoantigens genes may be selected from GGTA1, CAMH, B4GalNT2, Neu5Gc, ASGR1 and SLA, or any other xenoantigen that would be useful to reduce immunological reactions, especially the possibility of hyperacute organ rejection, as would be understood by a skilled worker.

In a sixth aspect the ~~invention~~disclosure provides pig cell lines that are PERV-C and PERV-A/C negative and gene edited to inactivate or delete one or more xenoantigens and optionally to express one or more human genes, produced by the method of the fifth aspect. The cell lines can be used to clone donor pigs for xenotransplantation of whole organs, tissues and/or cells into human recipients.

In a seventh aspect the ~~invention~~disclosure provides donor pigs that are DPF, PERV-C and PERV-A/C negative and gene edited to inactivate or delete one or more xenoantigens and optionally to express one or more human genes, produced by the method of the fifth aspect. These donor pigs represent a novel pig strain, designated NZeno-1. These pigs also preferably have a very low number of full-length and potentially functional PERV-A and PERV-B sequences of between 1-10, preferably between 1-5, and most preferably between 1-2.

In another aspect, the present disclosure provides organs, tissues and/or cells from the pigs as described herein for use in xenotransplantation.

In yet another aspect, the present disclosure provides a use of an organ, tissue and/or cells from the pigs as described herein in the manufacture of a medicament for treating a patient suffering from or predisposed to a disease, disorder or condition associated with a deficiency in or absence of organ function, said use comprising that the organ, tissue and/or cells is to be transplanted to a patient in need thereof.

In an ~~eighth aspect~~ example the ~~invention~~disclosure provides tissues and/or cells from the pigs ~~of the second, fourth, and seventh aspects~~as described herein for use in xenotransplantation.

In an ~~ninth aspect~~ example the ~~invention~~disclosure provides whole organs from the pigs as described herein ~~of the seventh aspect~~ for use in xenotransplantation.

The organs, tissues and/or cells for use in xenotransplantation may be selected from the group consisting of kidney, liver, lung, heart, brain, pancreas, muscle, blood, bone, testes and ovary.

The tissue and/or cells for use in xenotransplantation may be selected from pancreatic islets, hepatocytes, non-parenchymal liver cells, gall bladder epithelial cells, gall bladder endothelial cells, bile duct epithelial cells, bile duct endothelial cells, hepatic vessel epithelial cells, hepatic vessel endothelial cells, sinusoid cells, choroid plexus cells, fibroblasts, Sertoli cells, adrenal chromaffin cells and muscle cells.

In ~~a tenth~~another ~~aspect~~example the ~~invention~~disclosure provides a method of treating a patient suffering from or predisposed to a disease, disorder or condition associated with a deficiency in or

absence of organ function, said method comprising transplanting tissue and/or cells ~~of the eighth aspect~~as described herein, or whole organs as described herein~~of the ninth aspect~~ to a patient in need thereof.

It is intended that reference to a range of numbers disclosed herein (for example, 1 to 10) also incorporates reference to all rational numbers within that range (for example, 1, 1.1, 2, 3, 3.9, 4, 5, 6, 6.5, 7, 8, 9 and 10) and also any range of rational numbers within that range (for example, 2 to 8, 1.5 to 5.5 and 3.1 to 4.7) and, therefore, all sub-ranges of all ranges expressly disclosed herein are hereby expressly disclosed. These are only examples of what is specifically intended and all possible combinations of numerical values between the lowest value and the highest value enumerated are to be considered to be expressly stated in this application in a similar manner.

As used herein the term '(s)' following a noun means the plural and/or singular form of that noun.

The term 'comprising' as used in this specification and claims means 'consisting at least in part of'. When interpreting statements in this specification and claims which include the term 'comprising', other features besides the features prefaced by this term in each statement can also be present. Related terms such as 'comprise' and 'comprised' are to be interpreted in a similar manner.

As used herein the term 'and/or' means 'and' or 'or', or where the context allows both.

The ~~invention~~disclosure consists in the foregoing and also envisages constructions of which the following gives examples only.

As used herein the terms 'PERV-C negative' and 'PERV-A/C negative' means no full length proviral genome identified in pigs selected by the method of the ~~invention~~disclosure. This can be distinguished from the terms "PERV-C negative" and "PERV-A/C negative" when assessed by conventional PCR which can only detect the presence of a small part of env region (PERV-C (A/C) positive) or absence of this region (PERV-C (A/C) negative) of PERV-C and recombinant PERV-A/C genomic sequences.

As used herein the term 'PERV-null' means the inability to produce infectious PERV particles.

The term "porcine" is used interchangeably with the terms "pig" and "swine" and refers to mammals in the family Suidae. Such mammals include wholly or partially inbred swine, preferably those members of the Auckland Island pig herd described herein.

The term "recipient" as used herein refers to a human suffering from or predisposed to a disease, disorder or condition associated with a deficiency in or absence of organ, tissue or cell function.

The term "gene editing" or "genome editing" is a type of genetic engineering in which DNA is inserted, deleted, modified or replaced in specific targeted sites in the genome of a living organism. Gene editing techniques include the use of engineered nucleases, or 'molecular scissors' that create site-specific double stranded breaks at desired locations in the genome. The induced double-strand breaks are repaired through non-homogenous end-joining or homologous recombination, resulting in targeted mutations or gene edits. Engineered nucleases that can be used include zinc finger nucleases, transcription activator-like effector nucleases, engineered meganucleases and the clustered regularly interspaced short palindromic repeats (CRISPR/Cas-9) system.

This ~~invention~~disclosure may also be said broadly to consist in the parts, elements and features referred to or indicated in the specification of the application, individually or collectively, and any or all combinations of any two or more said parts, elements or features, and where specific integers are mentioned herein which have known equivalents in the art to which this ~~invention~~disclosure relates, such known equivalents are deemed to be incorporated herein as if individually set forth.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will now be described by way of example only and with reference to the accompanying drawings in which:

Figure 1 shows full PERV-C sequence BLAST hits against the *Sus scrofa* Male1 genome sequence;

Figure 2 shows a sequence alignment of selected regions of the *Sus scrofa* Male1 genome with known PERV sequences. Disagreements to the PERV-C reference KC116219 are highlighted in black;

Figure 3 shows a Maximum Likelihood phylogenetic reconstruction of 8 selected hits of *Sus scrofa* Male1 (black) with 9 known PERV sequences (red). The tree was rooted with the PERV-B sequence. Numbers at the nodes indicate the ML support values. The scale bar represents the number of expected substitutions per site;

WHAT WE CLAIM IS:

1. A method of selecting pigs suitable as donors for xenotransplantation of tissues and/or cells, or as foundation pigs for further manipulation, said method comprising the step:

   a) providing a designated pathogen-free (DPF) pig herd having a low PERV copy number of between 4 and 40;

   b) testing the PERV status of individual pigs of the herd using complete genome sequencing (CGS);

   c) identifying individual pigs that have PERV-C and PERV-A/C negative status; and

   d) selecting said PERV-C and PERV-A/C negative pigs as donor pigs for xenotransplantation, or as foundation pigs for further manipulation.

2. The method of claim 1 further comprising identifying the PERV-A and PERV-B status of the PERV-C negative pigs of step d) and further selecting pigs that have a very low number of functioning PERV-A and PERV-B sequences of between 1-10, preferably between 1-5, and most preferably between 1-2.

3. The method of claim 1 or 2 wherein the pigs are Auckland Island (AI) pigs.

4. The method of any one of claims 1-3, wherein the pigs are PERV-null.

5. Pigs selected by the method of any one of claims 1-4.

6. A method of breeding a herd of pigs that have no functional PERV genomic sequences suitable as donors for xenotransplantation, or as foundation pigs for further manipulation, said method comprising the steps:

   a) selecting PERV-C and PERV-A/C negative male and female pigs using the CGS selection method of any one of claims 1-4;

   b) analysing the chromosomal location of any functioning PERV-A and PERV-B gene sequences in the male pigs of step a);

   c) selecting male pigs that have functioning PERV-A and/or PERV-B present in the Y-chromosome only;

d) breeding the male pigs of step c) with the female pigs of step a) to produce progeny; and

e) selecting female progeny that will lack the paternal functional PERV-A and/or PERV-B as future breeding stock to produce a herd of pigs that have no functional PERV genomic sequences and that are suitable as donors of tissues and/or cells for xenotransplantation, or as foundation pigs for further manipulation.

7. A pig herd bred by the method of claim 6.

8. A method of providing donor pigs suitable for xenotransplantation of whole organs, tissues and/or cells into a human recipient comprising the steps:

(a) selecting PERV-C and PERV-A/C negative donor pigs using the CGS method of any one of claims 1-4,

(b) establishing a PERV-C and PERV-A/C negative cell line from the pigs of step (a);

(c) gene editing cells of said cells of step (b) to eliminate or deactivate one or more xenoantigen genes;

(d) optionally gene editing selected isolated cells of step (c) to express one or more human genes selected from A20, CD39, CD46, CD47, CD55, CD59, hemoxygenase-1 (HO-1), HLA-E, HLA-G, thrombomodulin (TM), CTLA4-Ig, and LEA29Y;

(e) establishing a gene-edited cell line from the pig cells of step (c) or (d);

(f) carrying out somatic cell nuclear transfer from one or more of said gene edited cells of step (e) into an oocyte from a PERV-C and PERV-A/C negative pig in vitro;

(g) culturing to blastocyst stage;

(h) transferring said blastocyst(s) to a female PERV-C and PERV-A/C negative pig and growing to full term; and

(i) providing donor pigs that are PERV-C and PERV-A/C negative and gene edited for one or more xenoantigens.

9. The method of claim 8, wherein the one or more xenoantigen genes is selected from GGTA1, CAMH, B4GalNT2, Neu5Gc, ASGR1 and SLA.

10. A pig cell line that is PERV-C and PERV-A/C negative and gene edited for inactivation or deletion of one or more xenoantigens and optionally for insertion of one or more human genes, produced by the method of claim 8(a)-(d) or 9.

11. Donor pigs that are PERV-C and PERV-A/C negative and gene edited for inactivation or deletion of one or more xenoantigens and optionally gene edited for expression of one or more human genes, produced by the method of claim 8 or 9.

12. Organs, tissues and/or cells from the pigs of any one of claims 5, 7 and 11 for use in xenotransplantation.

13. Use of an organ, tissue and/or cells from the pigs of any one of claims 5, 7 and 11 in the manufacture of a medicament for treating a patient suffering from or predisposed to a disease, disorder or condition associated with a deficiency in or absence of organ function, said use comprising that the organ, tissue and/or cells is to be transplanted to a patient in need thereof.

~~13~~14. The ~~organs, tissues and/or cells for~~ use ~~in xenotransplantation~~ of claim ~~12~~13, selected from the group consisting of kidney, liver, lung, heart, brain, pancreas, muscle, blood, bone, testes and ovary.

~~14~~15. The ~~tissue and/or cells for~~ use ~~in xenotransplantation~~ of claim ~~12~~ 13 or ~~13~~14, selected from pancreatic islets, hepatocytes, non-parenchymal liver cells, gall bladder epithelial cells, gall bladder endothelial cells, bile duct epithelial cells, bile duct endothelial cells, hepatic vessel epithelial cells, hepatic vessel endothelial cells, sinusoid cells, choroid plexus cells, fibroblasts, Sertoli cells, adrenal chromaffin cells and muscle cells.

~~15. A method of treating a patient suffering from or predisposed to a disease, disorder or condition associated with a deficiency in or absence of organ function, said method comprising transplanting an organ, tissue and/or cells of any one of claims 11-14 to a patient in need thereof.~~