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

The present 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 one aspect, the present 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 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;

wherein the pigs are Auckland Island (AI) pigs.

In an example, the present 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 disclosure.

In an example the disclosure provides pigs selected by the method as described herein.

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 as described herein, were located on the Y-chromosome only so could be used to breed out functioning PERV. In another aspect, the present disclosure 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 method as described herein;

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.

In an example the present 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 as described herein;

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 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 yet another aspect the disclosure provides a pig herd bred by the method as described herein.

The foundation pigs selected by the CGS selection method as described hereinthe disclosure, or bred by the method as described hereinthe disclosure can be further manipulated using gene editing technology to eliminate and/or deactivate one or more xenoantigens selected from GGTA1, CMAH, 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 another aspect, the present disclosure 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 method as described herein,

(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 the oocyte of step (f) to blastocyst stage to be transferred to a female PERV-C and PERV-A/C negative pig and growing to full term; and

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

In an example the 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 as described herein;

(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, CMAH, 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 yet another aspect the 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 as described herein. The cell lines can be used to clone donor pigs for xenotransplantation of whole organs, tissues and/or cells into human recipients.

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

In an example the disclosure provides whole organs from the pigs as described herein 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.

g) culturing to blastocyst stage;

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

i) providing a novel strain of donor pigs that are PERV-C and PERV-A/C negative and gene edited for one or more xenoantigens, and optionally gene edited to express one or more human genes.

The one or more xenoantigen genes may be selected from GGTA1, CMAH, B4GalNT2, Neu5Gc, ASGR1 and SLA, and other xenoantigen genes that would be beneficial to deactivate, to improve acceptance by the recipient's immune system as would be understood by a skilled worker.

The present invention also provides pig cell lines produced by step e) of the above method. Examples of pig cell lines of the present invention are set out in examples 4-8 below. Specifically, cell lines from DPF AI pigs selected by the CSG method of the invention (i.e. PERV-C and PERV-A/C negative) were produced that have been gene edited using CRISPR Cas-9 to disrupt GGTA1 (examples 4 and 7); that have been gene edited using CRISPR Cas-9 to disrupt CMAH (example 5); and that have been gene edited using CRISP Cas-9 to disrupt both GGTA1 and CMAH (examples 6 and 8). The cell lines of the present invention can be used to clone donor pigs using known somatic cell transfer techniques (example 9). The cloned donor pigs can then be used for xenotransplantation of whole organs, tissues and/or cells into human recipients. The cloned donor pigs represent a novel pig strain (NZeno-1) having PERV-C and PERV-A/C negative genomic status, a very low number (1-2) of full-length potentially functional genomic PERV-A and PERV-B sequences, having gene edits for one or more xenoantigens, and optionally having one or more gene edits to express human immune-compatible genes. The NZeno-1 donor pigs are also DPF and PERV-null making them particularly suitable for whole organ, tissue and/or cell xenotransplantation as they have been selected and manipulated to minimise the risk of hyperacute organ rejection.

The NZeno-1 novel donor pig strain of the present invention, produced by the combined method of CGS selection for PERV-C and PERV-A/C negative status and low (1-2) PERV A and B, being DPF and PERV-null, and having one or more of GGTA1, CMAH, B4GalT2, Neu5Gc, ASGR1 and/or SLA eliminated or deactivated via gene editing technology, are far superior to any currently available pigs in terms of their suitability as donors for xenotransplantation.

As discussed above, the current thinking in terms of producing pigs as a source of organs, tissues and/or cells for xenotransplantation is to use CRISPR Cas-9 technology to gene edit not only the xenoantigens but also all of the genome wide PERV genes. This would, however require dozens of gene edits being carried out on donor pig cells (usually fibroblasts) prior to somatic cell nuclear transfer and cloning of the resulting embryos. For example, Yang et al (2015) succeeded in inactivating 62 PERV sequences in an immortalised pig cell line and Niu et al (2017) succeeded in inactivating 25 PERV genes to produce piglets with inactive PERVs. Additional gene edits on top of the PERV gene edits would be required to inactivate the xenoantigens (eGenesis talk about introducing "double digit" gene edits to reduce the likelihood of organ rejection (Weintraub, 2019)).

As is now know, CRISPR Cas-9 technology can result in unwanted DNA deletions and rearrangements near its target site on the genome. CRISPR Cas-9 gene editing relies on the Cas-9 enzyme to cut DNA at a particular target site. The cell then attempts to reseal this break using DNA repair mechanisms. The repair mechanisms do not always work perfectly and sometimes segments of DNA will be deleted or rearranged or other unwanted changes can occur. Clearly the more gene edits that are carried out on a single cell, the more the chances of these unwanted edits occurring increases.

The present method produces PERV-C and PERV-A/C negative pigs, without having to carry out gene editing and only contemplates using gene editing to eliminate or deactivate a minimum number of xenoantigen genes thereby avoiding having to produce cloned animals having dozens of gene edits which may harm the animal and/or have unforeseen genetic consequences.

The present invention therefore provides PERV-C and PERV-A/C negative pigs selected by the CGS selection method of the invention, preferably also having a very low copy number of active PERV-A and PERV-B of 1-2, as donor pigs for xenotransplantation of tissues and/or cells or as foundation pigs for further manipulation.

The foundation pigs of the invention may be further manipulated or modified by undergoing gene editing to eliminate or inactivate one or more xenoantigens selected from GGTA1, CMAH, B4GalNT2, Neu5Gc, ASGR1 and SLA, and optionally 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, to produce a novel NZeno-1 strain.

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;

   wherein the pigs are Auckland Island (AI) pigs.

2. The method of claim 1 further comprising identifying the PERV-A and PERV-B status of the PERV-C and PERV-A/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 unable to produce infectious PERV particles.

4. 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 method of any one of claims 1-3;

   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.

5. A pig herd bred by the method of claim 4.

6. 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 method of any one of claims 1-3,

(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 the oocyte of step (f) to blastocyst stage to be transferred to a female PERV-C and PERV-A/C negative pig and growing to full term; and

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

7. The method of claim 6, wherein the one or more xenoantigen genes is selected from GGTA1, CMAH, B4GalNT2, Neu5Gc, ASGR1 and SLA.

8. 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 6(a)-(d) or 7.

9. 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 6 or 7.

10. Organs, tissues and/or cells from the pigs of claim 5 or 9 for use in xenotransplantation.

11. Use of an organ, tissue and/or cells from the pigs of claim 5 or 9 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.

12. The use of claim 11, wherein the organ, tissue and/or cells are selected from the group consisting of kidney, liver, lung, heart, brain, pancreas, muscle, blood, bone, testes and ovary.

13. The use of claim 11 or 12, wherein the organ, tissue and/or cells are 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.