

A prerequisite for any allogeneic T-cell product which uses an  $\alpha\beta$ TCR is the production of a clonal T-cell population. This is required to mitigate the risk of graft versus host disease (GvHD). iT-cells with restricted expression of a defined  $\alpha\beta$ TCR have been successfully produced from clones of hiPSC that were transduced with lentiviral vectors encoding a specific TCR [Minagawa, A., et al., *Enhancing T Cell Receptor Stability in Rejuvenated iPSC-Derived T Cells Improves Their Use in Cancer Immunotherapy*. Cell Stem Cell, 2018. **23**(6): p. 850-858 e4]. Related studies have suggested that restriction of  $\alpha\beta$ TCR expression in differentiated iT-cells is driven by TCR allelic exclusion, which prevents erroneous rearrangement of endogenous TCR genes. However, due to the phenomenon of promoter silencing, lentiviral transduction is an inefficient method at promoting stable transgene expression in hiPSC and differentiated cells [Zou, J., et al., *Oxidase-deficient neutrophils from X-linked chronic granulomatous disease iPS cells: functional correction by zinc finger nuclease-mediated safe harbor targeting*. Blood, 2011. **117**(21): p. 5561-72]. In addition, the development of a TiPSC derived from a recombinant heterologous T-cell receptor expressing T-cell would require additional interventions to prevent expression of the native TCR present in the initial T-cell material.

An alternative approach is to genetically engineer the hiPSC to prevent endogenous TCR expression in differentiated iT-cells. This is a challenging approach as it requires the inactivation of multiple genes in both alleles, for example TCR constant domains (TRBC1/2 and TRAC) or possibly genes involved in TCR gene rearrangement (e.g RAG1/2) in conjunction with the heterologous T-cell receptor knock-in. It is however difficult and unpredictable to ascertain what loci to target that will permit TCR expression in differentiated iT-cells. Here we present data based on a minimal editing strategy that targeted insertion of a recombinant heterologous T-cell receptor sequence permits its expression in differentiated iT-cells.

## Summary

According to the present disclosure there is provided a modified induced pluripotent stem cell iPSC or haemogenic lineage cell comprising at least one heterologous nucleic acid sequence encoding a heterologous T-cell receptor (TCR) integrated at or into a locus in the cell genome.

According to the disclosure the at least one heterologous nucleic acid sequence encoding a heterologous TCR can be an expressible heterologous nucleic acid sequence. For example the nucleic acid sequence may possess one or more encoding open reading frame and/or one or more regulatory nucleic acid sequences capable of enabling and/or regulating the

expression of the encoding gene or genes or one or more encoding open reading within the cell, for example sequences enabling the transcription and/or post transcriptional modification and/or translation of the encoded sequence or gene. Suitable regulatory sequences can comprise any of, transcriptional or translational start, stop or termination sequences, operator sequence, promoter sequence, enhancer sequence, terminal repeats, ribosome binding site, cap sequence, polyA tail sequence.

Preferably the modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to the disclosure expresses or presents the at least one heterologous TCR encoded by a heterologous TCR coding sequence, preferably expressed or presented at the cell surface. Preferably the at least one heterologous TCR is presented at the cell surface as a membrane bound functional TCR, preferably a membrane anchored heterodimeric TCR protein, preferably an alpha;beta TCR (optionally gamma : delta), preferably comprising variable alpha ( $\alpha$ ) and beta ( $\beta$ ) chains, preferably expressed as a complex with invariant CD3 chains molecules. Preferably the at least one heterologous TCR is capable of binding or specifically binding to a cancer and/or tumour antigen or peptide thereof and/or to tumour and/or cancer cells (e.g. expressing a cancer and/or tumour antigen or peptide thereof) and/or to peptides or antigenic peptides therefrom. Preferably the binding of the TCR promotes activation of the modified induced pluripotent stem cell iPSC or haemogenic lineage cell as herein described and/or activation of T cell activity as herein described.

According to the present disclosure the modified haemogenic lineage cell may be derived from the modified induced pluripotent stem cell iPSC. For example an unmodified induced pluripotent stem cell iPSC may be modified or engineered, for example recombinantly engineered, to comprise the at least one heterologous nucleic acid sequence encoding a heterologous T-cell receptor (TCR) integrated at or into a locus in the cell genome, for example to produce the modified induced pluripotent stem cell which may then be differentiated into a modified haemogenic lineage cell. Alternatively an unmodified haemogenic lineage cell may be derived from an unmodified induced pluripotent stem cell iPSC, for example by a process of differentiation, and then may be modified or engineered to comprise the at least one heterologous nucleic acid sequence encoding a heterologous T-cell receptor (TCR) integrated at or into a locus in the cell genome.

According to the disclosure the induced pluripotent stem cell iPSC or haemogenic lineage cell, or the modified induced pluripotent stem cell iPSC or haemogenic lineage cell may be selected from any one of;

- (a) a mesoderm cell, optionally which may express any one or more of the mesodermal markers, Brachyury, Goosecoid, Mixl1, KDR (also known as FLK1 or VEGFR2), FoxA2, GATA6 or PDGF alpha R,
- 5 (b) a haemogenic endothelial cell, optionally which may be (a) CD34+ or (b) CD34+ CD73- or (c) CD34+ CD73- CXCR4- (CD184-),
- (c) a haematopoietic progenitor cell, optionally which may be (a) CD34+ or (b) CD34+ CD45+ or (c) CD34+ and/or CD45+ in combination with any one or more of CD117+, CD133+, CD45+, FLK+, CD38-, (d) CD34+, CD133+, CD45+, FLK1+, CD38-
- 10 (d) a progenitor T cell, optionally which may be (a) CD5+ and/or CD7+, or (b) CD5+ and/or CD7+ in combination with any one or more of: CD44+, CD25+, CD2+, CD45+, CD3-, CD4-, CD8-,
- (e) a double positive T cell (DP T cell), optionally which may be (a) CD4+ CD8+ (b) CD4+ CD8+ in combination with any one or more of CD3+, CD28+, CD45+,
- 15 (f) a single positive T cell (SP T cell) optionally which may be (a) CD4+ (b) CD8+ (b) CD4+ or CD8+ in combination with any one or more of CD3+, CD28+, CD45+,
- (g) a mature T cell, optionally alpha beta T cell, gamma delta T cell, NKT cell, T helper cell (TH) which are CD4+, cytotoxic T cell (TC) which are CD8+, or
- 20 (h) an induced pluripotent stem cell iPSC, optionally a human induced pluripotent stem cell iPSC derived or created from a CD34+ progenitor cell, optionally isolated from umbilical cord blood, further optionally using the pEB-C5 and pEB-TG episomal plasmids.

Accordingly the induced pluripotent stem cell iPSC, is an induced pluripotent stem cell, and/or a human induced pluripotent stem cell iPSC, preferably derived or created from a

25 CD34+ progenitor cell, optionally preferably isolated from umbilical cord blood, further optionally isolated using pEB-C5 and/or pEB-TG episomal plasmids.

In examples of the disclosure, the iPSC may be modified to reduce or eliminate RAG1 expression, for example by inactivation or knock-out of the RAG1 gene.

The modified pluripotent stem cell iPSC or haemogenic lineage cells may be cells of the

30 lymphoid lineage including T cells, Natural Killer T (NKT) cells, and precursors thereof including embryonic stem cells, and pluripotent stem cells (e.g, those from which lymphoid

cells may be differentiated). T cells can be lymphocytes that mature in the thymus and are chiefly responsible for cell-mediated immunity and also involved in the adaptive immune system. According to the present disclosure the T cells can include, but are not limited to, helper T cells, cytotoxic T cells, memory T cells (including central memory T cells, stem-cell-like memory T cells (or stem-like memory T cells), and two types of effector memory T cells: e.g. , TEM cells and TEMRA cells, Regulatory T cells (also known as suppressor T cells), Natural killer T cells, Mucosal associated invariant T cells, and gamma-delta T cells. Cytotoxic T cells (CTL or killer T cells) are a subset of T-lymphocytes capable of inducing the death of infected somatic or tumour cells. Preferably, the T cells are optionally a CD4<sup>+</sup>T cell or a CD8<sup>+</sup>T cell or population of such T-cells, optionally CD4<sup>+</sup> T cells; or CD8<sup>+</sup> T cells, or a mixed population of CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells.

According to the present disclosure the heterologous nucleic acid sequence encoding the heterologous TCR is integrated at or into a locus in the cell genome, i.e. the genome of the induced pluripotent stem cell iPSC or haemogenic lineage cell. The locus may be a specific and/or fixed position on a chromosome for example where a particular gene or genetic marker is located, it may be the specific and/or fixed position of a gene or genetic marker or genetic element and/or a specific fixed position within a gene or genetic element of a gene such as, for example, an intron or exon of the gene or a regulatory element of a gene (e.g. transcriptional or translational start, stop or termination sequences, operator sequence, promoter sequence, enhancer sequence, terminal repeats, ribosome binding site, cap sequence, polyA tail sequence).

According to the disclosure, the heterologous nucleic acid sequence encoding a heterologous TCR can be integrated at or into one or both alleles of the locus in the cell genome. For example where the locus is a gene or endogenous gene or a specific and/or fixed position on a chromosome, and/or the position where a particular gene or endogenous gene of the induced pluripotent stem cell iPSC or haemogenic lineage cell is located, then the heterologous nucleic acid sequence encoding a heterologous TCR may be integrated at either one or both alleles of the locus.

Accordingly, the locus may be a gene or is within a gene encoding an endogenous protein of the cell or the induced pluripotent stem cell iPSC or haemogenic lineage cell.

According to the disclosure the heterologous nucleic acid sequence encoding a heterologous TCR may be integrated adjacent to or within a gene encoding an endogenous protein of the cell or the induced pluripotent stem cell iPSC or haemogenic lineage cell. Optionally the heterologous nucleic acid sequence encoding a heterologous TCR may be integrated within

an intron or exon of a gene or nucleic acid sequence encoding an endogenous protein of the cell or the induced pluripotent stem cell iPSC or haemogenic lineage cell, optionally a 5' exon or 3' exon, preferably 3' exon.

According to the disclosure the heterologous nucleic acid sequence encoding a  
5 heterologous TCR may be integrated within the 3' exon before the TAG stop sequence of the gene encoding the endogenous protein. Preferably the integration of the heterologous nucleic acid sequence encoding a heterologous TCR, is non-disruptive to the production of the endogenous protein. For example, the production of the endogenous protein by the modified cell should provide all or substantially all of the endogenous protein sequence,  
10 which may be translated and/or produced and/or processed equivalently to the native protein. For example the gene encoding the endogenous protein may still be capable of transcription and/or post transcriptional processing and/or translation produce the endogenous protein sequence or substantially all of the endogenous protein sequence which may optionally be post translationally processed and/or trafficked in the cell.  
15 Preferably the integration of the heterologous nucleic acid sequence encoding a heterologous TCR, does not result in the deletion or substitution of the gene encoding the endogenous protein, and/or the deletion and/or inactivation of the produced endogenous protein, it may result in the concatenation of the heterologous nucleic acid sequence encoding a heterologous TCR with the nucleic acid sequence or gene encoding the  
20 endogenous protein, optionally such that a fusion gene results and/or the gene products are produced as a fusion protein.

According to the disclosure the modified induced pluripotent stem cell iPSC or haemogenic lineage cell may comprise a fusion sequence between the nucleic acid encoding the heterologous TCR and the nucleic acid encoding the endogenous protein, for example as  
25 generated by the integration of the nucleic acid encoding the heterologous TCR, for example a fusion gene or sequence or multicistronic fusion gene or sequence between the nucleic acid encoding the heterologous TCR and the nucleic acid encoding the endogenous protein, for example wherein the expression of the heterologous TCR is linked to the expression of the endogenous protein and/or wherein the heterologous TCR may co-transcribed and/or co-  
30 translated and/or co-expressed with the gene product of the gene at the locus, e.g. the nucleic acid encoding the endogenous gene.

Accordingly the disclosure provides a modified induced pluripotent stem cell iPSC or haemogenic lineage cell in which the nucleic acid encoding the heterologous TCR may be connected to a nucleic acid encoding an endogenous protein by a nucleic acid sequence

enzymatic cleavage site, preferably a furin cleavage site, preferably RAKR and/or ribosome skip sequence, preferably T2A and/or P2A skip sequence.

Accordingly, the fusion protein may be processed in the cell to release the nascent, free or native heterologous TCR, optionally by cleavage of the enzyme cleavage site or furin cleavage site and/or removal the skip sequence peptides optionally performed in the endoplasmic reticulum of the cell.

The present disclosure provides a modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to the disclosure wherein the heterologous TCR is expressed and/or presented in the cell as a nascent heterologous TCR comprising a TCR alpha chain and TCR beta chain, preferably expressed and/or presented at the cell surface. The nascent protein may be separated from the fusion protein and thereby from the endogenous protein for example during post translational processing in the cell, such is in the cell endoplasmic reticulum or golgi, it is then optionally trafficked and/or presented at the cell surface as a membrane bound functional TCR, preferably a membrane anchored heterodimeric TCR protein, preferably an alpha;beta TCR optionally capable of binding or specifically binding to a cancer or tumour antigen or peptide thereof and/or to tumour and/or cancer cells and/or tumour and/or cancer tissue and/or to peptides or antigenic peptides therefrom and/or promoting activation of the modified induced pluripotent stem cell iPSC or haemogenic lineage cell as herein described and/or activating T cell activity as herein described.

According to the present disclosure the endogenous protein can be a protein that is trafficked to the cell surface, optionally via the secretory pathway and/or to the plasma membrane of the cell. Preferably the endogenous protein is a membrane protein and/or transmembrane protein, optionally a receptor protein, preferably a receptor protein tyrosine phosphatase (PTP), preferably CD45 or protein tyrosine phosphatase receptor type C or PTPRC.

According to the disclosure the least one heterologous nucleic acid sequence encoding a heterologous T-cell receptor (TCR) can be integrated at or into a locus in the cell genome where the locus is or is in a gene encoding a membrane protein or transmembrane protein, optionally a receptor protein, preferably a receptor protein tyrosine phosphatase (PTP), preferably CD45 or protein tyrosine phosphatase receptor type C or PTPRC. Preferably the locus is or is in the PTPRC (CD45) gene on chromosome 1.

According to the disclosure the least one heterologous nucleic acid sequence encoding a heterologous T-cell receptor (TCR) can be integrated at or into a locus in the cell genome where the locus or site of integration is at exon 33 of the PTPRC (CD45) gene, preferably in the PTPRC (CD45) gene on chromosome 1, optionally before the TAG stop codon or immediately adjacent to and/or before the TAG stop codon.

The present disclosure provides a modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to the present disclosure, wherein the fusion gene or sequence or multicistronic fusion gene or sequence comprises the nucleic acid encoding the heterologous TCR and nucleic acid encoding PTPRC with an intervening nucleic acid sequence encoding a peptide comprising an enzymatic cleavage site, preferably a furin cleavage site, and/or nucleic acid sequence which mediates ribosome-skipping, preferably selected from a T2A and/or P2A skip sequence and wherein the nucleic acid sequence encoding the heterologous TCR comprises the coding sequence of a TCR $\alpha$  and TCR $\beta$  chain, preferably with an intervening nucleic acid sequence encoding a peptide comprising an enzymatic cleavage site, preferably a furin cleavage site, and/or nucleic acid sequence which mediates ribosome-skipping, preferably selected from T2A and/or P2A skip sequence.

In one aspect, the present disclosure refers to a modified induced pluripotent stem cell iPSC or haemogenic lineage cell comprising at least one heterologous nucleic acid sequence encoding a heterologous T-cell receptor (TCR) integrated at or into a locus in the cell genome.

In another aspect the present disclosure refers to a nucleic acid construct or vector comprising a nucleic acid region encoding the heterologous TCR according to the disclosure and at least one homology region comprising a nucleic acid region homologous to a nucleic acid region at the locus in the cell genome for integration of the nucleic acid region encoding the heterologous TCR.

In another aspect, the present disclosure refers to a process of producing a modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to the disclosure comprising introducing the nucleic acid construct or vector according to the disclosure into an unmodified induced pluripotent stem cell iPSC or haemogenic lineage cell, optionally as defined in the disclosure, under conditions to permit integration of the nucleic acid sequence encoding a heterologous T-cell receptor (TCR) at or into a locus in the cell genome and optionally isolating the modified induced pluripotent stem cell iPSC or haemogenic lineage cell.

In another aspect, the present disclosure refers to a pharmaceutical composition comprising the modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to the disclosure and a pharmaceutically acceptable carrier.

In another aspect, the present disclosure refers to use of the modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to the disclosure, or pharmaceutical composition of the disclosure, in the manufacture of a medicament for the treatment of cancer and/or tumour in an individual or subject, optionally wherein the treatment is cancer

immunotherapy therapy and/or adoptive T cell therapy, optionally allogenic adoptive T cell therapy.

5 In yet another aspect, the present disclosure refers to a method of treating, preventing or delaying the progression of cancer and/or tumour in an individual or subject comprising administering to the individual the modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to the disclosure, or pharmaceutical composition of the disclosure, optionally wherein the treatment is cancer immunotherapy therapy and/or adoptive T cell therapy, optionally allogenic adoptive T cell therapy.

10 In yet another aspect, the present disclosure refers to a kit comprising (a) the modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to the disclosure, or pharmaceutical composition of the disclosure, and a package insert comprising instructions for use thereof for treating, preventing or delaying the progression of cancer and/or tumour in an individual or subject, or (b) the modified induced pluripotent stem cell iPSC or haemogenic lineage cell according to the disclosure, or pharmaceutical composition of the disclosure, and a package insert comprising instructions for use thereof for treating, preventing or delaying the progression of cancer and/or tumour in an individual or subject, in combination with one or more further therapeutic agent optionally administered or for administration separately, sequentially or simultaneously.

#### **TCR binding**

20 According to the invention the heterologous TCR may bind or specifically bind to an antigen or peptide antigen which is any of:

(a) a cancer and/or tumour antigen or peptide antigen thereof, or

(b) a cancer and/or tumour antigen or peptide antigen thereof associated with a cancerous condition and/or presented by tumour or cancer cell or tissue.

25 According to the present invention the heterologous TCR may bind or specifically and/or selectively bind to an antigen or peptide antigen thereof, for example to a cancer and/or tumour antigen or peptide antigen thereof, optionally presented by a cancer and/or tumour and/or in complex with a peptide presenting molecule, for example major histocompatibility complex (MHC) or an HLA, alternatively presented without a peptide presenting molecule  
30 (for example as an endogenously expressed cancer and/or tumour cell surface antigen or peptide antigen). The cancer and/or tumour antigen or peptide antigen thereof can be an antigen or peptide antigen thereof expressed by cancer and/or tumour cells or tissue at higher level than on normal cells or tissue, preferably a significantly higher level, for example 10, 100, 1000, 10,000, 100,000, 1000,000 or greater times higher, preferably the antigen or

- peptide antigen thereof is not expressed by normal cells or tissue. The cancer and/or tumour antigen or peptide antigen thereof may be any of a cancer-testis antigen, NY-ESO-1, MART-1 (melanoma antigen recognized by T cells), WT1 (Wilms tumor 1), gp100 (glycoprotein 100), tyrosinase, PRAME (preferentially expressed antigen in melanoma), p53,
- 5 HPV-E6 / HPV-E7 (human papillomavirus), HBV, TRAIL, DR4, Thyroglobin, TGFBI frameshift antigen, LAGE-1A, KRAS, CMV (cytomegalovirus), CEA (carcinoembryonic antigen), AFP ( $\alpha$ -fetoprotein), a MAGE, melanoma associated antigen or member of the