

T-CELL RECEPTOR AND USES THEREOF

5 [0001] This invention relates to polypeptides and polynucleotides encoding a T-cell receptor TCR) and its use in the treatment of Epstein-Barr virus positive (EBV+) tumours such as nasopharyngeal carcinoma, NKT cell Lymphoma, Hodgkin's Lymphoma, post-transplant lymphoproliferative disease, Diffuse large B-cell lymphoma and some gastric cancers.

BACKGROUND

10 [0002] Nasopharyngeal carcinoma (NPC) is unusually common throughout Southeast Asia, especially in southern China where it is the third most common cancer in men with annual incidence rates of up to 28 cases/100,000 men (1). Early stage disease responds well to radiotherapy (+/- chemotherapy), but a study of 2687 patients treated in Hong Kong reported that over half of these patients presented with advanced disease (Stage III-IV) have
15 a 5-year disease-specific survival rate of only 72% (2). Survivors are also at risk of treatment-related toxicities, including secondary malignancies (3). Therefore, there is clear need to develop improved therapies for this cancer.

[0003] Epstein-Barr virus (EBV) is consistently detected in malignant cells of patients with undifferentiated NPC and is strongly implicated in the pathogenesis of this and other human
20 tumours (4). Despite its oncogenic potential, EBV is ubiquitous in the human population and it normally persists as an asymptomatic life-long infection under the control of virus-specific T cells (4). The presence of this virus within NPC therefore raises the possibility of a T-cell-based therapy for this disease.

[0004] Treatments based on infusing tumour-specific T-cells have yielded impressive
25 clinical responses in some cancers. Indeed, some of the earliest data supporting this approach came from a trial targeting EBV+ lymphomas. Infusing EBV-specific polyclonal T-cell lines is highly effective as a therapeutic and prophylactic treatment for rare EBV+ lymphomas that occur in transplant recipients (5). However, to extend this treatment to more common EBV+ tumours, such as NPC, two issues must be addressed. Firstly, polyclonal T-
30 cell lines initially used to treat EBV+ lymphomas were reactivated *in vitro* using the autologous EBV-transformed lymphoblastoid cell line (LCL). Within an LCL (and most post-transplant EBV+ lymphomas), the virus expresses at least six nuclear antigens, EBNA-1, -2, -3A, -3B, -3C, -LP, and two latent membrane proteins, LMP1 and LMP2. Of these, members of the EBNA3 family are immunodominant antigens for CD8+ T-cells. However, in
35 NPC, EBV protein expression is restricted to EBNA1, LMP1 (variable) and LMP2.

Nevertheless, attempts to treat NPC by infusing LCL-reactivated T-cell lines have yielded objective responses in a minority of patients (6-9). Low frequencies of LMP2-specific T-cells were detectable within some infused cell preparations, and these may have mediated antitumour effects, but the procedure is clearly suboptimal since the majority of virus-specific T-cells targeted EBV genes not expressed in the tumour (7,9). Secondly, generating T-cells by LCL-reactivation takes over 2 months of *in vitro* culture, including the time required to establish an LCL and the subsequent selective expansion of EBV- specific effector cells. This is labour intensive and does not always generate detectable T-cell responses specific for NPC-associated EBV antigens (7-9). More recently, selective reactivation of T cells targeting NPC-associated EBV antigens has been attempted using recombinant viral vectors or peptides (10-12), but again this requires several weeks of *in vitro* culture and/or often results in products with very low frequencies of tumour-specific T-cells.

[0005] Therefore, there is a need for a quicker and easier approach to the provision of a medicament for the treatment or amelioration of EBV+ tumours, particularly those which express LMP2 and are HLA A*1101. Suitably EBV+ tumours may include nasopharyngeal carcinoma, NKT cell lymphoma, Hodgkin's Lymphoma, post-transplant lymphoproliferative disease, Diffuse large B-cell lymphoma and some gastric cancers. Preferably, NPC.

BRIEF SUMMARY OF THE DISCLOSURE

[0006] In one aspect, the present invention provides a T-cell receptor comprising:

- a. an alpha chain comprising an amino acid sequence as set forth in SEQ ID No.1 or a functional equivalent thereof; and/or
- b. a beta chain comprising an amino acid sequence as set forth in SEQ ID No.2 or a functional equivalent thereof.

[0007] Suitably, the alpha chain and beta chain of the T-cell receptor may be joined by a linker, such as a porcine linker.

[0008] Suitably, the T-cell receptor may comprise an amino acid sequence as set forth in SEQ ID No. 3 or a functional equivalent thereof.

[0009] Suitably, the T-cell receptor may comprise an amino sequence which is at least 95% identical to the amino acid sequence as set forth SEQ ID No. 1, SEQ ID No. 2 and/or SEQ ID No. 3.

[0010] Suitably, the T-cell receptor may comprise the sequence as set forth in any one of: SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3 except for one or several modifications. Preferably, the one or several modifications are substitutions and may preferably be made within a variable region.

[0011] Suitably, the T-cell receptor of the present invention may include a cysteine residue at a position equivalent to position 48 of the variable region of the alpha chain (shown in bold and underline in Figure 1) and a cysteine residue at a position equivalent to position 57 of the variable region of the beta chain (shown in bold and underline in Figure 2).

5 **[0012]** Suitably, the T-cell receptor may be encoded by a nucleotide sequence comprising:

a. any one of SEQ ID No.s 4 to 6;

b. a nucleotide sequence equivalent to any one of SEQ ID No.s 4 to 6 as a result of the degeneracy of the genetic code;

10 c. a nucleotide sequence encoding a T-Cell receptor functionally equivalent to a T-cell receptor encoded by a nucleotide sequence comprising any of SEQ ID No.s 4 to 6 and having at least 90% identity thereto; or

d. a nucleotide sequence equivalent to a nucleotide sequence in accordance with c. above as a result of the degeneracy of the genetic code.

15 **[0013]** In a further aspect, the present invention relates to an immune-mobilising monoclonal TCR against cancer (ImmTac) comprising a soluble TCR in accordance with the present invention. Suitably, the ImmTac may comprise an anti-CD3 scFv.

[0014] In another aspect, the present invention provides a nucleic acid sequence comprising:

a. any one of SEQ ID No.s 4 to 6;

20 b. a nucleotide sequence equivalent to any one of SEQ ID No.s 4 to 6 as a result of the degeneracy of the genetic code;

c. a nucleotide sequence encoding a TCR-receptor functionally equivalent to a T-cell receptor encoded by a nucleotide sequence comprising any of SEQ ID No.s 4 to 6 and having at least 90% identity thereto; or

25 d. a nucleotide sequence equivalent to a nucleotide sequence in accordance with c. above as a result of the degeneracy of the genetic code.

[0015] In a further aspect, the present invention provides a vector comprising a nucleic acid sequence of the present invention.

30 **[0016]** In another aspect, the present invention provides a host cell transformed or transfected with a nucleic acid sequence of the present invention, or a vector of the present invention; preferably the host cell is a T-cell such as CD8⁺ or CD4⁺ T-cells. Suitably, the host cell may be an isolated T-cell from a patient to be treated.

[0017] In yet another aspect, the present invention provides a composition comprising:

- a. a T-cell receptor of the present invention;
- b. an ImmTac of the present invention;
- c. a nucleic acid sequence of the present invention;
- d. a vector of the present invention; or
- 5 e. a host cell of the present invention.

[0018] In a further aspect, the present invention provides a T-cell receptor of the present invention; an ImmTac of the present invention, or a composition of the present invention for use in the treatment or prevention of an EBV+ tumour. Suitably, the EBV+ tumour may express LMP2 and is HLA A*1101. Suitably, EBV+ tumours may include nasopharyngeal carcinoma, NKT cell lymphoma, Hodgkin's Lymphoma, post-transplant lymphoproliferative disease, Diffuse large B-cell lymphoma and some gastric cancers. Preferably, the EBV+ tumour is nasopharyngeal carcinoma, preferably undifferentiated nasopharyngeal carcinoma.

[0019] Suitably, the T-cell receptor or pharmaceutical composition may be formulated to provide a dosage of at least 10^8 or at least 10^9 T-cells comprising the T-cell receptor.

[0020] In another aspect, the present invention provides use of a T-cell receptor of the present invention, or a composition of the present invention in the manufacture of a medicament for the treatment or prevention of an EBV+ tumour. Suitably, the EBV+ tumour may express LMP2 and is HLA A*1101. Suitably, EBV+ tumours may include nasopharyngeal carcinoma, NKT cell lymphoma, Hodgkin's Lymphoma, post-transplant lymphoproliferative disease, Diffuse large B-cell lymphoma and some gastric cancers. Preferably, the EBV+ tumour is nasopharyngeal carcinoma, preferably undifferentiated nasopharyngeal carcinoma.

[0021] Suitably, the T-cell receptor or pharmaceutical composition may be formulated to provide a dosage of at least 10^8 or at least 10^9 T-cells comprising the T-cell receptor

[0022] In a further aspect, the present invention provides an *in vitro* method of producing T-cells, comprising a T-cell receptor of the present invention comprising: transfecting or transforming a sample of T-cells (e.g. CD8⁺ or CD4⁺ T-cells) with a nucleic acid sequence of the present invention.

[0023] In another aspect, the present invention provides a method of treating or preventing an EBV+ tumour in a subject, comprising administering a therapeutically effective amount of:
a) T-cell receptors of the present invention; b) ImmTacs of the present invention; or c) a composition of the present invention. Suitably, the EBV+ tumour may express LMP2 and is HLA A*1101. Suitably, EBV+ tumours may include nasopharyngeal carcinoma, NKT cell

lymphoma, Hodgkin's Lymphoma, post-transplant lymphoproliferative disease, Diffuse large B-cell lymphoma and some gastric cancers. Preferably, the EBV+ tumour is nasopharyngeal carcinoma, preferably undifferentiated nasopharyngeal carcinoma.

[0024] Suitably, T-cells autologous to the subject may be transformed or transfected with a T-cell receptor of the present invention or composition of the present invention, and a therapeutically effective amount of the transformed T-cells are administered. Thus, autologous T-cells comprising the T-cell receptor of the present invention may be administered to the subject.

[0025] Suitably, at least 10^8 or 10^9 T-cells may be administered. Preferably, the administration is by infusion.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] Embodiments of the invention are further described hereinafter with reference to the accompanying drawings, in which:

Figure 1 shows the amino acid sequence (SEQ ID No. 1) of the alpha chain of the TCR of the present invention. The sequence in bold represents the joining region between the (preceding) constant region and the (proceeding) variable region. "C" denotes position 48 of the variable region where the amino acid was changed to cysteine.

Figure 2 shows the amino acid sequence (SEQ ID No. 2) of the beta chain of the TCR of the present invention. The sequence in bold represents the joining region between the (preceding) constant region and the (proceeding) variable region. "C" denotes position 57 of the variable region where the amino acid was changed to cysteine.

Figure 3 shows the full-length amino acid sequence (SEQ ID No. 3) of a TCR of the present invention. The sequence in bold represents a porcine linker which joins the preceding alpha chain to the proceeding beta chain.

Figure 4 shows a codon optimised nucleotide sequence (SEQ ID No. 4) encoding the alpha chain of a TCR of the present invention represented by SEQ ID No. 1. The nucleotide sequence of the joining region between the (preceding) constant region and the (proceeding) variable region is shown in bold.

Figure 5 shows a codon optimised nucleotide sequence (SEQ ID No. 5) encoding the beta chain of a TCR of the present invention represented by SEQ ID No. 2. The nucleotide sequence of the joining region between the (preceding) constant region and the (proceeding) variable region is shown in bold. Suitably, reference to SEQ ID No. 5 throughout refers to the sequence shown in Figure 5 without the underlined stop codon.

Figure 6 shows a codon optimised nucleotide sequence (SEQ ID No. 6) encoding a full length TCR of the present invention represented by SEQ ID No. 3. The nucleotide sequence of the linker between the (preceding) nucleotide sequence encoding the alpha chain and the (proceeding) nucleotide sequence encoding the beta chain is shown in bold.

Figure 7 shows the characterisation an A*1101-restricted SSC-specific CD8+ cytotoxic T-cell clone. (a) Avidity for SSC peptide was determined by cytotoxicity assay (E:T = 3:1). (b) Response to LMP2 expressed in A*1101-matched or -mismatched LCLs carrying EBV strains from Caucasian or Chinese populations was measured by IFN γ production. Target cells alone produced <100 pg/ml IFN γ . Responder:stimulator ratio = 1:10. Results show mean+SD and are representative of 3 separate experiments.

Figure 8 shows the expression and function of wild-type SSC-specific TCR. (a) Design of the pMP71 retroviral expression vector. (b) SSC-specific TCR expression on transduced PBMCs from a patient with advanced NPC (TCR-T) compared to that with mock-transduced cells (Mock-T). Values shown refer to percentage of pentamer+, CD8+ or CD4+ cells. (c) Avidity for SSC peptide of TCR- transduced T-cells (TCR-T) and T-cell clone 85 was measured by ELISA for IFN γ release. Mock-transduced T-cells (mock-T) were included as a control. Responder:stimulator ratio = 1:4. Results show mean $\pm$ SD and are representative of 3 repeat experiments. (d) TCR-transduced (but not mock-transduced) T-cells lyse autologous fibroblasts expressing LMP2 from a recombinant vaccinia vector (closed symbols) but not fibroblasts infected with a control vaccinia vector (open symbols). Data representative of 3 separate experiments.

Figure 9 shows the optimization of the TCR gene construct. (a) SSC-specific TCR expression 3 days post-transduction with wild-type TCR (WT TCR), a codon-optimised version (coTCR) or a codon-optimised TCR incorporating an additional disulphide bond (coTCRcys). (b) Intensity of pentamer staining for the different TCR constructs. (c) Avidity for SSC peptide of T-cells transduced with each of the TCR constructs was compared using an ELISA for IFN γ release. T-cell input numbers were adjusted to ensure equivalent numbers of transduced effectors were tested for each TCR construct. Responder:stimulator ratio = 1:3. Results show mean $\pm$ SD. Mock-transduced T-cells (Mock-T) were included as a control. All results shown are representative of at least 3 separate experiments.

Figure 10 shows the function of coTCRcys-expressing CD8+ and CD4+ T-cells. (a) Response of transduced T-cell clones to LMP2 expressed in A*1101-matched or -mismatched LCLs was measured by IFN γ production. Target cells alone produced <100pg/ml IFN γ . Responder:stimulator ratio = 1:10. Results show mean+SD and are representative of 7 clones for each subset. (b) Proliferation of coTCRcys-expression T-cells

measured by CFSE staining after stimulation with T2-A*1101 cells alone (dotted line) or T2-A*1101 cells pulsed with SSC peptide (solid line). (c) Cytotoxic activity of coTCRcys-transduced CD8+ and CD4+ T-cell clones against HONE1 cells expressing LMP2 +/- pulsed with SSC peptide or HONE1 cells alone. Results show mean±SD and are representative of 4 clones for each subset.

Figure 11 shows coTCRcys-expressing CD4+ T-cells produce multiple cytokines following stimulation with T2-A*1101 cells pre-pulsed with SSC peptide. (a) IL2 production by coTCRcys-T-cells stimulated with T2-A*1101+SSC (solid line) compared with coTCRcys-T-cells stimulated with T2-A*1101 alone (dashed line), or mock-T-cells stimulated with T2-A*1101+SSC (grey area). (b) The percentage of these IL2-producing coTCRcys-T-cells that also produced TNFα and/or IFNγ. All data shown were gated on CD4+ T-cells. Thresholds for positive cytokine staining were determined from coTCRcys-T-cells stimulated with T2-A*1101 alone. Results are representative of 5 separate experiments.

Figure 12 shows coTCRcys-transduced T cells control tumour growth *in vivo*. NSG mice were injected with A*1101+LMP2+ MDA-MB-231 tumour cells then treated with T-cell infusions (6 mice per group). Tumour size, measured by calipers (a) or bioluminescence (b) showed significant inhibition of tumour growth by coTCRcys-transduced T-cells compared with mock-T-cells. Bioluminescence images were taken 17 days after T-cell infusion.

Figure 13 shows the functional testing of coTCRcys-transduced T-cells from patients with advanced NPC. (a) IFNγ production following stimulation with A*1101+ targets infected with a recombinant modified vaccinia vector expressing LMP2 (MVA LMP2) or empty vector (MVA control). MVA LMP2 infected targets were also tested after pulsing with SSC peptide. Mock-transduced T-cells from the same donors were used as controls. Target cells alone produced <10pg/ml IFNγ. (b) Cytotoxic activity of coTCRcys- or mock-transduced T-cells from a patient with advanced NPC when co-cultured with NPC cell lines (HK1/A*1101 and c666.1/A*1101) (effector:target = 6:1). Targets were infected with recombinant vaccinia vector expressing LMP2 (vacc LMP2) or with empty vector (vacc control). Some vacc LMP2-infected targets were pre-pulsed with SSC peptides. All results shown represent mean±SD and are representative of 3-5 separate experiments.

DETAILED DESCRIPTION

[0027] In one aspect, the present invention relates to a HLA A*1101-restricted T-cell receptor (TCR) with optimised expression. Suitably, the TCR may be used to rapidly and reliably generate high avidity T-cells specific to an EBV+ tumour, preferably to a tumour expressing LMP2. Preferably, to an EBV+ tumour which expresses LMP2 and is HLA A*1101.

[0028] Suitably, EBV+ tumours may include nasopharyngeal carcinoma, NKT cell lymphoma, Hodgkin's Lymphoma, post-transplant lymphoproliferative disease, Diffuse large B-cell lymphoma and some gastric cancers. Preferably, the EBV+ tumour is nasopharyngeal carcinoma, preferably undifferentiated nasopharyngeal carcinoma.

5 **[0029]** Advantageously, 40% of nasopharyngeal carcinoma (NPC) patients carry this HLA allele. Suitably, the TCR may be used to rapidly and reliably generate high avidity T-cells specific to the NPC-associated viral protein LMP2 which may be used to treat NPC.

[0030] In one aspect, the present invention provides a TCR comprising:

- 10 a. an alpha chain comprising an amino acid sequence as set forth in SEQ ID No.1 or a functional equivalent thereof; and/or
- b. a beta chain comprising an amino acid sequence as set forth in SEQ ID No.2 or a functional equivalent thereof.

15 **[0031]** The alpha chain and beta chain of the T-cell receptor may be joined by a linker. Suitably the linker will join the alpha and beta chains and allow a disulphide bridge to form between position equivalent to 48 of the variable region of the alpha chain as depicted in SEQ ID No. 1 and a position equivalent to position 57 of the variable region of the beta chain as depicted in SEQ ID No. 2.

20 **[0032]** Suitably, the T-cell receptor may comprise an amino acid sequence as set forth in SEQ ID No. 3 or a functional equivalent thereof. SEQ ID No. 3 comprises the codon optimised amino acid sequence of the alpha chain as set forth in SEQ ID No. 1 and the codon optimised amino acid sequence of the beta chain as set forth in SEQ ID No. 2 linked by a porcine linker shown in bold.

25 **[0033]** Suitably, the T-cell receptor may comprise an amino sequence which is at least 95% identical to the amino acid sequence as set forth SEQ ID No. 1, SEQ ID No. 2 and/or SEQ ID No. 3. Suitably, such T-cell receptors may be functionally equivalent to a T-cell receptor as depicted in SEQ ID No. 3.

30 **[0034]** Suitably, the T-cell receptor may comprise the sequence as set forth in any one of: SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3 except for one or several modifications. Preferably, the one or several modifications are substitutions and may preferably be made within a variable region.

[0035] Suitably, the T-cell receptor may comprise the sequence as set forth in any one of: SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3 except for 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 substitutions. Suitably, the substitutions may be chosen to not have a detrimental effect on

the activity of the TCR to generate LMP2-specific T-cells that can be used to treat an EBV+ tumour, such as NPC.

[0036] Suitably, The T-cell receptor of the present invention may comprise a cysteine residue at a position equivalent to position 48 of the variable region of the alpha chain (shown in bold and underline in Figure 1) and a cysteine residue at a position equivalent to position 57 of the variable region of the beta chain (shown in bold and underline in Figure 2).

[0037] Suitably, the TCR of the present invention may be a soluble TCR. Soluble TCRs can in some embodiments be conjugated to immunostimulatory peptides and/or proteins, and/or moieties such as, but not limited, to CD3 agonists (e.g., anti-CD3 antibodies). The CD3 antigen is present on mature human T-cells, thymocytes, and a subset of natural killer cells. It is associated with the TCR and is involved in signal transduction of the TCR. Antibodies specific for the human CD3 antigen are well known. One such antibody is the murine monoclonal antibody OKT3, which was the first monoclonal antibody approved by the FDA. OKT3 is reported to be a potent T-cell mitogen (Van Waave, 1980; U.S. Pat. No. 4,361,539) and a potent T-cell killer (Wong, 1990). Other antibodies specific for the CD3 antigen have also been reported (see PCT International Patent Application Publication No. WO 2004/106380; U.S. Patent Application Publication No. 2004/0202657; U.S. Pat. No. 6,750,325; U.S. Pat. No. 6,706,265; Great Britain Patent Publication GB 2249310A; Clark et al., 1989; U.S. Pat. No. 5,968,509; U.S. Patent Application Publication No. 2009/0117102). Immune mobilising mTCR Against Cancer (ImmTAC; Immunocore Limited, Milton Park, Abingdon, Oxon, United Kingdom) are bifunctional proteins that combine affinity monoclonal T-cell receptor (mTCR) targeting with a therapeutic mechanism of action (i.e., an anti-CD3 scFv).

[0038] In one embodiment, the present invention provides an ImmTac comprising a soluble TCR of the present invention. Suitably, the ImmTac may comprise an anti-CD3 scFv. ImmTACs (immune-mobilising monoclonal TCRs against cancer) which are a class of bispecific reagents, comprising soluble monoclonal T-cell receptors, which have been engineered to possess extremely high affinity for cognate tumour antigen. In this way, ImmTACs overcome the problem of low affinity tumour-specific T-cells imposed by thymic selection, and provide access to the large number of antigens presented as peptide-HLA complexes. Once bound to tumour cells the anti-CD3 effector end of the ImmTAC drives recruitment of polyclonal T-cells to the tumour site, leading to a potent redirected T-cell response and tumour cell destruction.

[0039] Suitably, the T-cell receptor may be encoded by a nucleotide sequence comprising:

a. any one of the codon optimised sequences as set forth in SEQ ID No.s 4 to 6;

b. a nucleotide sequence equivalent to any one of SEQ ID No.s 4 to 6 as a result of the degeneracy of the genetic code;

c. a nucleotide sequence encoding a TCR-receptor functionally equivalent to a T-cell receptor encoded by a nucleotide sequence comprising any of SEQ ID No.s 4 to 6
5 and having at least 90% identity thereto; or

d. a nucleotide sequence equivalent to a nucleotide sequence in accordance with c. above as a result of the degeneracy of the genetic code.

[0040] Advantageously, the T-cell receptors of the present invention can be utilised in T-cell receptor (TCR) gene transfer, an approach that is rapid, reliable and capable of
10 generating large quantities of T cells ($>10^8$ - 10^{10} cells/patient) with specificity to LMP2 (which is e.g. a nasopharyngeal carcinoma(NPC) associated viral protein), regardless of the patient's pre-existing immune repertoire. For example, retroviral transductions may require only 48 hours of culture of preactivate T-cells. Further, large numbers of autologous T-cells can be obtained from leukaphoresis of a blood sample of a subject. Hence, it may be
15 possible to engineer 10^8 - 10^9 transformed or transfected T-cells for infusion in a few days. Such levels of T-cells greatly exceed the doses used to successfully treat patients with NPC by adoptive therapy with LCL-reactivated T-cells (7).

[0041] Without wishing to be bound by theory, it is believed that T-cells transduced with a T-cell receptor of the present invention (such as a TCR as set forth in SEQ ID No. 3) contain
20 a mixture of naïve, central memory and effector memory cells, which suggest that they should persist and display greater antitumor responses *in vivo* (30).

[0042] Suitably, CD8⁺ or CD4⁺ T-cells may be transfected with a vector comprising a nucleic acid sequence encoding a TCR of the present invention. Suitably, the host cell may be an isolated T-cell from a patient to be treated. Suitably, a mixture of T-cells may be
25 isolated from a blood sample by leukaphoresis.

[0043] Suitably, in the second medical uses and methods of treatment of the present invention the medicament may comprise at least 10^8 T-cells expressing a TCR of the present invention. Suitably, the medicament may comprise at least 10^9 or at least 10^{10} or at least 10^{11} cells, preferably said T-cells may be CD8⁺ and/or CD4⁺ T-cells.

30 [0044] Suitably the second medical uses and methods of treatment of the present invention the medicament may be in the form of a bi-specific immunotherapeutic agents such as ImmTACs (Immune mobilising TCRs against cancer) (Liddy, et al. (2012) Nat Med 18: 980- 987) or BiTEs (Bispecific T-cell engaging antibodies) (Baeuerle, et al. (2009). Curr Opin Mol Ther 11(1): 22-30).

[0045] In another aspect, the present invention provides a method of treating or preventing an EBV+ tumour (such as nasopharyngeal carcinoma) in a subject comprising administering a therapeutically effective amount of: a) T-cell receptors of the present invention; b) ImmTacs of the present invention; or c) a composition of the present invention. Suitably, T-cells autologous to the subject may be transformed or transfected with a T-cell receptor of the present invention or a composition of the present invention, and a therapeutically effective amount of the transformed T-cells are administered. Thus, autologous T-cells comprising the T-cell receptor of the present invention may be administered to the subject. Suitably, the patient population may be of Chinese origin. Preferably, the administration is by infusion.

[0046] Unless otherwise defined, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[0047] Exemplary techniques used in connection with recombinant DNA, oligonucleotide synthesis, tissue culture and transformation (e.g., electroporation, lipofection), enzymatic reactions, and purification techniques are known in the art. Many such techniques and procedures are described, e.g., in Sambrook et al. Molecular Cloning: A Laboratory Manual (3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2001)), among other places.

DEFINITIONS

[0048] In accordance with the present invention, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

[0049] The term "functional equivalent" refers to a variant TCR specific to LMP2 which has at least 80% or at least 85% or at least 90% or at least 95% or at least 97% or at least 99% or 100% of the avidity antigen specific function of a TCR having an amino acid sequence as set forth in SEQ ID No.s 1-3 (preferably SEQ ID No. 3) or encoded by the nucleic acid sequence as set forth in SEQ ID No.s 4-6 (preferably SEQ ID No. 3). In one embodiment, the avidity antigen -specific function is measured in relation to one or more of the following: proliferation, cytotoxicity, cytokine release or inhibition of LMP2⁺ tumour growth. In one embodiment, a functional equivalent is measured in specificity to LMP2 and inhibition of LMP2⁺ tumour growth.

[0050] The terms "nucleic acid molecule" and "polynucleotide" may be used interchangeably, and refer to a polymer of nucleotides. Such polymers of nucleotides may contain natural and/or non-natural nucleotides, and include, but are not limited to, DNA,

RNA, and PNA. "Nucleic acid sequence" refers to the linear sequence of nucleotides that comprise the nucleic acid molecule or polynucleotide.

[0051] The terms "polypeptide" and "protein" are used interchangeably to refer to a polymer of amino acid residues. Such polymers of amino acid residues may contain natural
5 or non-natural amino acid residues, and include, but are not limited to, peptides, oligopeptides, dimers, trimers, and multimers of amino acid residues. Both full-length proteins and fragments thereof are encompassed by the definition. The terms also include post-expression modifications of the polypeptide, for example, glycosylation, sialylation, acetylation, phosphorylation, and the like. Additionally, in the context of the present
10 invention, a "polypeptide" refers to a protein which includes modifications, such as deletions, additions, and substitutions (generally conservative in nature), to the native sequence, as long as the protein maintains the desired activity. These modifications may be deliberate, e.g. occurring by site-directed mutagenesis, or may be accidental, such as through mutations of hosts which produce the proteins or errors due to PCR amplification.

[0052] A "native sequence" polypeptide comprises a polypeptide having the same amino acid sequence as a polypeptide found in nature. Thus, a native sequence polypeptide can have the amino acid sequence of naturally occurring polypeptide from any mammal. Such native sequence polypeptide can be isolated from nature or can be produced by recombinant or synthetic means. The term "native sequence" polypeptide specifically encompasses
20 naturally occurring truncated or secreted forms of the polypeptide (e.g., an extracellular domain sequence), naturally occurring variant forms (e.g., alternatively spliced forms) and naturally occurring allelic variants of the polypeptide.

[0053] A polypeptide "variant" means a biologically active polypeptide having at least about 80% amino acid sequence identity with the native sequence polypeptide after aligning
25 the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Such variants include, for instance, polypeptides wherein one or more amino acid residues are added, or deleted, at the N- or C-terminus of the polypeptide. In some embodiments, a variant will have at least about 80% amino acid sequence identity. In some embodiment, a variant will have at least about 90% amino acid sequence identity. In some embodiment, a variant will have at least about 95% amino acid sequence identity with the native sequence polypeptide. In some embodiment, a variant will have at least about 97% or 98% or 99% amino acid sequence identity with the native sequence polypeptide.

[0054] As used herein, "Percent (%) amino acid sequence identity" and "homology" with
35 respect to a peptide, polypeptide or antibody sequence are defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues

in the specific peptide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or MEGALIGN™ (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0055] The terms "inhibition" or "inhibit" refer to a decrease or cessation of any phenotypic characteristic or to the decrease or cessation in the incidence, degree, or likelihood of that characteristic. Preferably, by "reduce" or "inhibit" is meant the ability to cause a decrease of 20% or greater; preferably to cause a decrease of 50% or greater. Preferably, by "reduce" or "inhibit" is meant the ability to cause an overall decrease of 75%, 85%, 90%, 95%, or greater.

[0056] The terms "subject" and "patient" are used interchangeably herein to refer to a human. In some embodiments, methods of treating other mammals, including, but not limited to, rodents, simians, felines, canines, equines, bovines, porcines, ovines, caprines, mammalian laboratory animals, mammalian farm animals, mammalian sport animals, and mammalian pets, are also provided. In some instances, a "subject" or "patient" refers to a subject or patient in need of treatment for a disease or disorder.

[0057] Throughout the description and claims of this specification, the words "comprise" and "contain" and variations of them mean "including but not limited to", and they are not intended to (and do not) exclude other moieties, additives, components, integers or steps. Throughout the description and claims of this specification, the singular encompasses the plural unless the context otherwise requires. In particular, where the indefinite article is used, the specification is to be understood as contemplating plurality as well as singularity, unless the context requires otherwise.

[0058] Features, integers, characteristics, compounds, chemical moieties or groups described in conjunction with a particular aspect, embodiment or example of the invention are to be understood to be applicable to any other aspect, embodiment or example described herein unless incompatible therewith. All of the features disclosed in this specification (including any accompanying claims, abstract and drawings), and/or all of the steps of any method or process so disclosed, may be combined in any combination, except combinations where at least some of such features and/or steps are mutually exclusive. The invention is not restricted to the details of any foregoing embodiments. The invention extends to any novel one, or any novel combination, of the features disclosed in this

specification (including any accompanying claims, abstract and drawings), or to any novel one, or any novel combination, of the steps of any method or process so disclosed.

[0059] The reader's attention is directed to all papers and documents which are filed concurrently with or previous to this specification in connection with this application and which are open to public inspection with this specification, and the contents of all such papers and documents are incorporated herein by reference.

[0060] **EXAMPLES**

[0061] **MATERIALS AND METHODS**

[0062] **Cells and Cell lines**

10 Peripheral blood mononuclear cells (PBMC) were isolated from heparinised blood by density gradient centrifugation on lymphoprep (Axis Shield, Oslo, Norway). LCLs were generated using Caucasian (B95.8) or Chinese (CKL) prototype 1 EBV strains (15). Phoenix amphotropic packaging cells were kindly provided by Gary Nolan (Stanford University). The T2 cell line transduced with HLA A*1101 gene was kindly provided by M. Masucci (Karolinska Institute, Stockholm, Sweden). NPC cell lines HK1 (16) and c666.1 (17) were transduced with retrovirus (pQCXIH and pQCXIN respectively; Clontech, CA) into which we had cloned the gene encoding HLA A*1101. These cell lines were then cultured under drug selection using 20µg/ml Hygromycin or 50µg/ml G418 (Life technologies, UK), respectively. Though originally described as an NPC cell line, and used here because it naturally expresses HLA A*1101, HONE-1 now appears to be a HeLa-related somatic cell hybrid (18). The breast cancer cell line MDA-MB-231 (19) was transduced with three retroviruses (pQCXIH, pLXSN and pMSCV) carrying genes encoding HLA A*1101, LMP2 and luciferase respectively, and cultured under drug selection using 300µg/ml Hygromycin, 600µg/ml G418 and 1µg/ml puromycin. All of the above cells lines were cultured in RPMI1640 (Sigma) containing 10% foetal bovine serum (FBS; PAA, Pasching Austria), 2mM glutamine, 100IU/ml penicillin, and 100pg/ml streptomycin (standard medium). Fibroblasts were grown from a skin biopsy cultured in DMEM (Sigma, UK) supplemented as described above. All T-, B- and fibroblast cell lines were derived from healthy donors or NPC patients of known HLA type. All cancer cell lines were authenticated by short tandem repeat analysis and passaged for fewer than 6 months before experiments. The use of human materials for this study was approved by the National Research Ethics Service, U.K., and the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee. Work was conducted according to the declaration of Helsinki protocols and all donors provided written informed consent.

35 [0063] **Synthetic peptides and recombinant vaccinia viruses**

Peptides were synthesized using Fluorenylmethoxycarbonyl chemistry by Alta Bioscience, Birmingham, U.K. Recombinant vaccinia and modified vaccinia Ankara viruses expressing LMP2 and corresponding control vectors have been described previously (20,21).

[0064] TCR gene cloning

- 5 RNA from the T-cell clone was isolated using an RNeasy mini kit (Qiagen, UK) and reverse transcribed. TCR- α and - β genes were then amplified with the BD SMART™ RACE cDNA Amplification Kit (BD Biosciences, San Jose, CA) according to the manufacturer's instructions using the following primers: TCR α constant region: 5'-agcacaggctgtcttacaatcttc-3' (SEQ ID No. 7); TCR β constant region: 5'-ggacacagattgggagcagg-3' (SEQ ID NO. 8). TCR genes were subcloned into the pCR2.1 (Life Technologies) vector and sequenced. The TCR- α (TCRVA22) and - β (TRBV4.01) chains were then cloned into a retroviral pMP71-PRE vector (22) (kindly provided by C. Baum, Hannover, Germany) separated by a 2A peptide linker from porcine teschovirus. Modified TCR genes were designed and produced by GeneArt (Regensburg, Germany).
- 10

15 **[0065] Retroviral transduction of human T-cells**

- [0066]** Phoenix amphotropic packaging cells were transfected with pMP71 retroviral vector and pCL amphi (Imgenex) using FuGENE HD (Roche) according to manufacturer's instructions and retroviral supernatant were harvested 48 hours later. PBMCs were pre-activated for 48 hours using anti-CD3 antibody (OKT3; 30ng/ml) and interleukin-2 (IL2; 600U/ml; Chiron, Emeryville, CA) in standard medium containing 1% human AB serum (TCS Biosciences, Buckingham, UK). These cells were then transduced with retroviral supernatant (or mock-transduced with conditioned supernatant from non-transfected phoenix cells) using retronectin-coated (Takara, Shiga, Japan) 6- well plates according to manufacturer's instructions. Cells were then maintained in standard medium containing 1% human AB serum and IL2 (100U/ml).
- 20
- 25

[0067] Flow cytometry

- [0068]** Cells were stained for 10 minutes at room temperature with a HLA-A*1101/SSC pentamer (5 μ g/ml; ProImmune, Oxford, U.K.) according to the manufacturer's instructions. Cells were then washed and stained on ice for 30 minutes with Pro5 Fluorotag (APC or R-PE-labelled; ProImmune) and saturating concentrations of anti-CD3 (PE-conjugated), anti-CD4 (FITC-conjugated) (Pharmingen) and anti-CD8 (tricolor- or ECD-conjugated) (Caltag) antibodies. For intracellular cytokine staining T cells were stimulated for two hours with T2-A11 cells pre-pulsed with or without SSC peptide (5 μ g/ml). Brefeldin A (10 μ g/ml, Sigma) was then added and cells cultured for another 5 hours.
- 30

[0069] Cells were then stained with pentamer and antibodies to surface markers (CD4-FITC, CD8-ECD, BD Pharmingen) as described above. After treatment with fixation and permeabilisation buffers (E- bioscience, San Diego, CA) according to the manufacturer's instructions, cells were incubated for 30 minutes at 4°C with anti-cytokine antibodies (IL2-PE, IFN γ -PECy7 and TNF α -APC) or an isotype- and concentration-matched control antibody (BD Pharmingen), then washed twice in PBS. Cells were analysed using an LSRII cytometer (Becton Dickinson, Franklin Lakes, NJ) and FlowJo software (Tree Star, Ashland, OR).

[0070] CFSE labelling

10 T cells were washed twice with PBS and incubated with 2.5 μ M Carboxyfluorescein succinimidyl ester (CFSE) for 10 minutes at 37°C. The labelling reaction was quenched by addition of RPMI-1640 containing 10% FBS. Cells were washed, resuspended in standard growth medium at 2 $\times$ 10⁶ cells/ml, co-cultured for 5 days with T2-A*1101 cells pre-pulsed with SSC peptide (10 μ g/ml), then analysed by flow cytometry as described above.

15 **[0071] IFN γ release assay**

Stimulator cells (5 $\times$ 10⁴/well) were co-cultured in triplicate with T cells at responder:stimulator ratios as indicated. Cells were incubated at 37°C/5% CO₂ in 100 μ l/well of Iscove's modified dulbecco's medium (Life Technologies) supplemented with 10% FBS and IL2 (25U/ml). After 18 hours, culture supernatants were tested for secreted IFN γ using an ELISA (Pierce
20 Endogen, Rockford, IL) according to the manufacturer's instructions.

[0072] Cytotoxicity assays

Chromium release assays, using vaccinia-infected or peptide-pulsed targets, were set up at known effector:target ratios (2500 targets/well) and harvested after 5 or 8 hours. These protocols have been described in detail previously (23).

25 **[0073] In vivo tumour protection experiments**

[0074] 6-8 week old female NSG mice (Charles River Laboratories) were inoculated subcutaneously on the flank with MDA-MB-231 cells expressing A*1101, LMP2 and luciferase (5 $\times$ 10⁶ cells/mouse) in matrigel (BD Biosciences). One day later, mice received 107 TCR-transduced (or mock- transduced) T cells intravenously. Intraperitoneal injections
30 of 104 units IL2 were given on days 2, 4, 7, 9 and 11. Tumour growth was measured in a blinded fashion with callipers and bioluminescence imaging (IVIS Spectrum, Caliper Life Sciences). All experiments were performed under UK Home Office authorization.

[0075] RESULTS

[0076] Expression and function of a wild-type HLA A*1101-restricted LMP2-specific TCR

[0077] EBV-specific T cells from a healthy Chinese donor were reactivated *in vitro* with the autologous LCL and cloned by limiting dilution as previously described (23). Clones
 5 were screened for reactivity to the A*1101-restricted LMP2 epitope SSC and clone 85 was selected. The avidity of this CD8⁺ clone for SSC peptide was determined using a cytotoxicity assay with A*1101⁺ targets pulsed with titrated concentrations of peptide. The clone displayed high avidity, with clear recognition of target cells pulsed with only 10⁻¹⁰M peptide (Fig.7a). When tested for IFN γ production in response to A*1101-matched and -
 10 mismatched LCL targets, a clear A*1101-restricted response was observed (Fig.7b). Importantly, this clone recognised not only A*1101⁺ LCLs carrying the standard EBV strain B95.8 (derived from a Caucasian population) but also those carrying EBV strains from the Chinese population, which is the most at risk of NPC.

[0078] Genes encoding TCR- α and - β chains from clone 85 were isolated and cloned
 15 into the same MP71 retroviral expression vector separated by a 2A peptide-linker from porcine teschovirus to ensure equimolar expression of these chains (Fig.8a). Activated T cells from healthy donors and NPC patients were then transduced with the recombinant retrovirus and surface expression of SSC- specific TCR determined using an A*1101/SSC pentamer. Figure 8b shows results with T cells from a patient with advanced NPC. SSC-
 20 specific T cells are rare/undetectable in most NPC patients and healthy virus carriers (as indicated by mock-transduced cells), but 3 days post transduction with recombinant retrovirus, surface expression of SSC-specific TCR was clearly detectable in 13.6% of CD8⁺ T cells. Note that 12% of CD4⁺ T cells also expressed this TCR following transduction. These data are representative of those from 9 healthy donors and 5 NPC
 25 patients.

[0079] Functional testing of this wild-type TCR began using transduced polyclonal T cells to explore their ability to produce IFN γ in response to T2:A*1101 cells pulsed with SSC peptide at titrating concentrations. TCR-transduced T cells clearly recognised peptide-pulsed targets with as low as 10⁻¹⁰M peptide, whereas mock-transduced T cells
 30 did not respond at any peptide concentration tested (Fig.8c). Testing Clone 85, from which the TCR genes were derived, at the same input cell number as SSC-specific effectors within the transduced T cells yielded almost identical results (Fig.8c). Transduced T cells also mediated specific cytotoxic function when tested against autologous fibroblasts expressing LMP2 protein from a recombinant vaccinia vector, compared with that against
 35 fibroblasts infected with the empty control vector (fig.8d).

[0080] Optimisation of the TCR gene construct.

We generated two variants of our wild-type SSC-specific TCR, a codon-optimised version (coTCR) and a codon-optimised TCR in which amino acid residue 48 of the TCR- α chain and residue 57 of the TCR- β chain were both changed to cysteine, thus introducing a second disulfide bond (coTCRcys) (25). A series of experiments then compared expression and function of these two variants with wild-type SSC-specific TCR (WT TCR). The main difference observed was TCR surface expression. Pentamer staining of CD8⁺ T cells, transduced with increasing volumes of the three retroviral supernatants produced in parallel, showed similar expression of WT TCR and coTCR, but a clear increase was observed with the coTCRcys construct (Fig.9a). Similar results were obtained with CD4⁺ T cells (data not shown). Not only was the coTCRcys receptor expressed on a greater proportion of T cells, but the levels of expression on individual cells were increased (fig.9b). Staining transduced cells with an antibody to V β 4.1 showed similar results to the same cells stained with the SSC pentamer (not shown) suggesting that there is little if any mispairing between this exogenous β -chain and the endogenous α -chains.

[0081] Although expression was improved with coTCRcys, when an equivalent number of transduced effectors were tested for each TCR construct, T-cell function was unaffected (Fig.9c). Although codon optimisation alone (coTCR) affected neither surface expression nor functional activity (Fig.9), other studies have shown that despite such lack of *in vitro* effects, codon optimisation can nevertheless improve both frequency of TCR-modified T cells detectable post-infusion and antitumour activity *in vivo* (26,27). Analysing the differentiation status of coTCRcys-transduced cells showed that they contained a mixture of mainly naïve, central-memory and effector-memory cells (not shown).

[0082] Functional analysis of coTCRcys in CD8⁺ and CD4⁺ T cells

Having optimised expression of the SSC-specific TCR, we then determined the ability of coTCRcys-transduced T-cells to recognise LMP2 protein expressed at physiological levels in an LCL. For this we used cloned populations of TCR-transduced cells to study the functional activity in CD8⁺ cells, which can have direct antitumour effects *in vivo*, and CD4⁺ cells, which can help generate and maintain effective CD8⁺ responses and can also be cytotoxic. To ensure SSC-specific CD8⁺ clones had been engineered and were not naturally occurring effectors, we used PCR to detect the retroviral construct (data not shown). Both engineered CD8⁺ and CD4⁺ cells responded by IFN γ production in an A*1101-restricted manner when tested against a panel of A*1101-matched and -mismatched LCLs (Fig.10a). Thus this TCR can function in a CD8-independent manner.

[0083] Using CFSE-labelling, we explored the ability of coTCRcys-transduced T-cells to proliferate following antigen encounter. Both engineered CD8⁺ and CD4⁺ T cells underwent several rounds of division following stimulation with SSC peptide-loaded T2-A*1101 cells

(compared to T2-A*1101 alone) (Fig.10b). Furthermore, both engineered CD8⁺ and CD4⁺ T cells were cytotoxic, lysing A*1101-positive HONE1 cells expressing LMP2 from a recombinant vaccinia vector with or without addition of the SSC peptide (Fig.10c).

5 [0084] An increased frequency of CD4 T cells with multifunctional capacity for cytokine production is associated with improved control of some infections (28). Using intracellular staining we showed coTCRcys-transduced CD4⁺ T cells can simultaneously produce multiple cytokines (IL2, IFN γ , TNF α) following antigen-specific stimulation (Fig.11).

[0085] ***In vivo* studies with an LMP2⁺ epithelial tumour model**

10 We engineered another human epithelial tumour (MDA-MB-231) to co-express LMP2 and A*1101 as well as luciferase for bioluminescence imaging. Immunodeficient mice carrying this tumour were treated with coTCRcys-expressing T cells. Flow cytometric analysis showed the infused T cells contained a CD4:CD8 ratio of 3:2, with 50% CD4 and 60% CD8 T cells expressing the SSC-specific TCR. Tumour growth in these mice was significantly reduced compared to that in control mice that received mock-transduced T
15 cells (Fig.12).

[0086] **TCR-transduction of T cells from patients with advanced NPC and recognition of NPC cell lines**

[0087] We sought to determine whether coTCRcys-transduced T cells from patients with advanced NPC could respond to NPC cell lines expressing LMP2. All NPC tumours are
20 EBV⁺, with the exception of c666.1, an NPC cell line established *in vitro* that has lost the EBV genome; c666.1 does not even express the LMP2 protein. Therefore having introduced the restricting HLA allele into c666.1 by retroviral transduction (c666.1/A*1101) we expressed LMP2 from a recombinant modified vaccinia (Ankara) vector with or without addition of the SSC peptide. Transduced T cells from two advanced NPC patients clearly
25 responded by producing IFN γ in an antigen-specific manner to LMP2-expressing c666.1/A*1101 cells. Similar levels of response were seen with antigen-loaded A*1101-matched fibroblasts and HONE1 cells (Fig.13a). These T cells were also tested for cytotoxic activity towards NPC cell lines and here we included a second NPC line HK1, which again had to be transduced to express A*1101 (HK1/A*1101). Transduced (but not
30 mock- transduced) T cells lysed both HK1/A*1101 and c666.1/A*1101 cells in an LMP2-specific manner (Figure 13b).

[0088] We sought to determine whether coTCRcys-transduced T cells from patients with advanced NPC could respond to NPC cell lines expressing LMP2. All NPC tumours are
35 EBV⁺, with the exception of c666.1, an NPC cell line established *in vitro* that has lost the EBV genome; c666.1 does not even express the LMP2 protein. Therefore having

introduced the restricting HLA allele into c666.1 by retroviral transduction (c666.1/A*1101) we expressed LMP2 from a recombinant modified vaccinia (Ankara) vector with or without addition of the SSC peptide. Transduced T cells from two advanced NPC patients clearly responded by producing IFN γ in an antigen-specific manner to LMP2-expressing c666.1/A*1101 cells. Similar levels of response were seen with antigen-loaded A*1101-matched fibroblasts and HONE1 cells (Fig.13a). These T cells were also tested for cytotoxic activity towards NPC cell lines and here we included a second NPC line HK1, which again had to be transduced to express A*1101 (HK1/A*1101). Transduced (but not mock- transduced) T cells lysed both HK1/A*1101 and c666.1/A*1101 cells in an LMP2-specific manner (Figure 13b).

[0089] Discussion

That NPC is responsive to EBV-specific T cell-based therapies is apparent from studies using adoptive T-cell therapy (6-9). However, current approaches to generate such cells for infusion are both time consuming and unreliable. We have utilised TCR gene transfer, a technology that can reliably generate large quantities of specific T cells in a few days, regardless of the patient's pre-existing immune response. Having identified a T-cell clone with high avidity for the HLA A*1101-restricted LMP2 epitope SSC, we cloned the genes encoding the TCR and through retroviral-mediated gene transfer expressed them in T cells from healthy donors and advanced NPC patients. T cells from healthy donors engineered to express a modified form of the TCR responded in an antigen-specific manner by proliferating, generating cytokines (IFN γ , TNF α and IL2), lysing target cells and inhibiting LMP2+ tumour growth in vivo. TCR-transduced T cells from advanced NPC patients could also recognise NPC cell lines expressing the LMP2 protein.

[0090] As described in the methods, retroviral transduction requires only 48 hours of culture to preactivate T cells, and scaling up the process by starting with large numbers (10^9 - 10^{10}) of T cells available from leukapheresis of patients, it should be possible to engineer $>10^8$ - 10^9 T-cells for infusion in a few days. Including a few days more for in vitro expansion, trials of TCR gene transfer have infused 10^9 - 10^{11} T cells per patient (13,14). This greatly exceeds the dose used to successfully treat patients with NPC by adoptive therapy with LCL-reactivated T cells (7), in which patients received only 4×10^7 - 4×10^8 cells/m ^{2} , and LMP-specific and SSC-specific T cells comprised $<1\%$ and $<0.05\%$ of this product, respectively (29). T cells transduced with the coTCRCys receptor contained a mixture of naïve, central memory and effector memory cells (Supplementary Fig.S2). The presence of less differentiated T cells suggests that they should persist and display greater antitumour responses in vivo (30).

[0091] We focussed on an A*1101-restricted TCR because this HLA allele is very common in the populations most at risk for NPC. Indeed, approximately 40% of NPC patients are A*1101+ (31,32) and are therefore available for treatment with an A*1101-restricted SSC-specific TCR.

5 **[0092]** Encouragingly, several studies have also reported that A*1101 is associated with decreased risk of NPC (31,32), supporting our hypothesis that SSC peptide is a good target for T-cell therapy. Furthermore, transiently boosting of T-cell responses to this epitope in A*1101+ NPC patients using SSC peptide-pulsed dendritic cells is safe and can induce partial clinical responses (33). The SSC epitope sequence, originally identified using
10 standard laboratory strain B95.8, is largely conserved in EBV strains within the Southern Chinese population, including virus isolates from NPC tumours (23,34). In Northern China an S-T mutation in residue 9 of the epitope has been detected in 50% of NPC patients (35). However, from our previous studies we found no evidence that this mutation affects antigenicity of the epitope (23).

15 **[0093]** T cell-based therapies targeting a single epitope could lead to selection of tumour cells carrying epitope-loss EBV variants. However, this could be avoided by using multiple TCRs targeting additional epitopes in NPC-associated EBV proteins. Indeed several epitopes have already been described, some of which are again restricted through HLA class I and II alleles present at relatively high frequency in the Chinese population (23,36),
20 thereby increasing the number of patients available for a TCR gene transfer-based therapy. Combining TCR gene transfer with vaccination (37) could also amplify and broaden the EBV-specific T-cell response in vivo.

[0094] If T-cell therapy is to be effective for NPC, antigen-presenting function in the tumour cells must be intact. Results from immunohistochemical analysis of NPC tissues have
25 indicated that critical components of the HLA class I antigen-processing pathway may be downregulated in some NPC tumours (38). Furthermore there is evidence for other potential immune evasion mechanisms in NPC including the presence of regulatory T cells (39) and transforming growth factor beta (40). Nevertheless, results from in vitro studies on NPC cell lines (41), including data presented in this report, and the association of A*1101 with reduced
30 risk for NPC (31,32) suggest that the malignant cells can present antigen to T cells. More importantly, clinical responses following adoptive T-cell therapy (6-9) and vaccination (33) indicate that immune evasion mechanisms can be overcome at least in some patients. Indeed, effective delivery of large numbers of tumour-specific IFN γ -producing cytotoxic T cells may be sufficient to overwhelm immunosuppressive factors. Additional genetic
35 modifications of infused T cells, such as expression of a dominant negative TGF β receptor (42) may also help. If the patient's antigen-presenting function is compromised, successful

treatment may yet be possible by targeting stromal cells if they cross-present tumour antigens. Cross-presentation appears dependent on HLA binding affinity of the target epitope (43) which suggests that SSC (predicted affinity (IC50) = 14nM based on the Immune Epitope Database Analysis Resource) should be readily cross-presented, thereby
5 also reducing the risk of tumour relapse through escape variants.

[0095] TCR gene transfer has been tested in the clinic to treat advanced melanoma and synovial cell sarcoma (13,14). Combining these studies, objective clinical responses were seen in 22/87 patients treated. However, significant autoimmune reactions occurred in some patients in whom

10 **[0096]** TCRs targeted self-proteins expressed on normal cells (13). In this respect, NPC is an ideal setting to test the potential of TCR gene transfer since foreign- (viral) rather than self-antigens can be targeted using naturally occurring high affinity TCRs. EBV is present in some normal lymphocytes, but only 1-50/million circulating B cells and most of these lack viral protein expression (44). Therefore the risk of on-target toxicity with an EBV-specific
15 TCR is minimal.

[0097] TCR gene transfer carries a potential risk of off-target toxicity due to mispairing of TCR chains generating novel autoreactive receptor specificities (45). Although such toxicity has not yet been reported in clinical trials, and we found little evidence of mispairing at least with the exogenous β chain (Supplementary Fig.S1), we have incorporated several
20 approaches to reduce this risk with the coTCRcys receptor. Thus genes encoding the TCR α - and β -chains were cloned into a single retroviral vector with a 2A peptide-linker to ensure equimolar expression in the same T cell. Furthermore we incorporated a second disulphide bond between the α - and β -constant domains, which also improved TCR surface expression. To reduce this risk further, it is possible to knockdown expression of
25 endogenous TCR chains using shRNA (46). Nevertheless, it may be prudent to incorporate a suicide gene (47) for selective deletion of infused cells should autoimmunity develop.

[0098] Several studies have highlighted the importance of CD4+ T cells in controlling tumour growth (48,49) and the ability of our SSC-specific TCR to function in these cells is important for two reasons. Firstly, a concurrent antigen-specific CD4+ T-cell response aids
30 expansion and efficacy of cytotoxic CD8+ T cells (50). Indeed when NPC patients were immunised with dendritic cells expressing SSC peptide, CD8+ T-cell responses to this epitope were boosted but only temporarily (33). The implication was that boosting EBV-specific CD4+ T cells was also required. When stimulated with SSC peptide, CD4+ T cells transduced with coTCRcys produced cytokines, including IL2, indicating that they could help
35 sustain coTCRcys-transduced CD8+ T cells. Secondly, coTCRcys-transduced CD4+ T cells

were cytotoxic, indicating they might destroy NPC cells directly. Therefore, the ability of this TCR to function in both CD8 and CD4 T cells increases its potential for treating NPC.

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CLAIMS

1. A T-cell receptor comprising:
 - a. an alpha chain comprising an amino acid sequence as set forth in SEQ ID No.1 or a functional equivalent thereof; and/or
 - 5 b. a beta chain comprising an amino acid sequence as set forth in SEQ ID No.2 or a functional equivalent thereof.
2. The T-cell receptor in accordance with claim 1, wherein the alpha chain and beta chain joined by a linker.
3. The T-cell receptor in accordance with claim 1 or claim 2, wherein the linker is a
10 porcine linker.
4. The T-cell receptor in accordance any one of claims 1 to 3, wherein the T-cell receptor comprises an amino acid sequence as set forth in SEQ ID No. 3 or a functional equivalent thereof.
- 5 The T-cell receptor in accordance with any of the preceding claims, wherein the T-
15 cell receptor comprises an amino sequence which is at least 95% identical to the amino acid sequence as set forth SEQ ID No. 1, SEQ ID No. 2 and/or SEQ ID No. 3.
6. The T-cell receptor in accordance with any of the preceding claims, wherein the T-cell receptor comprises the sequence as set forth in any one of: SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3 except for one or several modifications.
- 20 7. The T-cell receptor in accordance with claim 6, wherein the one or several modifications are substitutions.
8. The T-cell receptor according to claim 6 or claim 7, wherein the one or several modifications are within a variable region.
9. The T-cell receptor in accordance with any of the preceding claims, wherein the T-
25 cell receptor comprises a cysteine residue at position 48 of the variable region of the alpha chain and a cysteine residue at position 57 of the variable region of the beta chain.
10. The T-cell receptor according to any one of the preceding claims wherein the T-cell receptor is encoded by a nucleotide sequence comprising:
 - a. any one of SEQ ID No.s 4 to 6;
 - 30 b. a nucleotide sequence equivalent to any one of SEQ ID No.s 4 to 6 as a result of the degeneracy of the genetic code;

- c. a nucleotide sequence encoding a T—cell receptor functionally equivalent to a T-cell receptor encoded by a nucleotide sequence comprising any of SEQ ID No.s 4 to 6 and having at least 90% identity thereto; or
 - d. a nucleotide sequence equivalent to a nucleotide sequence in accordance with c. above as a result of the degeneracy of the genetic code.
- 5 11. An immune-mobilising monoclonal TCR against cancer comprising a soluble TCR in accordance with any one of claims 1 to 10.
- 12. The immune-mobilising monoclonal TCR against cancer according to claim 11, wherein the immune-mobilising monoclonal TCR against cancer comprises an anti-CD3 scFv.
- 10 13. A nucleic acid sequence comprising:
 - a. any one of SEQ ID No.s 4 to 6;
 - b. a nucleotide sequence equivalent to any one of SEQ ID No.s 4 to 6 as a result of the degeneracy of the genetic code;
 - 15 c. a nucleotide sequence encoding a T-cell receptor functionally equivalent to a T-cell receptor encoded by a nucleotide sequence comprising any of SEQ ID No.s 4 to 6 and having at least 90% identity thereto; or
 - d. a nucleotide sequence equivalent to a nucleotide sequence in accordance with c. above as a result of the degeneracy of the genetic code.
- 20 14. A vector comprising a nucleic acid sequence in accordance with claim 13.
- 15. A host cell transformed or transfected with a nucleic acid sequence according to claim 13 or a vector according to claim 14.
- 16. A composition comprising:
 - a. a T-cell receptor in accordance with any one of claims 1 to 10;
 - 25 b. an immune-mobilising monoclonal TCR against cancer in accordance with claims 11 or claim 12;
 - c. a nucleic acid sequence in accordance with claim 13;
 - d. a vector in accordance with claim 14; or
 - e. a host cell in accordance with claim 15.
- 30 17. A T-cell receptor of any one of claims 1 to 10, an immune-mobilising monoclonal TCR against cancer in accordance with claims 11 or claim 12 or a composition of claim 16 for use in the treatment or prevention of an EBV+ tumour.

18. A T-cell receptor or composition for use in accordance with claim 17 wherein the EBV+ tumour is selected from the group consisting of: nasopharyngeal carcinoma, NKT cell lymphoma, Hodgkin's Lymphoma, post-transplant lymphoproliferative disease, Diffuse large B-cell lymphoma and gastric cancer.
- 5 19. A T-cell receptor or composition for use in accordance with claim 17 or claim 18, wherein the EBV+ tumour is nasopharyngeal carcinoma, such as undifferentiated nasopharyngeal carcinoma.
- 10 20. A T-cell receptor or composition for use in accordance with any one of claims 17 to 19, wherein the T-cell receptor or pharmaceutical composition is formulated to provide a dosage of at least 10^8 T-cells comprising the T-cell receptor.
21. Use of the T-cell receptor of any one of claims 1 to 10, the immune-mobilising monoclonal TCR against cancer in accordance with claims 11 or claim 12 or the composition of claim 16 in the manufacture of a medicament for the treatment or prevention of an EBV+ tumour.
- 15 22. Use in accordance with claim 21, wherein the EBV+ tumour is selected from the group consisting of: nasopharyngeal carcinoma, NKT cell lymphoma, Hodgkin's Lymphoma, post-transplant lymphoproliferative disease, Diffuse large B-cell lymphoma and gastric cancer.
- 20 23. Use in accordance with claim 21 or claim 22, wherein the EBV+ tumour is nasopharyngeal carcinoma, such as undifferentiated nasopharyngeal carcinoma.
24. Use in accordance with any one of claims 21 to 23, wherein the T-cell receptor or composition is formulated to provide a dosage of at least 10^8 T-cells comprising the T-cell receptor.
- 25 25. An *in vitro* method of producing T-cells comprising a T-cell receptor in accordance with any one of claims 1 to 10 comprising: transfecting or transforming a sample of T-cells with a nucleic acid sequence according to claim 13 or a vector according to claim 14.
26. A method of treating or preventing an EBV+ tumour in a subject comprising administering a therapeutically effective amount of:
- a. the T-cell receptors in accordance with claim 1;
 - 30 b. the immune-mobilising monoclonal TCRs against cancer in accordance with claim 11; or
 - c. the composition of claim 16
- to the subject.

27. The method according to claim 26, wherein the EBV+ tumour is selected from the group consisting of: nasopharyngeal carcinoma, NKT cell lymphoma, Hodgkin's Lymphoma, post-transplant lymphoproliferative disease, Diffuse large B-cell lymphoma and gastric cancer.
- 5 28. The method according to claim 26, wherein the EBV+ tumour is nasopharyngeal carcinoma.
29. The method according to claim 26, wherein autologous T-cells comprising the T-cell receptor of claim 1 are administered to the subject.
30. The method according to claim 29, wherein at least 10^8 T-cells are administered.
- 10 31. The method according to claim 26, wherein the administration is by infusion.
32. A T-cell receptor in accordance with claim 1 and as substantially taught herein with reference to the Examples and Figures.
33. An immune-mobilising monoclonal TCR against cancer in accordance with claim 11 and as substantially taught herein with reference to the Examples and Figures.
- 15 34. A composition in accordance with claim 16 and as substantially taught herein with reference to the Examples and Figures.