SUMMARY ~~OF ASPECTS OF THE INVENTION~~

The present inventors have developed a cellular therapy to treat and/or prevent EBV-associated diseases which involves using TCR gene therapy to produce EBV-specific T cells.

The present inventors have assembled a T-cell receptor that is specific for the LMP-2 protein of EBV. They have also constructed a retroviral vector comprising the TCR α and β genes and used this to transduce human T cells. The cells were shown to express LMP2-specific TCR and show functional antigen specific activity.

In one aspect, there is provided a T-cell receptor (TCR) which binds to a peptide from latent membrane protein 2 (LMP-2) from the Epstein Barr Virus (EBV) having the amino acid sequence CLGGLLTMV (SEQ ID No. 1) when presented by a major histocampatability complex (MHC) molecule,

wherein the α chain comprises three complementarity determining regions (CDRs) having the following amino acid sequences:

CDR1α - TSDQSYG (SEQ ID No. 4)

CDR2α - QGSYDEQ (SEQ ID No. 5)

CDR3α - FCAMREGSGSARQLTFGSGTQLTVLPD (SEQ ID No. 2)

and wherein the β chain comprises three complementarity determining regions (CDRs) having the following amino acid sequences:

CDR1β - SSHAT (SEQ ID No. 6)

CDR2β - FNYEAQ (SEQ ID No. 7)

CDR3β - ASSLGPAGIQETQYFGPGTRLLVL (SEQ ID No. 3).

or variants of those sequences having up to three amino acid changes.

Thus, in a first ~~aspect~~example, the present ~~invention~~disclosure provides a T-cell receptor (TCR) specific for the LMP2 protein of Epstein Barr Virus.

The TCR may recognise the epitope CLGGLLTMV (SEQ ID No. 1) from LMP-2.

The TCR may be capable of binding to a peptide having the amino acid sequence CLGGLLTMV (SEQ ID No. 1) when presented by a major histocompatability complex (MHC) molecule.

The α chain and the β chain of the TCR each have three complementarity determining regions (CDRs). The α chain and the β chain of the TCR may have the following CDR3 sequences:

CDR3α - FCAMREGSGSARQLTFGSGTQLTVLPD (SEQ ID No. 2)

CDR3β - ASSLGPAGIQETQYFGPGTRLLVL (SEQ ID No. 3)

or a variant of those sequences having up to three amino acid changes.

The CDRs of the α chain may having the following amino acid sequences:

CDR1α - TSDQSYG (SEQ ID No. 4)

CDR2α - QGSYDEQ (SEQ ID No. 5)

CDR3α - FCAMREGSGSARQLTFGSGTQLTVLPD (SEQ ID No. 2)

or variants of those sequences having up to three amino acid changes.

The CDRs of the β chain may having the following amino acid sequences:

CDR1β - SSHAT (SEQ ID No. 6)

CDR2β - FNYEAQ (SEQ ID No. 7)

CDR3β - ASSLGPAGIQETQYFGPGTRLLVL (SEQ ID No. 3).

or variants of those sequences having up to three amino acid changes.

The TCR of the first ~~aspect~~example of the ~~invention~~disclosure may comprise the amino acid sequence shown as SEQ ID No. 8 or a variant thereof having at least 80% amino acid sequence identity.

The TCR of the first ~~aspect~~example of the ~~invention~~disclosure may comprise one or more mutations at the TCR α chain/β chain interface, such that when the TCR α chain and β chain as defined in any preceding claim are expressed in a T-cell, the frequency of mis-pairing between these chains and the endogenous TCR α chain and β chain is reduced.

For example, in the TCR of the first ~~aspect~~example of the ~~invention~~disclosure, the constant region domains of the α chain and β chain may each comprise an additional cysteine residue, enabling the formation of an extra disulphide bond between the α chain and the β chain.

The second ~~aspect~~example provides nucleotide sequences encoding all or a part of the TCR according to the first ~~aspect~~example of the ~~invention~~disclosure.

~~A first embodiment of the second aspect of the invention relates to~~In one aspect, there is provided a nucleotide sequence encoding the α chain of a TCR according to the ~~first aspect of the invention~~present invention.

The nucleotide sequence ~~of this first embodiment~~ may comprise bases 1-810 of the nucleotide sequence shown as SEQ ID No. 9 or a variant thereof having at least 80% sequence identity.

~~A second embodiment of the second aspect of the invention relates to~~In one aspect, there is provided a nucleotide sequence encoding the β chain of a TCR according to the ~~first aspect of the~~present invention.

The nucleotide sequence ~~of this second embodiment~~ may comprise bases 886-1812 of SEQ ID No. 9 or a variant thereof having at least 80% sequence identity.

~~A third embodiment of the second aspect of the invention relates to~~In one aspect, there is provided a nucleotide sequence encoding a TCR α chain linked to a TCR β chain.

The nucleotide sequence may comprise the TCR α and β genes linked by an internal self-cleaving sequence.

The nucleotide sequence ~~of this third embodiment~~ may comprise the sequence shown as SEQ ID No. 9 or a variant thereof having at least 80% sequence identity.

In one aspect, there is provided a vector comprising a nucleotide sequence as defined above.

In a third ~~aspect~~example, the present ~~invention~~disclosure provides a vector comprising a nucleotide sequence according to the ~~second aspect of the~~ present invention. The vector may, for example, be a retroviral vector.

In one aspect, there is provided a cell which comprises a nucleotide sequence as defined above.

In a fourth ~~aspect~~example, the ~~invention~~disclosure provides a cell which comprises a nucleotide sequence according to the ~~second aspect of the~~ present invention. The cell may, for example be a T-cell or a stem cell. The cell may be derived from a T-cell isolated from a subject.

~~In a fifth aspect the present invention provides~~In one aspect, there is provided a method for producing a cell according to the ~~fourth aspect of the~~present invention which comprises the step of transducing or transfecting a cell *in vitro* or *ex vivo* with a vector according to the ~~third aspect of the~~present invention.

In ~~a sixth aspect~~one example, the present ~~invention~~disclosure provides a method for treating and/or preventing a disease associated with EBV in a subject which comprises the step of adoptive transfer of a EBV-specific T-cell to the subject, wherein the EBV-specific T-cell is made by TCR gene transfer.

The T-cell comprises one or more heterologous nucleotide sequence(s) capable of encoding a EBV-specific TCR.

The TCR may be in accordance with the first ~~aspect~~example of the ~~invention~~disclosure.

In one aspect, there is provided the use of a EBV-specific T-cell as defined above in the manufacture of a medicament for treating and/or preventing EBV positive Hodgkin Lymphoma, EBV positive Nasopharyngeal Carcinoma or EBV positive post transplant lymphoproliferative disorder (PTLD) in a subject , wherein the EBV-specific T-cell is made by TCR gene transfer.

The method may be used to treat or prevent an EBV-associated disease such as EBV positive Hodgkin Lymphoma, EBV positive Nasopharyngeal Carcinoma or EBV positive post transplant lymphoproliferative disorder (PTLD).

~~The present invention also provides~~In one aspect, there is provided a vector according to the ~~third aspect of the~~present invention or a cell according to the ~~fourth aspect of the~~present invention for use in treating and/or preventing a disease associated with EBV in a subject.

~~The present invention also provides~~In one aspect, there is provided a pharmaceutical composition comprising a vector according to the ~~third aspect of the~~present invention or a cell according to the ~~fourth aspect of the~~present invention.

The present ~~invention~~disclosure also provides the use of a TCR according to the first ~~aspect~~example of the ~~invention~~disclosure, a nucleotide sequence according to the second ~~aspect~~example of the ~~invention~~disclosure, a vector according to the third ~~aspect~~example of the ~~invention~~disclosure, or a cell according to the fourth ~~aspect~~example of the ~~invention~~disclosure in the manufacture of a medicament for use in treating and/or preventing a disease associated with EBV in a subject.

DETAILED DESCRIPTION

T-CELL RECEPTOR