

TCR constructs specific for EBV-derived antigens

The present invention relates to the field of immunotherapy, in particular, of Epstein-Barr virus-associated diseases (EBV, also designated Human gammaherpesvirus 4), e.g., cancer or post-transplant lymphoproliferative disease, in particular, to adoptive T cell therapy or T cell receptor (TCR) gene therapy. The invention provides a combination of nucleic acids encoding at least two TCR constructs, or the respective proteins or host cells, wherein each TCR construct is capable of specifically binding to its respective epitope in the context of the respective MHC I, and wherein the epitopes are peptides from different antigens expressed by the same infective agent or cancer, e.g., EBV antigens. The invention also provides specific nucleic acids encoding a TCR alpha chain construct (TRA) and/or a TCR beta chain construct (TRB) of a TCR construct specific for an epitope in complex with a human MHC I, wherein the epitope is an epitope of an Epstein-Barr virus protein, wherein the TCR constructs are specific for epitopes from LMP2A, LMP1 or EBNA3C. Proteins encoded by said nucleic acids, corresponding host cells and pharmaceutical compositions and kits are also objects of the invention.

A TCR is a heterodimeric cell surface protein of the immunoglobulin super-family which is associated with invariant proteins of the CD3 complex involved in mediating signal transduction. TCRs exist in $\alpha\beta$ and $\gamma\delta$ forms, which are structurally similar, but have quite distinct anatomical locations and probably functions. The alpha and beta chains of native heterodimeric $\alpha\beta$ TCR are transmembrane proteins, which each comprises two extracellular domains, a membrane-proximal constant domain, and a membrane-distal variable domain. Each of the constant and variable domains includes an intra-chain disulfide bond.

The variable region of each TCR chain comprises variable and joining segments, and in the case of the beta chain also a diversity segment. Each variable region comprises three CDRs (Complementarity Determining Regions), highly polymorphic loops, embedded in a framework sequence, one being the hypervariable region named CDR3. There are several types of alpha chain variable ($V\alpha$) regions and several types of beta chain variable ($V\beta$) regions distinguished by their framework, CDR1 and CDR2 sequences, and by a partly defined CDR3 sequence. Unique TRAV or TRBV numbers are given to $V\alpha$ or $V\beta$ s by IMGT nomenclature. TCR specificity for the epitopes recognized is mainly determined by the CDR3 regions (Danska et al., 1990; Garcia et al., 2005.).

The use of adoptive TCR gene therapy allows equipping the patients' own T cells with desired specificities and generation of sufficient numbers of activated, non-exhausted T cells in a short period of time. The TCR may be transduced into all T cells or T-cell subsets such as CD8+ T cells, central memory T cells or T cells with stem cell characteristics, which may ensure better persistence and function upon transfer. TCR-engineered T cells may be infused into patients,

e.g., cancer patients that have, e.g., been rendered lymphopenic by chemotherapy or irradiation, inducing homeostatic expansion which greatly enhances engraftment and long term persistence of transferred T cells and is associated with higher cure rates.

TCR-based adoptive T cell therapy relies on classical TCR recognition of processed epitopes of antigens presented in the context of MHC molecules. Thus, a T cell expressing a specific TCR specific for an epitope in the context of a specific MHC can only be used for treatment of a patient expressing the respective MHC.

Epstein-Barr virus (EBV), a human herpesvirus, infects approx. 90% of the world's population. In healthy individuals, diseases caused by EBV are usually cleared by immune cells, wherein T cells play the most important role.

EBV-associated diseases are infectious mononucleosis and various non-malignant, premalignant, and malignant EBV-associated lymphoproliferative diseases such as post transplant lymphoproliferative disorder, Burkitt lymphoma, hemophagocytic lymphohistiocytosis, Hodgkin and non-Hodgkin lymphoma; non-lymphoid malignancies such as gastric cancer, lung cancer, and nasopharyngeal carcinoma; and conditions associated with human immunodeficiency virus such as hairy leukoplakia and central nervous system lymphomas. The virus is also associated with the childhood disorders of Alice in Wonderland Syndrome and acute cerebellar ataxia and, based on some evidence, higher risks of developing certain autoimmune diseases. About 200,000 cancer cases per year are thought to be attributable to EBV (Wikipedia).

Most of the EBV-associated cancers express only a limited number of EBV-specific antigens such as latent membrane proteins (LMP1, LMP2A) and nuclear proteins (EBNA1, EBNA3C). These antigens were shown to be interesting targets for TCR-based immunotherapy, e.g., TCR gene therapy or adoptive T cell therapy of EBV-associated diseases, e.g., post transplant lymphoproliferative disorder or cancer (Orentas et al., 2001; Jurgens et al., 2006; Hart et al., 2008; Simpson et al., 2011; Yang et al., 2011; Zheng et al., 2015; Cho et al., 2018; WO 2015/022520 A1; WO 2011/039508 A2).

However, most T cell-based immunotherapies targeting EBV-associated malignancies have been using natural EBV-specific T cells generated from third party donors or patients, where T cells have been expanded using EBV lymphoblastoid cell lines (LCL) or EBV peptide pools. Adoptive T cell therapies using EBV-specific TCR-engineered T cells have not been tested in clinical trials. TCR-engineered T cells have several advantages compared to natural EBV-specific T cells: 1) Efficacy: The introduced TCR is a pre-defined receptor with high affinity to EBV-positive tumor cells. Growing natural T cells from patient blood relies on the presence of EBV-specific T cells to grow out. However, a patient may lack effective T cells that can be expanded. 2) Feasibility: The success rate of manufacturing engineered T cells is above 95%, while procedures to grow natural T cells have success rates below 70%. 3) Costs: Vein-to-vein time is reduced to less than 21

days in engineered T cell processes compared to more than 40 days for expanding natural T cells.

To execute TCR gene therapy for patient populations with different MHC I (HLA) alleles it is necessary to identify TCRs which are restricted to different HLA alleles. Further, the identification of TCRs which are restricted to different HLA alleles is a pre-requisite for targeting EBV epitopes via two different HLA alleles that are expressed by the same cell.

The present invention addresses some of these problems, and provides novel, and preferably, advantageous immunotherapeutic agents useful for immunotherapy of cancer or infectious agents. This problem is solved by the subject matter of the claims.

Nucleic acids encoding TCR constructs

In one embodiment, the invention provides particular TCR constructs useful for therapy of EBV-associated diseases, and nucleic acids encoding them.

The invention provides a nucleic acid encoding a TCR alpha chain construct (TRA) and/or a TCR beta chain construct (TRB) of a TCR construct specific for an epitope in complex with a human MHC I, wherein the epitope is an epitope of an EBV protein,

- a) wherein the epitope has the sequence of SEQ ID NO: 1, the MHC I is HLA-A*02:01, and the TRA comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 13 and the TRB comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 18;
- b) wherein the epitope has the sequence of SEQ ID NO: 2, the MHC I is HLA-B*57:01, and the TRA comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 23 and the TRB comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 28;
- c) wherein the epitope has the sequence of SEQ ID NO: 3, the MHC I is HLA-C*15:02, and the TRA comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 33 and the TRB comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 38;
- d) wherein the epitope has the sequence of SEQ ID NO: 4, the MHC I is HLA-C*06:02, and the TRA comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 43 and the TRB comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 48;
- e) wherein the epitope has the sequence of SEQ ID NO: 5, the MHC I is HLA-B*44:02, and the TRA comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 53 and the TRB comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 58;
- f) wherein the epitope has the sequence of SEQ ID NO: 5, the MHC I is HLA-B*44:02, and the TRA comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 63 and the TRB comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 68;
- g) wherein the epitope has the sequence of SEQ ID NO: 6, the MHC I is HLA-B*07:02, and the TRA comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 73 and the TRB comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 78; and/or

- h) wherein the epitope has the sequence of SEQ ID NO: 7, the MHC I is HLA-B*07:02, and the TRA comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 83 and the TRB comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 88.

In the context of the present invention, "a" is understood to mean "one or more" unless expressly stated otherwise. Accordingly, for example, if the TCR construct of the invention contains both alpha and beta chain constructs, as preferred throughout the invention, it may be encoded by either one or two nucleic acids. The alpha and beta chain constructs together are capable of specifically binding to an epitope from an EBV protein in complex with a human MHC I. As intermediate products, the alpha and beta chain constructs are also subject matter of the invention by themselves. The invention also provides a single chain nucleic acid construct, wherein, e.g., TCR alpha and beta chain constructs are separated by a P2A element.

One nucleic acid of the invention encodes a TRA and/or a TRB of a TCR construct specific for an epitope in complex with a human MHC I, wherein the epitope has the sequence of SEQ ID NO: 1, the MHC I is HLA-A*02:01, and the TRA comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 13 and the TRB comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 18. SEQ ID NO: 1 is an epitope from the EBV protein LMP2A which is known in the art to be presented by cancer cells of an EBV-associated cancer type in the context of HLA-A2, e.g., HLA-A*02:01, i.e., a very common HLA-type (e.g., Orentas et al., 2001, see above). The TCR construct having the CDR3 sequences disclosed herein has a particularly high affinity or peptide sensitivity, as shown herein, compared to a TCR of the art, and thus is a preferred TCR construct of the invention.

Advantageously, said TCR has a high peptide sensitivity with a half maximum IFN- γ release with a peptide concentration of 10^{-8} mol/L or less, preferably, 10^{-9} mol/L or less. The analysis can be carried out by culturing TCR-engineered T cells with target cells (e.g., K562-HLA-A*02:01 cells loaded with peptide, preferably peptide of SEQ ID NO: 1) at an effector to target cell ratio of 1:1, e.g., as described for Fig. 15 or 16, preferably, for Fig. 15.

Optionally, the TRA comprises a CDR1 having at least 80% sequence identity to SEQ ID NO: 11, a CDR2 having at least 80% sequence identity to SEQ ID NO: 12 and a CDR3 having at least 90%, preferably, 100% sequence identity to SEQ ID NO: 13. Optionally, the TRB comprises a CDR1 having at least 80% sequence identity to SEQ ID NO: 16, a CDR2 having at least 80% sequence identity to SEQ ID NO: 17 and a CDR3 having at least 90%, preferably, 100% sequence identity to SEQ ID NO: 18.

The TRA may comprise the junction amino acids shown in SEQ ID NO: 14. The TRB may comprise the junction amino acids shown in SEQ ID NO: 19.

The TRA may comprise a variable region having at least 90%, optionally, at least 95% or 100% sequence identity to SEQ ID NO: 15. The TRB may comprise a variable region having at least 90%, optionally, at least 95% or 100% sequence identity to SEQ ID NO: 20.

A TCR construct comprising a TRA with a variable region of SEQ ID NO: 15 and a TRB with a variable region of SEQ ID NO: 20 has been shown to have particularly advantageous characteristics herein. It is also designated TCR06.

The variable region of the TRA of said TCR construct may be encoded by a nucleic acid having a sequence of SEQ ID NO: 91, and the variable region of the TRB of said TCR construct may be encoded by a nucleic acid having a sequence of SEQ ID NO: 92.

One nucleic acid of the invention encodes a TRA and/or a TRB of a TCR construct specific for an epitope in complex with a human MHC I, wherein the epitope has the sequence of SEQ ID NO: 2, the MHC I is HLA-B*57:01, and the TRA comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 23 and the TRB comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 28. SEQ ID NO: 2 is an epitope from the EBV protein LMP1 which is presented by cancer cells of an EBV-associated cancer type in the context of HLA-B*57:01. The TCR construct having the CDR3 sequences disclosed herein has a high affinity or peptide sensitivity, as shown herein.

Optionally, said TRA comprises a CDR1 having at least 80% sequence identity to SEQ ID NO: 21, a CDR2 having at least 80% sequence identity to SEQ ID NO: 22 and a CDR3 having at least 90%, preferably, 100% sequence identity to SEQ ID NO: 23. Optionally, said TRB comprises a CDR1 having at least 80% sequence identity to SEQ ID NO: 26, a CDR2 having at least 80% sequence identity to SEQ ID NO: 27 and a CDR3 having at least 90%, preferably, 100% sequence identity to SEQ ID NO: 28.

The TRA may comprise the junction amino acids shown in SEQ ID NO: 24. The TRB may comprise the junction amino acids shown in SEQ ID NO: 29.

The TRA may comprise a variable region having at least 90%, optionally, at least 95% or 100% sequence identity to SEQ ID NO: 25. The TRB may comprise a variable region having at least 90%, optionally, at least 95% or 100% sequence identity to SEQ ID NO: 30.

A TCR construct comprising a TRA with a variable region of SEQ ID NO: 25 and a TRB with a variable region of SEQ ID NO: 30 has been shown to have advantageous characteristics herein. It is also designated TCR50.

The variable region of the TRA of said TCR construct may be encoded by a nucleic acid having a sequence of SEQ ID NO: 93, and the variable region of the TRB of said TCR construct may be encoded by a nucleic acid having a sequence of SEQ ID NO: 94.

One nucleic acid of the invention encodes a TRA and/or a TRB of a TCR construct specific for an epitope in complex with a human MHC I, wherein the epitope has the sequence of SEQ ID NO: 3, the MHC I is HLA-C*15:02, and the TRA comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 33 and the TRB comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 38. SEQ ID NO: 3 is an epitope from the EBV protein LMP1 which is presently, for the first time, shown to be presented by cancer cells of an EBV-associated cancer type. It is presented in the context of HLA-C*15:02. The TCR construct having the CDR3 sequences disclosed herein has a high affinity or peptide sensitivity, as shown herein.

Optionally, said TRA comprises a CDR1 having at least 80% sequence identity to SEQ ID NO: 31, a CDR2 having at least 80% sequence identity to SEQ ID NO: 32 and a CDR3 having at least 90%, preferably, 100% sequence identity to SEQ ID NO: 33. Optionally, said TRB comprises a CDR1 having at least 80% sequence identity to SEQ ID NO: 36, a CDR2 having at least 80% sequence identity to SEQ ID NO: 37 and a CDR3 having at least 90%, preferably, 100% sequence identity to SEQ ID NO: 38.

The TRA may comprise the junction amino acids shown in SEQ ID NO: 34. The TRB may comprise the junction amino acids shown in SEQ ID NO: 39.

The TRA may comprise a variable region having at least 90%, optionally, at least 95% or 100% sequence identity to SEQ ID NO: 35. The TRB may comprise a variable region having at least 90%, optionally, at least 95% or 100% sequence identity to SEQ ID NO: 40.

A TCR construct comprising a TRA with a variable region of SEQ ID NO: 35 and a TRB with a variable region of SEQ ID NO: 40 has been shown to have advantageous characteristics herein. It is also designated TCR83.

The variable region of the TRA of said TCR construct may be encoded by a nucleic acid having a sequence of SEQ ID NO: 95, and the variable region of the TRB of said TCR construct may be encoded by a nucleic acid having a sequence of SEQ ID NO: 96.

One nucleic acid of the invention encodes a TRA and/or a TRB of a TCR construct specific for an epitope in complex with a human MHC I, wherein the epitope has the sequence of SEQ ID NO: 4, the MHC I is HLA-C*06:02, and the TRA comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 43 and the TRB comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 48. SEQ ID NO: 4 is an epitope from the EBV protein EBNA3C which is presently, for the first time, shown to be presented by cancer cells of an EBV-associated cancer type. It is presented in the context of HLA-C*06:02.

Optionally, said TRA comprises a CDR1 having at least 80% sequence identity to SEQ ID NO: 41, a CDR2 having at least 80% sequence identity to SEQ ID NO: 42 and a CDR3 having at least 90%,

preferably, 100% sequence identity to SEQ ID NO: 43. Optionally, said TRB comprises a CDR1 having at least 80% sequence identity to SEQ ID NO: 46, a CDR2 having at least 80% sequence identity to SEQ ID NO: 47 and a CDR3 having at least 90%, preferably, 100% sequence identity to SEQ ID NO: 48.

The TRA may comprise the junction amino acids shown in SEQ ID NO: 44. The TRB may comprise the junction amino acids shown in SEQ ID NO: 49.

Preferably, the TRA comprises a variable region having at least 90%, optionally, at least 95% or 100% sequence identity to SEQ ID NO: 45. Preferably, the TRB comprises a variable region having at least 90%, optionally, at least 95% or 100% sequence identity to SEQ ID NO: 50.

A TCR construct comprising a TRA with a variable region of SEQ ID NO: 45 and a TRB with a variable region of SEQ ID NO: 50 has been shown to have advantageous characteristics herein. It is also designated TCR64.

The variable region of the TRA of said TCR construct may be encoded by a nucleic acid having a sequence of SEQ ID NO: 97, and the variable region of the TRB of said TCR construct may be encoded by a nucleic acid having a sequence of SEQ ID NO: 98.

One nucleic acid of the invention encodes a TRA and/or a TRB of a TCR construct specific for an epitope in complex with a human MHC I, wherein the epitope has the sequence of SEQ ID NO: 5, the MHC I is HLA-B*44:02, and the TRA comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 53 and the TRB comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 58. SEQ ID NO: 5 is an epitope from the EBV protein EBNA3C which is presently, for the first time, shown to be presented by cancer cells of an EBV-associated cancer type. It is presented in the context of HLA-B*44:02.

Optionally, said TRA comprises a CDR1 having at least 80% sequence identity to SEQ ID NO: 51, a CDR2 having at least 80% sequence identity to SEQ ID NO: 52 and a CDR3 having at least 90%, preferably, 100% sequence identity to SEQ ID NO: 53. Optionally, said TRB comprises a CDR1 having at least 80% sequence identity to SEQ ID NO: 56, a CDR2 having at least 80% sequence identity to SEQ ID NO: 57 and a CDR3 having at least 90%, preferably, 100% sequence identity to SEQ ID NO: 58.

The TRA may comprise the junction amino acids shown in SEQ ID NO: 54. The TRB may comprise the junction amino acids shown in SEQ ID NO: 59.

Preferably, the TRA comprises a variable region having at least 90%, optionally, at least 95% or 100% sequence identity to SEQ ID NO: 55. Preferably, the TRB comprises a variable region having at least 90%, optionally, at least 95% or 100% sequence identity to SEQ ID NO: 60.

A TCR construct comprising a TRA with a variable region of SEQ ID NO: 55 and a TRB with a variable region of SEQ ID NO: 60 has been shown to have advantageous characteristics herein. It is also designated TCR25.

The variable region of the TRA of said TCR construct may be encoded by a nucleic acid having a sequence of SEQ ID NO: 99, and the variable region of the TRB of said TCR construct may be encoded by a nucleic acid having a sequence of SEQ ID NO: 100.

One nucleic acid of the invention encodes a TRA and/or a TRB of a TCR construct specific for an epitope in complex with a human MHC I, wherein the epitope has the sequence of SEQ ID NO: 5, the MHC I is HLA-B*44:02, and the TRA comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 63 and the TRB comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 68. As mentioned above, SEQ ID NO: 5 is an epitope from the EBV protein EBNA3C which is presently, for the first time, shown to be presented by cancer cells of an EBV-associated cancer type. It is presented in the context of HLA-B*44:02.

Optionally, said TRA comprises a CDR1 having at least 80% sequence identity to SEQ ID NO: 61, a CDR2 having at least 80% sequence identity to SEQ ID NO: 62 and a CDR3 having at least 90%, preferably, 100% sequence identity to SEQ ID NO: 63. Optionally, said TRB comprises a CDR1 having at least 80% sequence identity to SEQ ID NO: 66, a CDR2 having at least 80% sequence identity to SEQ ID NO: 67 and a CDR3 having at least 90%, preferably, 100% sequence identity to SEQ ID NO: 68.

The TRA may comprise the junction amino acids shown in SEQ ID NO: 64. The TRB may comprise the junction amino acids shown in SEQ ID NO: 69.

Preferably, the TRA comprises a variable region having at least 90%, optionally, at least 95% or 100% sequence identity to SEQ ID NO: 65. Preferably, the TRB comprises a variable region having at least 90%, optionally, at least 95% or 100% sequence identity to SEQ ID NO: 70.

A TCR construct comprising a TRA with a variable region of SEQ ID NO: 65 and a TRB with a variable region of SEQ ID NO: 70 has been shown to have advantageous characteristics herein. It is also designated TCR58.

The variable region of the TRA of said TCR construct may be encoded by a nucleic acid having a sequence of SEQ ID NO: 101, and the variable region of the TRB of said TCR construct may be encoded by a nucleic acid having a sequence of SEQ ID NO: 102.

One nucleic acid of the invention encodes a TRA and/or a TRB of a TCR construct specific for an epitope in complex with a human MHC I, wherein the epitope has the sequence of SEQ ID NO: 6, the MHC I is HLA-B*07:02, and the TRA comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 73 and the TRB comprises a CDR3 having at least 90% sequence identity

to SEQ ID NO: 78. SEQ ID NO: 6 is an epitope from the EBV protein EBNA3C which is presented by cancer cells of an EBV-associated cancer type in the context of HLA-B*07:02.

Optionally, said TRA comprises a CDR1 having at least 80% sequence identity to SEQ ID NO: 71, a CDR2 having at least 80% sequence identity to SEQ ID NO: 72 and a CDR3 having at least 90%, preferably, 100% sequence identity to SEQ ID NO: 73. Optionally, said TRB comprises a CDR1 having at least 80% sequence identity to SEQ ID NO: 76, a CDR2 having at least 80% sequence identity to SEQ ID NO: 77 and a CDR3 having at least 90%, preferably, 100% sequence identity to SEQ ID NO: 78.

The TRA may comprise the junction amino acids shown in SEQ ID NO: 74. The TRB may comprise the junction amino acids shown in SEQ ID NO: 79.

Preferably, the TRA comprises a variable region having at least 90%, optionally, at least 95% or 100% sequence identity to SEQ ID NO: 75. Preferably, the TRB comprises a variable region having at least 90%, optionally, at least 95% or 100% sequence identity to SEQ ID NO: 80.

A TCR construct comprising a TRA with a variable region of SEQ ID NO: 75 and a TRB with a variable region of SEQ ID NO: 80 has been shown to have advantageous characteristics herein. It is also designated TCR27.

The variable region of the TRA of said TCR construct may be encoded by a nucleic acid having a sequence of SEQ ID NO: 103, and the variable region of the TRB of said TCR construct may be encoded by a nucleic acid having a sequence of SEQ ID NO: 104.

One nucleic acid of the invention encodes a TRA and/or a TRB of a TCR construct specific for an epitope in complex with a human MHC I, wherein the epitope has the sequence of SEQ ID NO: 7, the MHC I is HLA-B*07:02, and the TRA comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 83 and the TRB comprises a CDR3 having at least 90% sequence identity to SEQ ID NO: 88. SEQ ID NO: 7 is an epitope from the EBV protein EBNA3C, and it comprises SEQ ID NO: 6 and a C-terminal T.

Optionally, said TRA comprises a CDR1 having at least 80% sequence identity to SEQ ID NO: 81, a CDR2 having at least 80% sequence identity to SEQ ID NO: 82 and a CDR3 having at least 90%, preferably, 100% sequence identity to SEQ ID NO: 83. Optionally, said TRB comprises a CDR1 having at least 80% sequence identity to SEQ ID NO: 86, a CDR2 having at least 80% sequence identity to SEQ ID NO: 87 and a CDR3 having at least 90%, preferably, 100% sequence identity to SEQ ID NO: 88.

The TRA may comprise the junction amino acids shown in SEQ ID NO: 84. The TRB may comprise the junction amino acids shown in SEQ ID NO: 89.

Preferably, the TRA comprises a variable region having at least 90%, optionally, at least 95% or 100% sequence identity to SEQ ID NO: 85. Preferably, the TRB comprises a variable region having at least 90%, optionally, at least 95% or 100% sequence identity to SEQ ID NO: 90.

A TCR construct comprising a TRA with a variable region of SEQ ID NO: 85 and a TRB with a variable region of SEQ ID NO: 90 has been shown to have advantageous characteristics herein. It is also designated TCR01.

The variable region of the TRA of said TCR construct may be encoded by a nucleic acid having a sequence of SEQ ID NO: 105, and the variable region of the TRB of said TCR construct may be encoded by a nucleic acid having a sequence of SEQ ID NO: 106.

In any of the TRA and/or TRB constructs of the invention, independently, the CDR1 and CDR3 may have at least 80%, at least 90% or 100% sequence identity to the recited sequences. Preferably, the CDR3 has 100% sequence identity to the recited defined CDR3. Typically, if the sequence is not identical, there is at most one amino acid exchange, deletion or insertion, typically, an amino acid exchange. The exchange may be a conserved substitution, i.e., one amino acid of a particular type (e.g., polar, apolar, acidic, basic, aromatic) is exchanged against another amino acid of the same type. Methods for affinity maturation are known in the art and further described below.

Optionally, the sequence identity to the recited CDR1 and CDR2 and CDR3 regions is 100% in the TRA or TRB, preferably, in both TRA and TRB of the TCR constructs of the invention.

A TCR alpha and/or beta chain construct of the invention may comprise all characteristics or domains corresponding to its native counterpart, i.e., a TCR alpha or beta chain, but this is not essential. Preferably, the TCR alpha and/or beta chain construct comprises at least a variable region, or a variable and a constant region, e.g., the variable and/or constant region having at least 60%, at least 70%, at least 80%, at least 90% or at least 95% sequence identity to a human variable or constant TCR region.

The TCR alpha chain constructs and/or the TCR beta chain constructs of the invention preferably comprise a constant region. For adoptive TCR therapy, it is preferred that the TCR construct comprises full length TCR alpha and beta chains comprising variable and constant regions, including transmembrane regions. The constant region may be a human constant region, a murine constant region or a chimeric constant region such as a minimally murine constant region. The TCR construct may be of essentially or exclusively human origin to minimize immunogenicity. To prevent pairing with endogenous TCR chains, the constructs of the invention however preferably contain one or more, e.g., 1-5, 1-10 or 1-20, preferably, 9 amino acid exchanges in comparison to a human sequence (Sommermeyer and Uckert, 2010). To this end, the constant region of the TCR alpha and beta chain construct may also be a murine constant

region (Cohen et al., 2006) or/and additional cysteines are provided to enable the formation of an additional disulfide bond between the TCR chains (Cohen et al., 2007, Kuball et al., 2007). In addition, codon modification of the TCR sequence may be used to enhance the functional expression of the transgenic TCR (Scholten et al., 2006) and/or a peptide (e.g. P2A) can be applied to link both TCR chains to achieve a stoichiometric expression of both chains (Leisegang et al., 2008), also resulting in an enhanced functional expression of the transgenic TCR.

The construct may also be a chimeric antigen receptor, or part of it, wherein, e.g. a human TCR variable region may be linked to a different immunoglobulin constant domain, e.g. an IgG constant domain, or to an antibody domain capable of specifically binding to an antigen such as LMP2A.

Single chain constructs (scTCR) are encompassed as well as heterodimeric TCR constructs. A scTCR can comprise a variable region of a first TCR chain construct (e.g., an alpha chain) and an entire (full-length) second TCR chain (e.g., a beta chain), or vice versa. Furthermore, the scTCR can optionally comprise one or more linkers which join the two or more polypeptides together. The linker can be, for instance, a peptide which joins together two single chains. Also provided is such a scTCR of the invention, which is fused to a cytokine, e.g., a human cytokine, such as IL-2, IL-7, IL-12 or IL-15.

To enable specific recognition of the epitope in the context of the MHC, in particular, for therapeutic purposes, the nucleic acid of the invention encodes one TCR alpha and one beta chain construct of a TCR construct of the invention, e.g., the TCR construct specific for the epitope of SEQ ID NO: 1, as described herein.

Typically, the nucleic acid sequences provided by the invention are codon-optimized for expression in human cells.

In the context of the invention, the nucleic acid can either be DNA or RNA. Preferably, it is DNA. The nucleic acid may, e.g., be a viral vector, or a non-viral vector, e.g., a transposon, a vector suitable for CRISPR/CAS based recombination or a plasmid suitable for *in vitro* RNA transcription. The nucleic acid of the invention preferably is a vector. Suitable vectors include those designed for propagation and expansion, or for expression or both, such as plasmids and viruses. The vector may be an expression vector suitable for expression in a host cell selected from the group comprising a human T cell or a human T cell precursor, preferably, a human T cell such as CD8⁺ T cell. Said CD8⁺ cell may be a central-memory T cell, effector-memory T cell, stem cell-like T cell or effector T cell, or a mixture of these. The vector may be a viral vector, e.g. a retroviral, in particular gamma-retroviral or lentiviral vector. Examples of suitable expression vectors include the retroviral vector MP71 (Engels et al., 2003). The expression vector comprises regulatory sequences, such as transcription and translation initiation and termination codons, which are specific to the type of host cell (e.g., bacterium, fungus, plant, or animal) into which the

vector is to be introduced and in which the expression of the nucleic acid of the invention shall be performed, typically, in the context of the invention, human CD8+ T cells. Furthermore, the vector of the invention may include one or more marker genes, which allow for selection of transduced or transfected hosts. The recombinant expression vector can comprise a native or, preferably, a heterologous promoter operably linked to the nucleotide sequence encoding the TCR construct of the invention, or to a nucleotide sequence which is complementary to or which hybridizes to the nucleotide sequence encoding the constructs of the invention. The selection of promoters includes, e.g., strong, weak, inducible, tissue-specific and developmental-specific promoters. The promoter can be a non-viral promoter or a viral promoter. Preferably, it is a heterologous promoter, i.e., a promoter not naturally linked to TCR in human T cells, such as long terminal repeat promoter, which is suitable for expression in human T cells. The inventive recombinant expression vectors can be designed for either transient expression, for stable expression, or for both. Also, the recombinant expression vectors can be made for constitutive expression or for inducible expression.

Proteins

The present invention also provides a protein, i.e., an alpha or beta chain construct, or, preferably, a TCR construct comprising both alpha and beta chain constructs capable of specifically binding an epitope from an EBV-protein described above in the context of the respective MHC I, as described herein, e.g., the TCR construct specific for the epitope of SEQ ID NO: 1, as described herein. The protein is encoded by a nucleic acid of the invention.

The terms "capable of specifically binding" or "recognizing" or "specific for" a given antigen, as used herein, are synonymous, and mean that the TCR construct can specifically bind to and immunologically recognize said epitope, preferably from an EBV protein, more preferably with high affinity. For example, a TCR may be considered to have "be able of specifically binding" to a peptide from an EBV protein if T cells expressing the TCR secrete at least about 200 pg/mL or more (e.g. 250 pg/mL or more, 300 pg/mL or more, 400 pg/mL or more, 500 pg/mL or more, 600 pg/mL or more, 700 pg/mL or more, 1000 pg/mL or more, 2,000 pg/mL or more, 2,500 pg/mL or more, 5,000 pg/mL or more) of interferon γ (IFN- γ) upon co-culture with target cells pulsed with a low concentration of the respective epitope, e.g., about 10^{-11} mol/L, 10^{-10} mol/L, 10^{-9} mol/L, 10^{-8} mol/L, 10^{-7} mol/L, 10^{-6} mol/L, 10^{-5} mol/L, but not without epitope or with an unrelated control peptide epitope. Preferably, this is tested with 10,000 TCR+CD8+ T cells and 20,000-50,000, preferably, 50,000 target cells expressing an appropriate HLA, e.g., with an assay as described below in the examples. Alternatively, or additionally, a TCR may be considered to have "antigenic specificity" for an epitope if T cells expressing the TCR secrete at least twice as much IFN- γ as the untransduced background level of IFN- γ upon co-culture with target cells pulsed with a low concentration of the appropriate peptide. Such "specificity" as described above can – for example – be analyzed with an ELISA.

A high affinity is correlated with a high peptide sensitivity, as described above for the TCR of the invention, e.g., with a half maximum IFN- γ release with a peptide concentration of 10^{-6} mol/L or less, preferably, 10^{-7} mol/L or less, 10^{-8} mol/L or less or 10^{-9} mol/L or less. Alternatively, affinity can be analyzed by methods well known to the skilled person, e.g. by BiaCore. A TCR affinity or T cell avidity of 100 μ M or higher, more preferably 10 μ M or higher is considered high affinity.

Based on the defined CDR3 and variable region sequences provided by the invention, it is possible to carry out affinity maturation of the TCR sequences (Chervin et al., 2008; Robbins et al., 2008). Non-synonymous nucleotide substitutions, which lead to amino acid exchanges in the CDR3 sequence, may lead to enhanced affinity of the TCR to target antigen. Furthermore, TCR sequence changes in other parts of the variable TRA and TRB regions may change affinity of the TCR to the peptide-MHC I complex. This may increase overall affinity of the TCR to the peptide-MHC, but harbors the risk of unspecific recognition and increased cross-reactivity (Linette et al., 2013). It is preferred that TCRs varying from the specific sequences provided retain exclusive specificity for the target antigen provided, i.e., that they are not cross-reactive, most importantly, that they do not have cross-reactivity for human self-peptides. Potential cross-reactivity of TCR can be tested against known self-peptides loaded on cells with the correct MHC allele (Morgan et al., 2013). Accordingly, it is preferred that adoptive transfer of T cells expressing the TCR construct of the invention has no or significant negative effects on healthy tissue.

The TCR construct according to the invention can also be provided in the form of a multimeric complex, comprising at least two scTCR molecules, wherein said scTCR molecules are each fused to at least one biotin moiety, and wherein said scTCRs are interconnected by biotin-streptavidin interaction to allow the formation of said multimeric complex. Also provided are multimeric complexes of a higher order, comprising more than two, e.g., four, scTCR of the invention.

The TCR construct of the invention can be modified to comprise a detectable label, such as, for instance, a radioisotope, a fluorophore (e.g., fluorescein isothiocyanate (FITC), phycoerythrin (PE)), a tag, such as a HIS-tag, an enzyme (e.g., alkaline phosphatase, horseradish peroxidase), or particles (e.g., gold particles or magnetic particles).

Host cells

The invention also provides a host cell comprising a nucleic acid and/or protein of the invention. The host cell can be a eukaryotic cell, e.g., plant, animal, fungi, or algae, or can be a prokaryotic cell, e.g., bacteria or protozoa. The host cell can be a cultured cell or a primary cell, i.e., isolated directly from an organism, e.g., a human. The host cell can be an adherent cell or a suspended cell, i.e., a cell that grows in suspension. For purposes of producing a recombinant TCR, polypeptide, or protein, the host cell is preferably a mammalian cell. Most preferably, the host cell is a human cell. While the host cell can be of any cell type, can originate from any type of tissue,

and can be of any developmental stage, the host cell preferably is a peripheral blood leukocyte (PBL) or a peripheral blood mononuclear cell (PBMC). More preferably, the host cell is a T cell or T cell precursor, in particular, a human T cell, which may be isolated from PBMC. The T cell can be any T cell, such as a cultured T cell, e.g. a primary T cell, or a T cell from a cultured T cell line, or a T cell obtained from a mammal, preferably, it is a T cell or T cell precursor from a human patient. The T cell can be obtained from numerous sources, such as blood, bone marrow, lymph node, the thymus, or other tissues or fluids. T cells can also be enriched for or purified. It can be, e.g., a tumor infiltrating cell (TIL), effector cell, central effector cell, memory T cell, naive T cell, and the like, preferably a central-memory T cell. Alternatively, the host cell may be another immune effector cell, e.g., an NK cell or a macrophage.

Preferably, in particular, in the context of therapy of a human, the T cell is a human T cell. The T cell preferably is a CD8+ cell (e.g., cytotoxic T cell). Preferably, the T cell is a T cell isolated from a human, e.g., a human patient, in particular, the patient who is to be treated. Alternatively, the T cell may be from a third-party donor who may be related or unrelated to the patient. Such T cells may be genetically engineered, e.g., in multiple ways. For example, by knock out of the endogenous TCR and/or MHC I. T cells may also be generated from autologous or third-party donor stem cells, wherein, optionally, the stem cells are not human embryonic stem cells.

Preferably, the host cell is a human CD8+ T cell comprising a nucleic acid of the invention which is an expression vector, wherein the nucleic acid encoding the TRA and/or TRB is operably linked to a heterologous promotor, wherein the host cell expresses a TCR construct of the invention.

The invention also provides a host cell expressing two or more different TCR constructs, e.g., two TCR constructs of the invention. Preferably, in this context, the TCR constructs are single chain TCR constructs in which the TCR alpha and beta chains of each TCR are combined by a linker to avoid mispairing between transgenic TCR chains. Said two single chain TCR constructs may be encoded on a single expression vector.

Pharmaceutical compositions

The invention also provides a pharmaceutical composition comprising

- a) a nucleic acid of the invention encoding a TCR construct capable of specifically binding to its respective epitope in the context of the respective MHC I; or
- b) a protein of the invention comprising a TCR construct capable of specifically binding to its respective epitope in the context of the respective MHC I (i.e., a TCR construct of the invention); or
- c) a host cell of the invention expressing a TCR construct capable of specifically binding to its respective epitope in the context of the respective MHC I.

Preferably, the TCR construct is the TCR construct specific for the epitope of SEQ ID NO: 1.

In another embodiment, the present invention provides a pharmaceutical composition, or a kit comprising at least two pharmaceutical compositions, comprising

- a) at least two nucleic acids, each encoding a TCR construct capable of specifically binding to its respective epitope in the context of the respective MHC I; or
- b) at least two proteins, each comprising a TCR construct capable of specifically binding to its respective epitope in the context of the respective MHC I; or
- c) at least two host cells, each expressing a TCR construct capable of specifically binding to its respective epitope in the context of the respective MHC I,

wherein the epitopes are peptides from different antigens expressed by the same cancer or infectious agent. Typically, immunotherapy will be carried out for treatment of a cancer, so the different antigens are preferably antigens expressed by a cancer, i.e., by cells of the same cancer. In this context, antigens are proteins comprising an epitope which may be presented on an MHC I. It is possible that different cancer cells of the same cancer type each express at least one antigen, and the TCR constructs are thus directed to different cancer cells of the same cancer, which may be useful, e.g., if the stage of the cancer is not known and/or if cells of different stages which express different antigens are to be treated. However, generally, it is beneficial if the different antigens are expressed by the same cancer cells.

The inventors have surprisingly found that it is advantageous to use a combination of at least two TCR constructs expressed by T cells for adoptive T cell therapy, in particular, two or more T cells each expressing a TCR construct capable of specifically binding to its respective epitope in the context of the respective MHC I.

Without intending to be bound by the theory, it is believed that attacking the cancer (or infectious agent) based on its expression of two or more antigens can help to sustain the attack and avoid immune evasion, e.g., through mutations, downregulation of one target antigen or antigen loss variants.

It is also possible to target a cancer or infectious agent with three or more TCR constructs, in particular, three or more host cells each comprising a nucleic acid encoding a TCR construct capable of specifically binding to its respective epitope in the context of the respective MHC I, wherein the epitopes are peptides from different antigens expressed by the same cancer or infectious agent.

The kit or pharmaceutical composition is for use in treatment of the cancer or infectious agent from which the epitopes targeted by the TCR constructs are derived.

The infection or cancer may be associated, e.g., with EBV. In this case, the different EBV antigens may be LMP2A, LMP1, EBNA1 or EBNA3C. Preferably, one of the EBV antigens targeted by one TCR construct of the invention is LMP2A. A second EPV antigen targeted by one TCR construct of the invention may be LMP1 or EBN3C, preferably, LMP1. If three EBV antigens are to be targeted, the antigens may be LMP2A, LMP1 and EBNA3C. An alternative or additional EBV antigen is EBNA1.

The present invention also provides a pharmaceutical composition a kit as mentioned above, comprising

- a) at least two nucleic acids of the invention, each encoding a TCR construct capable of specifically binding to its respective epitope in the context of the respective MHC I; or
- b) at least two proteins of the invention, each comprising a TCR construct capable of specifically binding to its respective epitope in the context of the respective MHC I; or
- c) at least two host cells of the invention, each expressing a TCR construct capable of specifically binding to its respective epitope in the context of the respective MHC I;

wherein, optionally, the epitopes are derived from different EBV proteins.

One or two or three TCR constructs used in the context of a pharmaceutical composition or kit of the invention may be TCR constructs of the present invention, as disclosed herein. Preferably, one of the TCR constructs used is the TCR construct disclosed herein recognizing the epitope of SEQ ID NO: 1 in HLA-A2 context.

Accordingly, the kit or pharmaceutical composition may comprise human CD8+ T cells comprising a nucleic acid encoding a TCR construct of the invention, e.g., recognizing the epitope of SEQ ID NO: 1 in HLA-A2 context, as defined herein. The TCR construct may be expressed from the nucleic acid under the control of a heterologous promotor.

In the kit or pharmaceutical composition of the invention comprising at least two nucleic acids encoding TCR constructs, at least two TCR constructs or at least two host cells each comprising a nucleic acid encoding a TCR construct, one, two or three of said TCR constructs may also be other TCR constructs, e.g., TCR constructs known in the art. For example, if the cancer is EBV-associated, one, two or three of the TCR constructs disclosed by Cho et al., 2018; Jurgens et al., 2006; Zheng et al., 2015; Simpson et al., 2011; Yang et al., 2011; Orentase et al., 2001; Hart et al., 2008; WO 2015/022520 A1; or WO 2011/039508 may be used, optionally, in combination with one of the TCR constructs provided herein.

Adoptive T cell therapy (option c) is preferred throughout the present application, wherein the host cell is a T cell, preferably, a human CD8+ T cell. Said host cell typically comprises a nucleic acid encoding the TCR construct under the control of a heterologous promotor.

However, gene therapy with a nucleic acid of the invention (option a) is also possible, wherein, e.g., a lentiviral vector may be used.

A TCR construct of the invention in protein form (option b) may also be used in therapy, e.g., for targeting a toxin to which it is linked to a cancer, for targeting a bacterial minicell to a cancer, which may comprise a therapeutic agent such as a toxin. A TCR construct of the invention in protein form may also be used for targeting a diagnostic agent to a cancer. The composition may thus also be a diagnostic composition.

The T cells each expressing its respective TCR construct may be contained in a kit, wherein, separately, each T cell is stored, e.g., in a pharmaceutically acceptable buffer. The components of a kit of the invention may be formulated for simultaneous administration or for administration in any sequence. The components may also be for repeated administration. Tran et al., 2014 describe a possible regimen of administration. Alternatively, they may be mixed before administration and administered together. Alternatively, the T cells each expressing its respective TCR construct may be contained in a single pharmaceutical composition. The same applies for nucleic acids or proteins of the invention.

The pharmaceutical compositions or kits of the invention typically are for intravenous administration. They may further comprise pharmaceutically acceptable carriers such as buffers, e.g., physiological saline or PBS. They may further comprise excipients, such as stabilizers such as SPGA, carbohydrates (e.g. sorbitol, mannitol, starch, sucrose, glucose, dextran), proteins such as albumin or casein, or protein containing agents such as bovine serum or skimmed milk.

T cells are typically administered to a patient in a concentration of 1×10^5 - 1×10^9 cells per kg. About 1×10^6 - 1×10^{11} cells may be administered to a patient as a single dose. These parameters may be adapted by the medical practitioner, depending, e.g., on the patient's age, sex, body weight and medical condition. Protocols disclosed, e.g., in Doran et al., 2019 may be adapted to use the host cells of the present invention.

The pharmaceutical composition of the invention or a kit comprising a pharmaceutical composition or a kit of the invention may be for use in the treatment of a patient expressing an MHC in the context of which the respective TCR recognizes the respective epitope, e.g., as disclosed herein. Average HLA (MHC I) distribution in different populations can be found, e.g., under <http://allelefrequencies.net/>. It is advantageous to test before treatment if a patient expresses the respective MHC I.

The patient may have a cancer or an infectious disease, in particular in context with the specific TCR constructs disclosed herein, an EBV-associated disease, e.g., selected from the group comprising Hodgkin's and non-Hodgkin lymphoma, Burkitt lymphoma, hemophagocytic lymphohistiocytosis, nasopharyngeal carcinoma, head and neck cancer, gastric cancer, lung cancer hairy leukoplakia, post-transplant lymphoproliferative disorder and central nervous system lymphoma. Preferably, the EBV-associated cancer is a type II malignancy, as exemplified by Hodgkin lymphoma and nasopharyngeal carcinoma, or a type III malignancy, as exemplified by hairy leukoplakia, post transplant lymphoproliferative disorder and central nervous system lymphoma (Orentas et al., 2001), as these cancers typically express high levels of LMP2A and LMP1. In type III malignancies, EBNA2C is additionally expressed.

Targeted cancer cells express the protein or, optionally, the proteins, from which the recognized epitope is derived, preferably, most of the cancer cells.

The patient typically is a mammalian patient. The patient may be a mouse, but, preferably, the patient is a human patient.

The invention also discloses a method of treating cancer or an infectious disease, e.g., an EBV-associated disease, preferably, an EBV-associated cancer, by administering an effective amount of a pharmaceutical composition or kit of the present invention to a patient in need thereof, e.g., a patient having said cancer or disease.

Pharmaceutical compositions and kits of the present invention may be used in combination with other agents, in particular, with other anti-cancer agents. For example, other anti-cancer agents may be TCR-engineered T cells expressing TCRs specific for other antigens expressed in the EBV-positive tumor (e.g. MAGE, NY-ESO, o. a.), checkpoint inhibitors or other immunotherapies as well as antibodies, small molecule inhibitors or other types of reagents.

One preferred medicinal use of the invention is immune therapy, preferably adoptive T cell therapy. The product and methods of the invention are particularly useful in the context of adoptive T cell therapy. The administration of the compounds of the invention can for example involve the administration, e.g., infusion of T cells of the invention into said patient. Preferably such T cells are autologous T cells of the patient which were *in vitro* transduced with a nucleic acid of the present invention.

Alternatively, the patient may also be administered a nucleic acid of the invention, in particular, an expression vector, for *in vivo* transduction of T cells.

Protein TCR constructs of the invention may also, e.g., be used for diagnostic purposes to find out if a subject expresses the respective protein, and, in particular, if the epitope recognized by the TCR construct in the context of the MHC I is presented. To this end, such constructs are

preferably labelled to facilitate detection. Preferably, a patient presenting such an epitope on the respective MHC I is treated by an adoptive T cell therapy of the invention.

The invention also relates to a method of preparing a host cell of the invention, comprising introducing an expression vector encoding a TCR construct of the invention into a suitable host cell, preferably, a human CD8+ T cell isolated from a patient.

The present invention is further illustrated in the following examples with reference to the accompanying figures and sequences, nevertheless, without being limited thereto. For the purposes of the present invention, all references as cited herein are incorporated by reference in their entirety.

Vaccines

In one embodiment, the invention provides a pharmaceutical composition, in particular, a vaccine composition, comprising a peptide comprising an epitope capable of being presented by a human MHC I, wherein the epitope is newly identified herein, or a nucleic acid (such as RNA) encoding such a peptide. The epitope may

- a) have at least 88% sequence identity to SEQ ID NO: 3, wherein the epitope is capable of being presented on HLA-C*15:02, and wherein the peptide comprises at most 25, preferably, at most 11 consecutive amino acids identical to the sequence of amino acids occurring in LMP1 of SEQ ID NO: 120;
- b) have at at least 88% sequence identity to SEQ ID NO: 4, wherein the epitope is capable of being presented on HLA-C*06:02, and wherein the peptide comprises at most 25, preferably, at most 11 consecutive amino acids identical to the sequence of amino acids occurring in EBNA3C of SEQ ID NO: 121; and
- c) have at at least 90% sequence identity to SEQ ID NO: 5, wherein the epitope is capable of being presented on HLA-B*44:02, and wherein the peptide comprises at most 25, preferably, at most 11 consecutive amino acids identical to the sequence of amino acids occurring in EBNA3C of SEQ ID NO: 121.

Preferably, the peptide of a) can be specifically recognized by TCR83 as defined herein.

Preferably, the peptide of b) can be specifically recognized by TCR64 as defined herein.

Preferably, the peptide of c) can be specifically recognized by TCR25 or 58 as defined herein.

Peptide vaccinations are well known in the state of the art. For example, peptide vaccines may be for use in administration to a subject in combination with an adjuvant, such as an aluminium salt, e.g., aluminium phosphate or aluminium hydroxide, squalene, e.g., MF59, liposomes, e.g., QS21 or monophosphoryl lipid A.

Such a pharmaceutical composition of the invention may comprise a peptide comprising an epitope of SEQ ID NO: 3, wherein the epitope is capable of being presented on HLA-C*15:02, and wherein the peptide comprises at most 25, at most 15, or preferably, at most 11 amino acids identical to the sequence of amino acids occurring in LMP1 of SEQ ID NO: 120.

Such a pharmaceutical composition of the invention may comprise a peptide comprising an epitope of SEQ ID NO: 2, wherein the epitope is capable of being presented on HLA-B*57:01, and wherein the peptide comprises at most 25, at most 15, or preferably, at most 11 consecutive amino acids identical to the sequence of amino acids occurring in LMP1 of SEQ ID NO: 120.

Such a pharmaceutical composition of the invention may comprise a peptide comprising an epitope of SEQ ID NO: 5, wherein the epitope is capable of being presented on HLA-B*44:02, and wherein the peptide comprises at most 25, at most 15, or preferably, at most 11 consecutive amino acids identical to the sequence of amino acids occurring in EBNA3C of SEQ ID NO: 121.

Nucleic acids encoding a peptide comprising an epitope, as defined herein, may also be used, e.g., in combination with a suitable adjuvant such as liposomes or CpG nucleotides.

Such a pharmaceutical composition of the invention may comprise a nucleic acid encoding a peptide comprising an epitope of SEQ ID NO: 3, wherein the epitope is capable of being presented on HLA-C*15:02, and wherein the peptide comprises at most 25, at most 15, or preferably, at most 11 amino acids identical to the sequence of amino acids occurring in LMP1 of SEQ ID NO: 120.

Such a pharmaceutical composition of the invention may comprise a nucleic acid encoding a peptide comprising an epitope of SEQ ID NO: 4, wherein the epitope is capable of being presented on HLA-C*06:02, and wherein the peptide comprises at most 25, at most 15, or preferably, at most 11 consecutive amino acids identical to the sequence of amino acids occurring in EBNA3C of SEQ ID NO: 121.

Such a pharmaceutical composition of the invention may comprise a nucleic acid encoding a peptide comprising an epitope of SEQ ID NO: 5, wherein the epitope is capable of being presented on HLA-B*44:02, and wherein the peptide comprises at most 25, at most 15, or preferably, at most 11 consecutive amino acids identical to the sequence of amino acids occurring in EBNA3C of SEQ ID NO: 121.

Any of said vaccine pharmaceutical compositions may be for use in vaccination against an EBV-associated disease selected from the group comprising Hodgkin's and non-Hodgkin lymphoma, Burkitt lymphoma, hemophagocytic lymphohistiocytosis, nasopharyngeal carcinoma, head and neck cancer, gastric cancer hairy leukoplakia, post transplant lymphoproliferative disorder and central nervous system lymphoma. The vaccination may be preventive vaccination, i.e., a

vaccination provided to a subject not yet having the disease or not yet having an EBV infection, with the intent of reducing the risk for the subject to develop the disease in case of EBV-contact. The subject may belong to a risk group for the disease, e.g., it may be a subject identified as having had EBV contact or as having a current EBV infection, wherein the subject does not have the disease (yet). Alternatively, the vaccination may be therapeutic vaccination. A patient having an EBV-associated disease may be treated with a therapeutic vaccination.

Advantageously, the HLA of the subject or patient is known, and the vaccine is administered to a patient who is capable of presenting the epitope on an MHC I molecule, e.g., for the epitope as defined in a), a patient who has HLA-C*15, in particular, HLA-C*15:02, for the epitope as defined in b), a patient who has HLA-B*57, in particular HLA-B*57:01, or for the epitope as defined in c), a patient who has HLA-B*44, in particular HLA-B*44:02.

Legends

Figure 1. Detection of T cell responses using a MHC class I K562 cell library. T cells expanded on EBV antigen-expressing dendritic cells were co-cultured with K562 cells of the MHC cell library. Screening for immunogenic EBV antigen-HLA combinations was performed by (A) analysing the expression of CD137 and (B) determining the amount of secreted IFN- γ by ELISA. (C) FACS sorting of CD137-positive T cells (11%) responding to K562-HLA-B*57:01-positive cells, which then were used to identify dominant TCR α - and TCR β chains. (MIN - no antigen stimulation, MAX - unspecific antigen stimulation, us - unstained T cells). This approach was applied for the identification and isolation of all further TCRs described herein.

Figure 2. TCR gene analysis. (A) Next generation sequencing-based TCR repertoire analysis of FACS-sorted T cells responding to EBV antigen-positive (LMP1/LMP2A/EBNA1) K562-HLA-B*57:01 cells. Indicated are parts of total reads assigned to each sequence cluster, which has a sequence representative indicated on the y-axis. TCR α - and TCR β chains with a frequency of >10% were utilized to construct single chain TCR-retroviruses to identify the functional TCR. (B) For one TCR (designated as TCR50) the different V segments of the most dominant TCR α - (TRAV) and TCR β (TRBV) chains, their frequency, and the sequences of the CDR-3 region (IMGT nomenclature) are shown (AMSDLYAGNNRKL: SEQ ID NO: 122, ALTFLRDDKII: SEQ ID NO: 123, VVMATGFQKLV: SEQ ID NO: 24, ASSQDARVSGANVLT: SEQ ID NO: 124, ASSVTSGSDEQF: SEQ ID NO: 125, ASSFSLGHSYEY: SEQ ID NO: 126). This approach was applied to all further TCRs described herein.

Figure 3. Identification of the functional TCR $\alpha\beta$ chain combination. TCR α - and TCR β chains with a frequency of >10% were used to construct single chain TCR-retroviruses for the identification of the functional TCR. For TCR50 TRAV8-2*01 and TRBV9*01 formed a functional TCR as TCR-engineered T cells specifically recognize one antigen (LMP1) in combination with one HLA (B*57:01). Other TCR α - and TCR β chain combinations resulted in unspecific antigen

recognition. The combinatorial approach to identify functional TCR α - and TCR β chain combinations, as exemplarily shown here for TCR50, was applied to all further TCRs described herein. (MIN - no antigen stimulation, MAX - unspecific stimulation).

Figure 4. Epitope mapping to identify the antigenic peptide recognized by TCR50. (A) Truncated versions (LMP1/2, LMP1/1) of the full-length LMP1 antigen were generated and expressed in K562-HLA-B*57:01 cells. (B) The antigen region harboring the immunogenic epitope was identified by measuring the amount of secreted IFN- γ by ELISA in the supernatant and is located between nucleotide (nt) 316 and 624. (MIN - no antigen stimulation, MAX - unspecific antigen stimulation, UT - untransduced T cells).

Figure 5. Identification of the immunogenic epitope of LMP1. The protein region identified as epitope-positive sequence (LMP1/2 nt 316-624) was used to select for candidate peptides applying the epitope prediction algorithm NetMHCpan4.0 for HLA-B*57:01. (A) 13 peptides, according to their peptide-MHC I binding affinity (bind level) classified as strong binder (SB) and weak binder (WB), respectively, were utilized for the identification of the epitope. (B) K562-HLA-B*57:01 cells were loaded with the selected peptides, co-cultured with TCR50-engineered T cells of two donors, and IFN- γ secretion was determined by ELISA. Four epitopes (highlighted in A) with the following amino acid sequences were recognized by TCR50-engineered T cells: **IALYLQQNWW (SEQ ID NO: 108)**, **IALYLQQNW (SEQ ID NO: 2)**, **IALYLQQNW (SEQ ID NO: 110)**, **ALYLQQNWW (SEQ ID NO: 116)**. SEQ ID NOs of the analyzed peptides are shown in Table 1 below. (MIN - no antigen stimulation, MAX - unspecific stimulation, SB - strong binder, WB - weak binder, UT - untransduced T cells).

Figure 6. Peptide titration of TCR50-engineered T cells. Untransduced (UT) and TCR50-transduced T cells (TCR50) of two donors were co-cultured with K562-HLA-B*57:01 cells loaded with titrated amounts of the indicated peptides (SEQ ID NO: 2, 108, 110, 116) and the amount of secreted IFN- γ in the supernatant was determined by ELISA. Peptide IALYLQQNW (9mer, SEQ ID NO: 2), was recognized at lowest concentrations and can therefore be considered as cognate EBV LMP1 epitope of TCR50. Interestingly and worth to mentioning, this epitope recognized by TCR50 was not ranked highest in the NetMHCpan4.0 prediction tool indicating that the prediction tool is not precise in forecasting relevant immunodominant epitopes.

Figure 7. TCR50-engineered T cells recognize LMP1-positive cells. Functional analysis of LMP1-specific TCR50-engineered T cells of two donors using K562-HLA-B*57:01 antigen-loaded cells, an EBV-associated cancer cell line (L591-B*57:01), and lymphoblastoid cell lines (WIN, DEM), respectively. T cell reactivity was determined by measuring the amount of secreted IFN- γ by ELISA at an effector to target (E:T) cell ratio of 1:1. (MIN - no antigen stimulation, MAX - unspecific stimulation, UT - untransduced T cells).

Figure 8. Functional analysis of the EBNA3C-reactive TCR01. (A) EBNA3C-specific TCR01-engineered T cells were co-cultured with K562-HLA-B*07:02 antigen-pulsed cells and EBV-positive cell lines. T cell functionality was determined by measuring the amount of secreted IFN- γ by ELISA at an effector to target (E:T) cell ratio of 1:1. (B) Truncated versions (EBNA3C/3, EBNA3C/2, EBNA3C/1) of the full-length EBNA3C antigen were generated and expressed in K562-HLA-B*07:02 cells. (C) The antigen region harboring the immunogenic epitope was identified by measuring the amount of secreted IFN- γ by ELISA in the supernatant and is located between nucleotide (nt) 2071 and 2979. (D) The protein region identified as epitope-positive sequence (EBNA3C nt 2071-2979) was used to select for candidate peptides applying the epitope prediction algorithm NetMHCpan4.0 for HLA-B*07:02. (E) 27 peptides according to their peptide-MHC I binding affinity (bind level) classified as strong binder (SB) and weak binder (WB), respectively, were utilized for the identification of the epitope (QPRAPIRPI (SEQ ID NO: 127), RPIPTRFPPPPM (SEQ ID NO: 128), RPRVESSHGPA (SEQ ID NO: 129), SPQPRAPI (SEQ ID NO: 130), SPQPRAPIRPI (SEQ ID NO: 131), SPQPRAPIRPIP (SEQ ID NO: 132), PQPRAPIRPI (SEQ ID NO: 133), QPRAPIRPIP (SEQ ID NO: 134), PRAPIRPI (SEQ ID NO: 135), APIRPIPTRF (SEQ ID NO: 136), FPPPPMPL (SEQ ID NO: 137), HGPARCSQAT (SEQ ID NO: 138), RPIPTRFPP (SEQ ID NO: 139), RPIPTRFP (SEQ ID NO: 140), IPTRFPPPPMP (SEQ ID NO: 141), PIPTRFPPPPM (SEQ ID NO: 142), IPTRFPPPPMPL (SEQ ID NO: 143), GPARCSQATA (SEQ ID NO: 144), FPPPPMPLQDSM (SEQ ID NO: 145), PMPLQDSM (SEQ ID NO: 146), RPIPTRFPPP (SEQ ID NO: 147), MPLQDSMAVG (SEQ ID NO: 148), PIPTRFPPPPMP (SEQ ID NO: 149), PMPLQDSMAV (SEQ ID NO: 150), PMPLQDSM (SEQ ID NO: 151), QPRAPIRPIPT (SEQ ID NO: 152), QPRAPIRP (SEQ ID NO: 153)). K562-HLA-B*07:02 cells were loaded with the selected peptides, co-cultured with TCR01-engineered T cells, and IFN- γ secretion was determined by ELISA. Epitopes (highlighted in D) were recognized by TCR01-engineered T cells. (F) K562-HLA-B*07:02 cells were loaded with titrated amounts of the indicated peptides or no peptide as control and peptide sensitivity of TCR01-engineered T cells was determined by measuring the amount of secreted IFN- γ by ELISA at an E:T cell ratio of 1:1. (MIN - no antigen stimulation, MAX - unspecific stimulation, SB - strong binder, WB - weak binder, UT - untransduced T cells).

Figure 9. Functional analysis of the EBNA3C-reactive TCR25. (A) EBNA3C-specific TCR25-engineered T cells were co-cultured with K562-HLA-B*44:02 antigen-pulsed cells or an EBV-associated cancer cell line. T cell functionality was determined by measuring the amount of secreted IFN- γ by ELISA at an effector to target (E:T) cell ratio of 1:1. (B) Truncated versions (EBNA3C/3, EBNA3C/2, EBNA3C/1) of the full-length EBNA3C antigen were generated and expressed in K562-HLA-B*44:02 cells. (C) The antigen region harboring the immunogenic epitope was identified by measuring the amount of secreted IFN- γ by ELISA in the supernatant and is located between nucleotide (nt) 1 and 567. (D) The protein region identified as epitope-positive sequence (EBNA3C nt 1-567) was used to select for candidate peptides applying the

epitope prediction algorithm NetMHCpan4.0 for HLA-B*44:02. (E) Four peptides according to their peptide-MHC binding affinity (bind level) classified as strong binder (SB) and weak binder (WB), respectively, were utilized for the identification of the epitope (AEGGVGWRHW (SEQ ID NO: 5), SERLVPEESY (SEQ ID NO: 155), WLLTSPSQSW (SEQ ID NO: 156), LLTSPSQSW (SEQ ID NO: 157)). K562-HLA-B*44:02 cells were loaded with the selected peptides, co-cultured with TCR25-engineered T cells, and IFN- γ secretion was determined by ELISA. One epitope (highlighted in D) with the amino acid sequence AEGGVGWRHW was recognized by TCR25-engineered T cells and can therefore be considered as cognate EBV EBNA3C epitope of TCR25. (F) K562-HLA-B*44:02 cells were loaded with titrated amounts of the indicated peptide or no peptide as control. Peptide sensitivity of TCR25-engineered T cells was determined by measuring the amount of secreted IFN- γ by ELISA at an E:T cell ratio of 1:1. (MIN - no antigen stimulation, MAX - unspecific stimulation, SB - strong binder, WB - weak binder, UT - untransduced T cells).

Figure 10. Functional analysis of the EBNA3C-reactive TCR27. (A) EBNA3C-specific TCR27-engineered T cells were co-cultured with K562-HLA-B*07:02 antigen-pulsed cells and EBV-positive cell lines. T cell functionality was determined by measuring the amount of secreted IFN- γ by ELISA at an effector to target (E:T) cell ratio of 1:1. (B) Truncated versions (EBNA3C/3, EBNA3C/2, EBNA3C/1) of the full-length EBNA3C antigen were generated and expressed in K562-HLA-B*07:02 cells. (C) The antigen region harboring the immunogenic epitope was identified by measuring the amount of secreted IFN- γ (ELISA) in the supernatant and is located between nucleotide (nt) 2071 and 2979. (D) The protein region identified as epitope-positive sequence (EBNA3C nt 2071-2979) was used to select for candidate peptides applying the epitope prediction algorithm NetMHCpan4.0 for HLA-B*07:02. (E) 27 peptides according to their peptide-MHC binding affinity (bind level) classified as strong binder (SB) and weak binder (WB), respectively, were utilized for the identification of the epitope. K562-HLA-B*07:02 cells were loaded with the selected peptides, co-cultured with TCR27-engineered T cells, and IFN- γ secretion was determined by ELISA. Epitopes (highlighted in D) were recognized by TCR27-engineered T cells. (F) K562-HLA-B*07:02 cells were loaded with titrated amounts of the indicated peptides or no peptide as control and peptide sensitivity of TCR27-engineered T cells was determined by measuring the amount of secreted IFN- γ by ELISA at an E:T cell ratio of 1:1. (MIN - no antigen stimulation, MAX - unspecific stimulation, SB - strong binder, WB - weak binder, UT - untransduced T cells, SEQ ID NOs cf. legend to Fig. 8).

Figure 11. Functional analysis of the EBNA3C-reactive TCR58. (A) EBNA3C-specific TCR58-engineered T cells were co-cultured with K562-HLA-B*44:02 antigen-pulsed cells or an EBV-associated cancer cell line. T cell functionality was determined by measuring the amount of secreted IFN- γ by ELISA at an effector to target (E:T) cell ratio of 1:1. (B) Truncated versions (EBNA3C/3, EBNA3C/2, EBNA3C/1) of the full-length EBNA3C antigen were generated and expressed in K562-HLA-B*44:02 cells. (C) The antigen region harboring the immunogenic epitope was identified by measuring the amount of secreted IFN- γ by ELISA in the supernatant

and is located between nucleotide (nt) 1 and 567. (D) The protein region identified as epitope-positive sequence (EBNA3C nt 1-567) was used to select for candidate peptides applying the epitope prediction algorithm NetMHCpan4.0 for HLA-B*44:02. (E) Four peptides according to their peptide-MHC binding affinity (bind level) classified as strong binder (SB) and weak binder (WB), respectively, were utilized for the identification of the epitope. K562-HLA-B*44:02 cells were loaded with the selected peptides, co-cultured with TCR58-engineered T cells, and IFN- γ secretion was determined by ELISA. One epitope (highlighted in D) with the amino acid sequence AEGGVGWRHW was recognized by TCR58-engineered T cells and can therefore be considered as cognate EBV EBNA3C epitope of TCR58. (F) K562-HLA-B*44:02 cells were loaded with titrated amounts of the indicated peptide or no peptide as control. Peptide sensitivity of TCR58-engineered T cells was determined by measuring the amount of secreted IFN- γ by ELISA at an E:T cell ratio of 1:1. (MIN - no antigen stimulation, MAX - unspecific stimulation, SB - strong binder, WB - weak binder, UT - untransduced T cells, SEQ ID NOs cf. legend to Fig. 9).

Figure 12. Functional analysis of the EBNA3C-reactive TCR64. (A) EBNA3C-specific TCR64-engineered T cells were co-cultured with K562-HLA-C*06:02 antigen-pulsed cells and EBV-positive cell lines. T cell functionality was determined by measuring the amount of secreted IFN- γ by ELISA at an effector to target (E:T) cell ratio of 1:1. (B) Truncated versions (EBNA3C/3, EBNA3C/2, EBNA3C/1) of the full-length EBNA3C antigen were generated and expressed in K562-HLA-C*06:02 cells. (C) The antigen region harboring the immunogenic epitope was identified by measuring the amount of secreted IFN- γ by ELISA in the supernatant and is located between nucleotide (nt) 568 and 1569. (D) The protein region identified as epitope-positive sequence (EBNA3C nt 568-1569) was used to select for candidate peptides applying the epitope prediction algorithm NetMHCpan4.0 for HLA-C*06:02. (E) 27 peptides according to their peptide-MHC binding affinity (bind level) classified as strong binder (SB) and weak binder (WB), respectively, were utilized for the identification of the epitope (RRYRRIYDL (SEQ ID NO: 158), FRKAQIQGL (SEQ ID NO: 4), AREAEVRFL (SEQ ID NO: 159), LRGKWQRRY (SEQ ID NO: 160), ERYAREAEV (SEQ ID NO: 161), SRRRRGACV (SEQ ID NO: 162), NLLDFVRFM (SEQ ID NO: 163), RRIYDLIEL (SEQ ID NO: 164), RRRRGACVV (SEQ ID NO: 165), VRFLRGKWQ (SEQ ID NO: 166), RRRGACVVY (SEQ ID NO: 167), QRRYRRIYD (SEQ ID NO: 168), VRFMGVMSS (SEQ ID NO: 169), YAREAEVRFL (SEQ ID NO: 170), NRVGADSIM (SEQ ID NO: 171), LHHIWQNLL (SEQ ID NO: 172), RRGKEHVI (SEQ ID NO: 173), YRRIYDLIE (SEQ ID NO: 174), RRYRRIYDLI (SEQ ID NO: 175), ARRGKEHV (SEQ ID NO: 176), QRRYRRIYDL (SEQ ID NO: 177), WQRRYRRIY (SEQ ID NO: 178), FLRGKWQRRY (SEQ ID NO: 179), RRGACVVYD (SEQ ID NO: 180), VYDDDVIEV (SEQ ID NO: 181), YAREAEVRF (SEQ ID NO: 182), GCQNAARTL (SEQ ID NO: 183)). K562-HLA-C*06:02 cells were loaded with the selected peptides, co-cultured with TCR64-engineered T cells, and IFN- γ secretion was determined by ELISA. One epitope (highlighted in D) with the amino acid sequence FRKAQIQGL was recognized by TCR64-engineered T cells and can therefore be considered as cognate EBV EBNA3C epitope of TCR64.

(F) K562-HLA-C*06:02 cells were loaded with titrated amounts of the indicated peptide or no peptide as control and peptide sensitivity of TCR64-engineered T cells was determined by measuring the amount of secreted IFN- γ by ELISA at an E:T cell ratio of 1:1. (MIN - no antigen stimulation, MAX - unspecific stimulation, SB - strong binder, WB - weak binder, UT - untransduced T cells).

Figure 13. Functional analysis of the LMP1-reactive TCR83. (A) LMP1-specific TCR83-engineered T cells were co-cultured with K562-HLA-C*15:02 antigen-pulsed cells or an EBV-associated cancer cell line. T cell functionality was determined by measuring the amount of secreted IFN- γ by ELISA at an effector to target (E:T) cell ratio of 1:1. (B) Truncated versions (LMP1/2, LMP1/1) of the full-length LMP1 antigen were generated and expressed in K562-HLA-C*15:02 cells. (C) The antigen region harboring the immunogenic epitope was identified by measuring the amount of secreted IFN- γ by ELISA in the supernatant and is located between nucleotide (nt) 316 and 624. (D) The protein region identified as epitope-positive sequence (LMP1 nt 316-624) was used to select for candidate peptides applying the epitope prediction algorithm NetMHCpan4.0 for HLA-C*15:02. (E) Four peptides according to their peptide-MHC binding affinity (bind level) classified as weak binder (WB) were utilized for the identification of the epitope (NSNEGRHHL (SEQ ID NO: 184), **QQNWWTLLV** (SEQ ID NO: 3), DSLPHPQQA (SEQ ID NO: 185), YLQQNWWTL (SEQ ID NO: 186)). K562-HLA-C*15:02 cells were loaded with the selected peptides, co-cultured with TCR83-engineered T cells, and IFN- γ secretion was determined by ELISA. One epitope (highlighted in D) with the amino acid sequence QQNWWTLLV was recognized by TCR83-engineered T cells and can therefore be considered as cognate EBV LMP1 epitope of TCR83. (F) K562-HLA-C*15:02 cells were loaded with titrated amounts of the indicated peptide or no peptide as control. Peptide sensitivity of TCR83-engineered T cells was determined by measuring the amount of secreted IFN- γ by ELISA at an E:T cell ratio of 1:1 (B). (MIN - no antigen stimulation, MAX - unspecific stimulation, WB - weak binder, UT - untransduced T cells).

Figure 14. Functional analysis of the LMP2A-reactive TCR06. (A) LMP2A-specific TCR06-engineered T cells were co-cultured with K562-HLA-A*02:01 antigen-pulsed cells or EBV-associated cancer cell lines. T cell functionality was determined by measuring the amount of secreted IFN- γ by ELISA at an effector to target (E:T) cell ratio of 1:1. (B) Truncated versions (LMP2A/2, LMP2A/1) of the full-length LMP2A antigen were generated and expressed in K562-HLA-A*02:01 cells. (C) The antigen region harboring the immunogenic epitope was identified by measuring the amount of secreted IFN- γ (ELISA) in the supernatant and is located between nucleotide (nt) 1006 and 1494. (D) The protein region identified as epitope-positive sequence (LMP1 nt 1006 -1494) was used to select for candidate peptides applying the epitope prediction algorithm NetMHCpan4.0 for HLA-A*02:01. (E) 14 peptides according to their peptide-MHC binding affinity (bind level) classified as strong binder (SB) and weak binder (WB), respectively, were utilized for the identification of the epitope (FMCLGGLLTM (SEQ ID NO: 187), MLLLVAGI

(SEQ ID NO: 188), NLFCMLLLI (SEQ ID NO: 189), LLIVAGILFI (SEQ ID NO: 190), NLFCMLLLIV (SEQ ID NO: 191), **MCLGGLTMMV** (SEQ ID NO: 192), **CLGGLTMMV** (SEQ ID NO: 1), LIVAGILFI (SEQ ID NO: 193), FIPNLFCML (SEQ ID NO: 194), IVAGILFIL (SEQ ID NO: 195), MLLIVAGIL (SEQ ID NO: 196), CMLLLIVAGI (SEQ ID NO: 197), PNLFCMLLLI (SEQ ID NO: 198), FIPNLFCMLL (SEQ ID NO: 199)). K562-HLA-A*02:01 cells were loaded with the selected peptides, co-cultured with TCR06-engineered T cells, and IFN- γ secretion was determined by ELISA. Epitopes (highlighted in D) were recognized by TCR06-engineered T cells and can therefore be considered as cognate EBV LMP2A epitopes of TCR06. Interestingly and worth to mentioning, that the two epitopes recognized by TCR06 were not ranked highest in the NetMHCpan4.0 prediction tool indicating that the prediction tool is not precise in forecasting relevant immunodominant epitopes. (F) K562-HLA-A*02:01 cells were loaded with titrated amounts of the indicated peptides or no peptide as control. Peptide sensitivity of TCR06-engineered T cells was determined by measuring the amount of secreted IFN- γ by ELISA at an E:T cell ratio of 1:1(B). (MIN – no antigen stimulation, MAX - unspecific stimulation, S - strong binder, WB - weak binder, UT - untransduced T cells).

Figure 15. Comparison of peptide sensitivity of LMP2A-specific TCR06 and TCRs provided in patents WO 2015/022520 A1 (PUBTCR1) and WO 2011/039508 A2 (PUBTCR2). The previously published wt EBV LMP2A TCR with a TRAV12-3*01/TRAJ41*01/TRAC alpha chain amino acid sequence (SEQ ID No: 2 of WO 2015/022520 A1) and a TRBV11-2*01/TRBD1/TRBJ2-7/TRBC beta chain amino acid sequence (SEQ ID No: 3 of WO 2015/022520 A1), also Fig. 1 of said document, is herein designated PUBTCR1. The previously published LMP2A TCR described in WO 2011/039508 A2, in particular, based on SEQ ID NO: 8 thereof, is designated PUBTCR2. K562-HLA-A*02:01 cells were loaded with titrated amounts of the indicated peptides CLGGLTMMV and MCLGGLTMMV, respectively, co-cultured with TCR06- or PUBTCR1- or PUBTCR2-engineered T cells, and peptide sensitivity of all TCRs was determined by measuring the amount of secreted IFN- γ by ELISA at an effector to target (E:T) cell ratio of 1:1. TCR06 has a higher peptide sensitivity in comparison to PUBTCR1 and PUBTCR2. UT - untransduced T cells.

Figure 16. Extended comparison of peptide sensitivity of LMP2A-specific TCR06 with TCRs provided in patents WO 2015/022520 A1 (PUBTCR1) and WO 2011/039508 A2 (PUBTCR2) and TCR-JC isolated by the applicants.

The previously published wt EBV LMP2A TCR with a TRAV12-3*01/TRAJ41*01/TRAC alpha chain amino acid sequence (SEQ ID NO: 2 of WO 2015/022520 A1) and a TRBV11-2*01/TRBD1/TRBJ2-7/TRBC beta chain amino acid sequence (SEQ ID NO: 3 of WO 2015/022520 A1), also Fig. 1 of said document, is herein designated PUBTCR1. The previously published LMP2A TCR described in WO 2011/039508 A2, in particular, based on SEQ ID NO: 8 thereof, is designated PUBTCR2. Another LMP2A-specific TCR, herein designated as TCR-JC, was isolated by the applicants. (A) K562-HLA-A*02:01 cells were loaded with titrated amounts of

the peptide CLGGLTMMV, co-cultured with TCR06-, PUBTCR1-, PUBTCR2- or TCR-JC-engineered T cells, and peptide sensitivity of all TCRs was determined by measuring the amount of secreted IFN- γ by ELISA at an effector to target (E:T) cell ratio of 1:10. (B) Sigmoidal 4PL curves (constrained model) based on data points from A. The sigmoidal regression shows a very good R^2 (approx. 0.95) for TCR06, PUBTCR1, and PUBTCR2. Data from TCR-JC shows a model fit of 0.81 (lack of upper plateau). (C) Based on data shown in B, the peptide sensitivity of the four TCRs was calculated and indicated as EC50 in 10^{-9} M (EC50 - mol/l of peptide needed to achieve 50% of the maximal IFN- γ release, SEM - standard error mean).

Figure 17. EBV-specific TCR-engineered T cells kill cancer cells. LMP1-specific TCR50-, LMP2A-specific TCR06-, and EBNA3C-specific TCR64-engineered T cells were co-cultured with L591 EBV⁺ tumor cells, which naturally express LMP1, LMP2A, and EBNA3C but were transfected with the respective MHC I allele. At indicated effector : target (E:T) cell ratios, data of triplicate wells were averaged and the percentage of surviving cells was calculated in relation to the values obtained from the samples co-cultured with untransduced T cells: % specific survival = $100 \times (\text{test value})/(\text{average background})$. (UT - untransduced T cells).

Figure 18. *In vivo* mouse models of tumor rejection (A) NOG mice received subcutaneously 5×10^6 K562-HLA-A*02:01 tumor cells and 24 h later intravenously 1×10^7 TCR06-engineered T cells. Data obtained from individual mice receiving TCR06-engineered T cells (n=10) from two donors are shown in comparison to untransduced T cells (n=6). (B) NSG mice received subcutaneously 5×10^6 K562-HLA-B*57:01 tumor cells and nine days later intravenously 5×10^6 TCR50-engineered T cells. In both models, tumor size was measured using a caliper and calculated.

Examples

Generation of EBV-specific TCR constructs and transgenic T cells and identification of epitopes

The inventors used an innovative method (Lorenz et al., 2018; WO2016/146618 A1) to generate EBV-specific TCRs, which recognize endogenously processed, immunogenic EBV epitopes presented by different MHC class I molecules.

Briefly, after selecting the EBV antigen of interest, e.g., LMP1, LMP2A or EBNA3C, the following experimental steps were performed:

- (i) Pulsing of professional antigen-presenting cells (preferably dendritic cells (DCs)) with *in vitro*-transcribed (ivt)RNA encoding the full-length sequence of the selected EBV antigen to stimulate autologous T cells. This procedure is completely unbiased and allows the DCs to select the best epitope of the antigen for expression, processing, and presentation on the cell surface in conjunction with the most suitable MHC class I (MHC I) molecule.

- (ii) Identification of EBV antigen-reactive T cells. This step was performed by means of a newly established MHC class I cell library, which is composed of single MHC I-expressing cell lines originated from K562 cells. This part is an important feature of the TCR isolation approach, because it is basic to take advantage of the broad MHC flexibility. For the identification of each TCR, the inventors selected up to six MHC I from the K562 cell library that correspond to MHC I alleles of the T cell donor, and transfected the cells with the relevant antigens used for priming in step (i). After co-culture of antigen-presenting K562 cells and antigen-stimulated T cells, responding T cells were identified by interferon-(IFN) γ release using an ELISA, and up-regulation of the T cell activation marker CD137 measured by flow cytometry. Subsequently, reactive CD8+ T cells were enriched by FACS sorting.
- (iii) Isolation of total RNA from FACS-sorted CD8+ T cells and PCR amplification of TCR α - and TCR β chain-specific sequences. Identification of dominant TCR α - and TCR β sequences by next generation sequencing.
- (iv) Re-expression of dominant (at least 10%) TCR α - and TCR β chain combinations using the γ -retrovirus vector MP71 (Engels et al., 2003; Leisegang et al., 2008; Sommermeyer and Uckert, 2010) in primary human T cells to identify the functional TCR $\alpha\beta$ chain combination. This was done by co-cultivation of TCR-engineered T cells with K562 cells carrying the proper MHC I molecule and expressing the full-length EBV antigen. Antigen recognizing TCR $\alpha\beta$ chain combinations were linked with a P2A element and recloned in the configuration of TCR β gene-P2A-TCR α gene into the MP71 vector. Constant TCR $\alpha\beta$ chain regions were replaced by their murine counterparts to enhance pairing of transgenic TCR chains. Subsequently, the complete TCR transgene cassette was codon optimized.
- (v) Identification of the antigenic peptide (epitope) of EBV, which is recognized by the TCR-engineered T cells. For this, the full-length antigen was C- or N-terminally truncated, cloned into the plasmid vector pcDNA3.1(-) and expressed in K562 cells carrying the proper MHC I molecule. Then, the remaining protein fragments were tested for their ability to furthermore present the epitope recognized by the TCR. Finally, candidate peptides of the corresponding protein region were identified by the epitope prediction algorithm NetMHCpan4.0 (<http://www.cbs.dtu.dk/services/NetMHCpan/>). Predicted peptides were generated and loaded onto K562 cells carrying the proper MHC I molecule and investigated in co-culture experiments for their ability to be recognized by TCR-engineered T cells. The peptides capable of stimulating most IFN- γ production are considered as cognate epitope.

Table 1: Peptides tested for one of the TCR (TCR50). The protein region identified as epitope-positive sequence (LMP1/2 nt 316-624) was used to select for candidate peptides applying the epitope prediction algorithm NetMHCpan4.0 for HLA-B*57:01. 13 peptides, according to their peptide-MHC binding affinity classified as strong binder (SB) and weak binder (WK), respectively, were utilized for epitope identification. Results are shown in Figure 5.

Epitope (SEQ ID NO:)	Length (aa)	Bind level	Affinity
WTLLVDLLW (107)	9	SB	12.25
IALYLQQNWW (108)	10	SB	14.84
IALYLQQNW (2)	9	SB	16.32
WWTLLVDLLW (109)	10	SB	32.51
IIALYLQQNW (110)	10	SB	57.84
WTLLVDLLWL (111)	10	SB	111.95
LAILIWMYY (112)	9	SB	231.02
LLFLAILIW (113)	9	SB	234.00
LLLFLAILIW (114)	10	WB	749.75
LAILIWMYYH (115)	10	WB	789.96
ALYLQQNWW (116)	9	WB	853.78
FLAILIWMYY (117)	10	WB	1515.23
NSNEGRHHL (118)	9	WB	12828.84

TCR constructs generated are characterized in Tables 2, 3 and 4 below.

Table 2: TCR summary

TCR	half-maximum IFN- γ	EBV-Antigen	Epitope	SEQ ID NO:	MHC I	Recognition of tumor cells
01	6×10^{-8}	EBNA3C	QPRAPIRPIPT	7	B*07:02	+
25	6×10^{-7}	EBNA3C	AEGGVGWRHW	5	B*44:02	+
27	6×10^{-8}	EBNA3C	QPRAPIRPIP	6	B*07:02	+
58	3×10^{-7}	EBNA3C	AEGGVGWRHW	5	B*44:02	+
64	7×10^{-6}	EBNA3C	FRKAQIQGL	4	C*06:02	+
50	3×10^{-8}	LMP1	IALYLQQNW	2	B*57:01	+
83	2×10^{-9}	LMP1	QQNWWTLLV	3	C*15:02	+
06	6×10^{-9}	LMP2A	CLGGLTMTV	1	A*02:01	+

TCR06, TCR50 and TCR83 have a high peptide sensitivity (half maximum IFN- γ release), in particular, TCR06.

Table 3: CDR sequences of preferred TCR constructs of the invention. CDR1 IMGT aa location: 27-38. CDR2 IMGT aa location: 56-65. CDR3 IMGT aa location: 105-117. Numbers in parentheses: SEQ ID NO:

TCR		CDR-1 aa seq	CDR-2 aa seq	CDR-3 aa seq
TCR 06	TRA	DSAIYN (11)	IQSSQRE (12)	AVLMDSNYQLI (13)
	TRB	WSHSY (16)	SAAADI (17)	ASSEDGMNTEAF (18)
TCR 50	TRA	SSYSPS (21)	YTSAATLV (22)	VVMATGFQKLV (23)
	TRB	SGDLS (26)	YYNGEE (27)	ASSVTSGSDEQF (28)

TCR 83	TRA	TSGFNG (31)	NVLDGL (32)	AAVNNAGNMLT (33)
	TRB	LGHDT (36)	YNNKEL (37)	ASSQGYGGPSTDTQY (38)
TCR 64	TRA	SVFSS (41)	VVTGGEV (42)	AGDVDGTASKLT (43)
	TRB	MDHEN (46)	SYDVKM (47)	ASSLLGSGALYEQY (48)
TCR 25	TRA	SSYSPS (51)	Y TSAATLV (52)	VAWDTGFQKLV (53)
	TRB	SNHLY (56)	FYNNEI (57)	ASKALADTQY (58)
TCR 58	TRA	NSASQS (61)	VYSSGN (62)	VASGDSSYKLI (63)
	TRB	SNHLY (66)	FYNNEI (67)	ASSDPLSTYNEQF (68)
TCR 27	TRA	TISGTDY (71)	GLTSN (72)	ILCGAGGTSYGKLT (73)
	TRB	MNHEY (76)	SMNVEV (77)	ASNVQGANNEQF (78)
TCR 01	TRA	TISGTDY (81)	GLTSN (82)	ILCGAGGTSYGKLT (83)
	TRB	MNHEY (86)	SMNVEV (87)	ASAIQGANNEQF (88)

Table 4: TRAV and TRBV segments and junction aa (IMGT location 104-118) of preferred TCR constructs of the invention. Numbers in parentheses: SEQ ID NO:

TCR		V segment	aa junction aa seq
TCR 06	TRA	TRAV21*01	CAVLMDSNYQLIW (14)
	TRB	TRBV10-2*02	CASSEDGMNTEAFF (19)
TCR 50	TRA	TRAV8-2*01	CVVMATGFQKLVF (24)
	TRB	TRBV9*01	CASSVTSGSDEQFF (29)
TCR 83	TRA	TRAV1-2*01	CAAVNNAGNMLTF (34)
	TRB	TRBV3-1*01	CASSQGYGGPSTDTQYF (39)
TCR 64	TRA	TRAV27*01	CAGDVDGTASKLTF (44)
	TRB	TRBV28*01	CASSLLGSGALYEQYF (49)
TCR 25	TRA	TRAV8-2*01	CVAWDTGFQKLVF (54)
	TRB	TRBV2*01	CASKALADTQYF (59)
TCR 58	TRA	TRAV12-1*01	CVASGDSSYKLIF (64)
	TRB	TRBV2*01	CASSDPLSTYNEQFF (69)
TCR 27	TRA	TRAV26-2*01	CILCGAGGTSYGKLTF (74)
	TRB	TRBV27*01	CASNVQGANNEQFF (79)
TCR 01	TRA	TRAV26-2*01	CILCGAGGTSYGKLTF (84)
	TRB	TRBV27*01	CASAIQGANNEQFF (89)

Functional characterization

The generated TCR-engineered T cells were functionally characterized using *in vitro* assays.

Firstly, the peptide sensitivity (half maximum IFN- γ release) of the isolated TCRs was analyzed in peptide titration experiments (cf. Fig. 6 and Lorenz et al., 2018). Briefly, peptides were loaded in titrating amounts onto K562 target cells, ranging from 10^{-5} mol/l to 10^{-13} mol/l. A co-culture assay was performed with TCR-engineered T cells at an effector to target (E:T) cell ratio of 1:1 (if not otherwise indicated), meaning 2.5×10^4 TCR-engineered T cells were co-cultured with 2.5×10^4

peptide-loaded K562 cells. The ability of TCR-transduced T cells to recognize their target epitope was assessed after 24 h by an IFN- γ ELISA.

Secondly, EBV antigen-expressing target cell lines (LCLs, EBV-associated cancer cell lines which endogenously process and present EBV epitopes and peptide-loaded K562 cells) were applied in co-culture experiments to determine the amount of IFN- γ released by TCR-engineered T cells (cf. Fig. 7 and Lorenz et al., 2018). In brief, TCR-engineered T cells and target cells (each 2×10^4) were co-cultured. After 24 h, T cell reactivity was determined by measuring the amount of secreted IFN- γ by ELISA. T cells stimulated with PMA and ionomycin were used as positive control, untransduced T cells as negative control.

According to the described isolation and characterization procedure and the identification of the epitopes recognized by TCR-engineered T cells, we have isolated in total eight EBV-specific TCRs, and the relevant, immunogenic peptides (epitopes) recognized by these TCRs. Some of the epitopes were already known in the art, others, in particular, peptides of SEQ ID NO: 3, 4 and 5, have been newly identified herein to be presented by the respective MHC I molecules.

Thirdly, EBV antigen-expressing L591 tumor cells which endogenously process and present epitopes of LMP1, LMP2A, and EBNA3C but where transfected with the relevant MHC I alleles were applied in co-culture experiments with TCR-engineered T cells to determine the killing capacity of TCR-engineered T cells.

Furthermore, the generated TCR-engineered T cells were functionally characterized using *in vivo* assays. Therefore, two models of immunocompromised mice (NOD, NSG) were applied. Tumor cells expressing both the relevant EBV antigen and MHC I molecule were injected subcutaneously into the animals and TCR-engineered T cells were transferred by tail vein injection into the mice.

Combination of TCRs for immunotherapy

The combination of TCR-engineered T cells which recognize different epitopes presented by distinct MHC I molecules is an interesting option to improve TCR gene therapy. This approach aims to overcome and prevent two problems of immunotherapy: (i) tumor outgrowth due to loss variants of a specific tumor antigen and (ii) tumor immune evasion due to downregulation of a specific MHC I molecule. Using such a combinatorial approach, the efficiency of TCR gene therapy will be enhanced.

To prove this concept, two or three TCRs, which are able to recognize endogenously processed EBV antigens naturally presented by different MHC I molecules, are selected, e.g. TCR06 (LMP2A, MHC A*02:01), TCR50 (LMP1, MHC B*57:01), and, optionally, TCR64 (EBNA3C, MHC C*06:02). TCR-engineered T cells are generated with these TCRs by retroviral transduction as

described. Tumor cells which naturally express the EBV antigens recognized by the TCR and which harbor the respective MHC I molecules (A*02:01, B*57:01, C*06:02) are used in co-culture experiments with TCR-engineered T cells.

Alternatively, such cells (e.g. K562 cells) are generated by transfection of the corresponding EBV antigen genes and the MHC I genes.

In the experiment, TCR-engineered T cells are used individually or in combinations, and will be co-cultured with tumor cells in an E:T ratio of 2:1. To evaluate the effectiveness of the single and combinatorial application of TCR-engineered T cells, IFN- γ secretion is determined by ELISA.

In addition, a cytotoxicity assay is used to analyze the capacity of the single and combinatorial application of TCR-engineered T cells to kill tumor cells.

In a further experiment, an *in vivo* mouse model is established. Tumor cells, e.g. K562 cells, expressing the respective EBV antigens and MHC I molecules, are s.c. injected into the flank of NSG mice (NOD.Cg-Prkdc^{scid} Il2rg^{tm1 Wjl}/Sz). After tumors are palpable, TCR-engineered T cells are i.v. injected, individually or in combination, to determine the efficacy of TCR gene therapy in terms of tumor rejection.

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