express T cell receptors (TCR) with a specificity for a defined epitope derived from an intracellular protein like MAGE-A3.

In an open –label test of an affinity-enhanced TCR against HLA-A*01:01-restricted MAGE-A3 (EVDPIGHLY; SEQ ID NO: 1) severe side effects occured. Studies revealed myocardial damage due to T cell infiltration of the heart tissue. Studies on the molecular level also revealed, that the TCR cross-reacted with a similar epitope of the striated muscle protein titin (Cameron, et al. Science Tranlational Medcine 5 (197): 1-11;2013; Linetteet al. *Blood*, *122*(6), 863–871; 2013).

Thus, there is still a need for highly efficient TCRs which target only the tumor specific/restricted antigen MAGE-A3 and therefore bear an exceptional potential for cancer immunotherapy. Thus, there is a need for such specific TCRs targeting MAGE-A3 having no or only very low cross-reactivity to healthy tissue.

OBJECTIVES AND SUMMARY

One objective of the present disclosure is the provision of a T cell receptor (TCR) which is specific for MAGE-A3. In particular, the TCR may specifically recognize the MAGE-A3 epitope having the amino acid sequence SEQ ID NO: 1 or a fragment thereof. Preferably the TCR specifically recognizes the HLA-A1 bound form of the amino acid sequence of SEQ ID NO: 1, more preferably the TCR specifically recognizes the amino acid sequence of SEQ ID NO: 1, which is presented by the HLA-A*01:01 encoded molecule.

The fragment of the epitope may be a protein sequence that is specific for this antigen, i.e. does not occur in another protein or peptide of a mammal, especially of a human. The fragment may be shorter than the sequence of the antigen, such as at least 5%, at least 10%, at least 30%, at least 50%, at least 70%, at least 90% shorter than the antigen. The fragment may have a length of at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15 or more amino acids.

In one aspect, disclosed herein is an isolated T cell receptor (TCR) specific for MAGE-A3.

In another aspect, disclosed herein is a multivalent TCR complex comprising a least one TCR as disclosed herein.

In another aspect, disclosed herein is a nucleic acid encoding a TCR as disclosed herein or encoding the isolated polypeptide as disclosed herein.

In another aspect, disclosed herein is a vector comprising the nucleic acid as disclosed herein, wherein the vector is preferably an expression vector, more preferably a retroviral vector or lentiviral vector.

In another aspect, disclosed herein is a cell expressing the TCR as disclosed herein.

In another aspect, disclosed herein is an antibody or antigen binding fragment thereof specifically binding to a portion of the TCR as disclosed herein, wherein the portion of the TCR comprises a) the CDR3 of the alpha chain of SEQ ID NO: 4 and/or the CDR3 of the beta chain of SEQ ID NO: 7 or; b) the CDR3 of the alpha chain of SEQ ID NO: 12 and/or the CDR3 of the beta chain of SEQ ID NO: 15 or; c) the CDR3 of the alpha chain of SEQ ID NO: 20 and/or the CDR3 of the beta chain of SEQ ID NO: 23.

In another aspect, disclosed herein is the use of the TCR, the polypeptide, the multivalent TCR complex, the nucleic acid, the vector, the cell, or the antibody as disclosed herein for use in therapy.

In another aspect, disclosed herein is a use of the TCR, the polypeptide, the multivalent TCR complex, the nucleic acid, the vector, the cell, or the antibody as disclosed herein in the manufacture of a medicament for treating cancer, wherein the cancer is selected from the group consisting of prostate cancer, uterine cancer, thyroid cancer, testicular cancer, renal cancer, pancreatic cancer, ovarian cancer, esophageal cancer, non-small-cell lung cancer, lung adenocarcinoma, squamous cell carcinoma, non-Hodgkin's lymphoma, multiple myeloma, melanoma, hepatocellular carcinoma, head and neck cancer, gastric cancer, endometrial cancer, cervical cancer, colorectal cancer, stomach adenocarcinoma, cholangiocarcinoma, breast cancer, bladder cancer, myeloid leukemia and acute lymphoblastic leukemia, carcinoma, sarcoma or osteosarcoma.

In a specific example, the TCR does not recognize the titin epitope of amino acid sequence SEQ ID NO: 54 or a fragment thereof. It is desired that there is no or substantially no cross-reactivty with the titin-derived epitope in order to avoid unwanted severe side effects.

It has been shown that a TCR described in the prior art directed against the epitope of SEQ ID NO: 1 led to severe side effects. It was also found that the adverse events arose due to the binding of the prior art TCR to the titin epitope of SEQ ID NO: 54, which is similar to the MAGE-A3 epitope of SEQ ID NO: 1. Therefore, it is highly important that a TCR, directed to MAGE-A3, in particular to the epitope of SEQ ID NO: 1, used for the treatment of humans is not activated by binding to the titin-derived epitopes, in particular to the titin epitope of SEQ ID NO: 54.

Preferably the TCR does not show cross-reactivity to other MAGE-A family members, specifically to MAGE-A6.

In a specific example the isolated TCR comprises

a) -a TCR α chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 2, a CDR2 having the amino acid sequence of SEQ ID NO: 3 and a CDR3 having the amino acid sequence of SEQ ID NO: 4, and

-a TCR β chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 5, a CDR2 having the amino acid sequence of SEQ ID NO: 6 and a CDR3 having the amino acid sequence of SEQ ID NO: 7; or

b) - a TCR α chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 10, a CDR2 having the amino acid sequence of SEQ ID NO: 11 and a CDR3 having the amino acid sequence of SEQ ID NO: 12, and

- a TCR β chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 13, a CDR2 having the amino acid sequence of SEQ ID NO: 14 and a CDR3 having the amino acid sequence of SEQ ID NO: 15; or

c) - a TCR α chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 18, a CDR2 having the amino acid sequence of SEQ ID NO: 19 and a CDR3 having the amino acid sequence of SEQ ID NO: 20, and

-a TCR β chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 21, a CDR 2 having the amino acid sequence of SEQ ID NO: 22 and a CDR 3 having the amino acid sequence of SEQ ID NO: 23.

The TCR further may be defined by

a) a variable TCR α region having an amino acid sequence which is at least 80% identical to SEQ ID NO: 8 and a variable TCR β region having an amino acid sequence which is at least 80% identical to SEQ ID NO: 9; or

b) a variable TCR α region having an amino acid sequence which is at least 80% identical to SEQ ID NO: 16 and a variable TCR β region having an amino acid sequence which is at least 80% identical to SEQ ID NO: 17; or

c) a variable TCR α region having an amino acid sequence which is at least 80% identical to SEQ ID NO: 24 and a variable TCR β region having an amino acid sequence which is at least 80% identical to SEQ ID NO: 25.

The TCR according to the disclosure is isolated and/or purified and may be soluble or membrane bound.

In some examples, the amino acid sequence of the TCR may comprise one or more phenotypically silent substitutions. In addition, the TCR of the disclosure can be labelled with a detectable label. Additionally, or alternatively, the amino acid sequence may be modified to comprise a therapeutic agent or pharmacokinetic modifying moiety. The therapeutic agent may be selected from the group consisting of an immune effector molecule, a cytotoxic agent and a radionuclide. The immune effector molecule may for example be a cytokine. The pharmacokinetic modifying moiety may be at least one polyethylene glycol repeating unit, at least one glycol group, at least one sialyl group or a combination thereof.

The TCR, in particular a soluble form of the TCR, according to the disclosure can be modified by attaching additional functional moieties, e.g. for reducing immunogenicity, increasing hydrodynamic size (size in solution) solubility and/or stability (e.g. by enhanced protection to proteolytic degradation) and/or extending serum half-life. Other useful functional moieties and modifications include “suicide” or “safety switches” that can be used to shut off or turn on effector host cells carrying an inventive TCR in a patient’s body or to shut off or turn on the transgenic TCR itself. TCRs with an altered glycosylation pattern are also envisaged herein.

It is also conceivable to add a drug or a therapeutic entity, such as a small molecule compound to the TCR, in particular to a soluble form of the inventive TCR.

The TCR, in particular a soluble form of the inventive TCR, can additionally be modified to introduce additional domains which aid in identification, tracking, purification and/or isolation of the respective molecule (tags).

In some examples, the TCR is of the single chain type, wherein the TCR α chain and the TCR β chain are linked by a linker sequence.

Another example of the disclosure refers to an isolated polypeptide comprising a functional portion of the TCR as described herein,
wherein the functional portion comprises the

a) the amino acid sequences SEQ ID NOs: 2, 3, 4, 5, 6, and 7, or
b) the amino acid sequences SEQ ID NOs: 10, 11, 12, 13, 14, and 15, or
c) the amino acid sequences SEQ ID NOs:18, 19, 20, 21, 22, and 23.

In specific examples, the functional portion comprises the TCR α variable chain and/or the TCR β variable chain.

Futher contemplated is a multivalent TCR complex comprising a least one TCR as described herein.

Thus, the disclosure refers to an isolated TCR as described herein, a polypeptide as described herein, a multivalent TCR complex as described herein, wherein IFN-γ secretion of a cell, e.g. T cell, which expresses the herein described TCR is induced by binding to the amino acid sequence of SEQ ID NO: 1, which is presented by the HLA-A*01:01 molecule.

Also contemplated is a nucleic acid encoding a TCR as described herein or encoding the isolated polypeptide as described herein.

Accordingly, another example refers to a vector comprising the nucleic acid as described herein, wherein the vector is preferably an expression vector, more preferably a retroviral vector or lentiviral vector.

Moreover, a further example defines a cell expressing the TCR as described herein.

Also envisaged is an antibody or antigen binding fragment thereof specifically binding to a portion of the TCR as described herein that mediates specificity for MAGE-A3, preferably wherein the portion of the TCR that mediates the MAGE-A3 specificity comprises

a) the CDR3 of the alpha chain of SEQ ID NO: 4 and/or the CDR3 of the beta chain of SEQ ID NO: 7 or;
b) the CDR3 of the alpha chain of SEQ ID NO: 12 and/or the CDR3 of the beta chain of SEQ ID NO: 15 or;
c) the CDR3 of the alpha chain of SEQ ID NO: 20 and/or the CDR3 of the beta chain of SEQ ID NO: 23.

Another example of the disclosure refers to a pharmaceutical composition comprising the TCR as described herein, the polypeptide as described herein, the multivalent TCR complex as described herein, the nucleic acid as described herein, the vector as described herein, the cell as described herein, or the antibody as described herein.

Typically, the pharmaceutical composition comprises at least one pharmaceutically acceptable carrier.

Another example refers to the TCR, the multivalent TCR complex, the nucleic acid, the vector, the cell, or the antibody as described herein for use as a medicament.

In particular, it is refered to the TCR, the multivalent TCR complex, the nucleic acid, the vector, the cell, or the antibody as described herein for use in the treatment of cancer, wherein the cancer is selected from the group consisting of prostate cancer, uterine cancer, thyroid cancer, testicular cancer, renal cancer, pancreatic cancer, ovarian cancer, esophageal cancer, non-small-cell lung cancer, lung adenocarcinoma, squamous cell carcinoma, non-Hodgkin's lymphoma, multiple myeloma, melanoma, hepatocellular carcinoma, head and neck cancer, gastric cancer, endometrial cancer, cervical cancer, colorectal cancer, stomach adenocarcinoma, cholangiocarcinoma, breast cancer, bladder cancer, myeloid leukemia and acute lymphoblastic leukemia, carcinoma, sarcoma or osteosarcoma.

CLAIMS

1. Isolated T cell receptor (TCR) specific for MAGE-A3.

2. Isolated TCR according to claim 1, wherein the TCR specifically recognizes the amino acid sequence SEQ ID NO: 1 or a fragment thereof.

3. Isolated TCR according to claim 1, wherein the TCR does not recognize the amino acid sequence SEQ ID NO: 54 or a fragment thereof.

4. Isolated TCR according to any one of the preceding claims, wherein the TCR specifically recognizes the HLA-A1 bound form of the amino acid sequence of SEQ ID NO: 1, preferably wherein the TCR specifically recognizes the amino acid sequence of SEQ ID NO: 1, which is presented by the HLA-A*01:01-encoded molecule.

5. Isolated TCR according to any one of the preceding claims, wherein the TCR comprises

a) -a TCR α chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 2, a CDR2 having the amino acid sequence of SEQ ID NO: 3 and a CDR3 having the amino acid sequence of SEQ ID NO: 4, and
-a TCR β chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 5, a CDR2 having the amino acid sequence of SEQ ID NO: 6 and a CDR3 having the amino acid sequence of SEQ ID NO: 7; or
b) -a TCR α chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 10, a CDR2 having the amino acid sequence of SEQ ID NO: 11 and a CDR3 having the amino acid sequence of SEQ ID NO: 12, and
-a TCR β chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 13, a CDR2 having the amino acid sequence of SEQ ID NO: 14 and a CDR3 having the amino acid sequence of SEQ ID NO: 15; or
c) -a TCR α chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 18, a CDR2 having the amino acid sequence of SEQ ID NO: 19 and a CDR3 having the amino acid sequence of SEQ ID NO: 20, and

-a TCR β chain comprising a CDR1 having the amino acid sequence of SEQ ID NO: 21, a CDR 2 having the amino acid sequence of SEQ ID NO: 22 and a CDR 3 having the amino acid sequence of SEQ ID NO: 23.

6. Isolated TCR according to any one of the preceding claims, wherein the TCR
a) a variable TCR α region having an amino acid sequence which is at least 80% identical to SEQ ID NO: 8 and a variable TCR β region having an amino acid sequence which is at least 80% identical to SEQ ID NO: 9; or
b) a variable TCR α region having an amino acid sequence which is at least 80% identical to SEQ ID NO: 16 and a variable TCR β region having an amino acid sequence which is at least 80% identical to SEQ ID NO: 17; or
c) a variable TCR α region having an amino acid sequence which is at least 80% identical to SEQ ID NO: 24 and a variable TCR β region having an amino acid sequence which is at least 80% identical to SEQ ID NO: 25.

7. Isolated polypeptide comprising a functional portion of the TCR of any of claims 1 to 6, wherein the functional portion comprises the

a) the amino acid sequences SEQ ID NOs: 2, 3, 4, 5, 6, and 7, or
b) the amino acid sequences SEQ ID NOs: 10, 11, 12, 13, 14, and 15, or
c) the amino acid sequences SEQ ID NOs: 18, 19, 20, 21, 22 and 23.

8. Multivalent TCR complex comprising a least one TCR as claimed in any one of claims 1 to 6.

9. Isolated TCR according to claims 1 to 6, isolated polypeptide according to claim 7, multivalent TCR complex according to claim 8, wherein IFN-γ secretion is induced by binding to the amino acid sequence of SEQ ID NO: 1, which is presented by the HLA-A*01:01-encoded molecule.

10. Nucleic acid encoding a TCR according to any one of claims 1 to 6 or encoding the isolated polypeptide according to claim 7.

11. Vector comprising the nucleic acid of claim 10, wherein the vector is preferably an expression vector, more preferably a retroviral vector or lentiviral vector.

12. Cell expressing the TCR according to claims 1 to 6.

13. Antibody or antigen binding fragment thereof specifically binding to a portion of the TCR according to claims 1 to 6 , wherein the portion of the TCR comprises

a) the CDR3 of the alpha chain of SEQ ID NO: 4 and/or the CDR3 of the beta chain of SEQ ID NO: 7 or;

b) the CDR3 of the alpha chain of SEQ ID NO: 12 and/or the CDR3 of the beta chain of SEQ ID NO: 15 or;

c) the CDR3 of the alpha chain of SEQ ID NO: 20 and/or the CDR3 of the beta chain of SEQ ID NO: 23.

14. The TCR according to claims 1 to 6, the polypeptide according to claim 7, the multivalent TCR complex according to claim 8, the nucleic acid according to claim 10, the vector according to claim 11, the cell according to claim 12, or the antibody according to claim 13 for use in therapy.

15. Use of the TCR according to claims 1 to 6, the polypeptide according to claim 7, the multivalent TCR complex according to claim 8, the nucleic acid according to claim 10, the vector according to claim 11, the cell according to claim 12, or the antibody according to claim 13 in the manufacture of a medicament for treating cancer, wherein the cancer is selected from the group consisting of prostate cancer, uterine cancer, thyroid cancer, testicular cancer, renal cancer, pancreatic cancer, ovarian cancer, esophageal cancer, non-small-cell lung cancer, lung adenocarcinoma, squamous cell carcinoma, non-Hodgkin's lymphoma, multiple myeloma, melanoma, hepatocellular carcinoma, head and neck cancer, gastric cancer, endometrial cancer, cervical cancer, colorectal cancer, stomach adenocarcinoma, cholangiocarcinoma, breast cancer, bladder cancer, myeloid leukemia and acute lymphoblastic leukemia, carcinoma, sarcoma or osteosarcoma.