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(54) Title: TUMOR SPECIFIC T-CELL RECEPTORS

(57) Abstract: The present invention relates to a method for the production of novel T-cell receptors (TCR) which provide a reduced risk of adverse events in immune therapy, specifically in adoptive T cell transfer. The TCRs produced according to the method of the invention are specific for tumor cells and do not react with healthy tissue. Furthermore provided are nucleic acids encoding the TCR of the invention, vectors and host cells comprising the TCRs of the invention as well as their use is the treatment of tumorous diseases.

TUMOR SPECIFIC T-CELL RECEPTORS

FIELD OF THE INVENTION

The present invention pertains to a method for the production of novel T-cell receptors (TCR) which provide a reduced risk of adverse events in immune therapy, specifically in adoptive T cell transfer. The TCRs produced according to the method of the invention are specific for tumor cells and do not react with healthy tissue. Furthermore provided are nucleic acids encoding the TCR of the invention, vectors and host cells comprising the TCRs of the invention as well as the use of these compounds in the treatment of tumorous diseases.

DESCRIPTION

Despite remarkable technological advancements in the diagnosis and treatment options available to patients diagnosed with cancer, the prognosis still often remains poor and many patients cannot be cured. Immunotherapy holds the promise of offering a potent, yet targeted, treatment to patients diagnosed with various tumors, with the potential to eradicate the malignant tumor cells without damaging normal tissues. In theory the T cells of the immune system are capable of recognizing protein patterns specific for tumor cells and to mediate their destruction through a variety of effector mechanisms. Adoptive T-cell therapy is an attempt to harness and amplify the tumor-eradicating capacity of a patient's own T cells and then return these effectors to the patient in such a state that they effectively eliminate residual tumor, however without damaging healthy tissue. Although this approach is not new to the field of tumor immunology, still many drawbacks in the clinical use of adoptive T cell therapy impair the full use of this approach in cancer treatments.

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 αβ and γδ forms, which are structurally similar but have quite distinct anatomical locations and probably functions. The extracellular portion of native heterodimeric αβTCR consists of two polypeptides, each of which has 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 domains contain the highly polymorphic

loops analogous to the complementarity determining regions (CDRs) of antibodies. The use of TCR gene therapy overcomes a number of current hurdles. It allows equipping patients' own T cells with desired specificities and generation of sufficient numbers of T cells in a short period of time, avoiding their exhaustion. The TCR will be transduced into central memory T cells or T cells with stem cell characteristics, which may ensure better persistence and function upon transfer. TCR-engineered T cells will be infused into cancer patients rendered lymphopenic by chemotherapy or irradiation, allowing efficient engraftment but inhibiting immune suppression. Transgenic mice expressing human MHC molecules and a diverse human TCR repertoire serve as a tool to rapidly analyze whether peptide antigens are immunogenic, i.e. are they efficiently processed and presented by MHC molecules, do they efficiently induce T cell responses following immunization (Li et al. 2010 Nat Med).

Using the human TCR transgenic mouse, any human peptide sequence not encoded by the mouse genome is suitable for immunization and will yield TCRs with optimal affinity. Optimal affinity means that the T cells are restricted to human self-MHC molecules and recognize the peptide antigen as foreign, e.g. represent the non-tolerant repertoire. By using peptide/MHC multimers, specific T cells of the transgenic mice can be sorted, human TCRs isolated, e.g. by single cell PCR, the TCRs optimized for efficient expression while avoiding mispairing with endogenous TCR and used for transduction of patients' T cells with viral vectors (Uckert et al. 2008 Cancer Immunol Immunother; Kammertoens T et al. 2009 Eur J Immunol).

The key problem of ATT is to target the right antigen to prevent tumor recurrence and toxic side effects. This sounds simple given the large number of putative tumor antigens. However, most are tumor-associated (self) antigens (TAAs). TAAs are also expressed by normal cells. Expression by rare but vital cells has usually not been analyzed. Moreover, TAA expression may be heterogeneous within the tumor/metastases of a given individual. Thus, targeting TAAs bears the risk of ineffective long-term responses and destruction of normal tissues.

Clinical trials with TCR (or chimeric antibody receptor; CAR)-engineered T cells, e.g. directed against Melan-A/MART-1, gp100, HER-2 and carcinoembryonic antigen, support this assumption.

Morgan RA and colleagues present a case report on a patient with ERBB2 overexpressing cancer that was treated by infusing T cells transduced with a chimeric antigen receptor recognizing ERBB2 into the patient. After 15 minutes of the infusion the patient experienced respiratory distress and dramatic pulmonary infiltrate. The patient died after 5 days. This dramatic outcome underlines the problem of toxic adverse effects in the context of adoptive T cell therapy.

Another approach makes use of the immunization of mice, the subsequent isolation of the T-cells and T cell receptors from these cells, in order to transduce autologous peripheral lymphocytes of a tumor patient. The transduced lymphocytes were expanded and then re-infused. Although tumor regression was observed, the patients still showed the destruction of normal cells (Johnson LA et al. 2009 Blood).

Parkhurst and colleagues (2010 Mol Ther) genetically engineered autologous T lymphocytes of patients suffering from metastatic colorectal cancer refractory to standard treatments. The T lymphocytes were altered to express a murine T cell receptor directed at the carcinoembryonic antigen (CEA). Again the report shows regression of the tumor, however with severe transient inflammatory colitis as side effect in all patients.

Thus, for many, if not most, tumor associated antigens substantial toxicity by effective adoptive T cell therapy is predictable.

In view of the above described major drawbacks in the background art, it is the objective of the present invention to provide novel approaches for adoptive T-cell therapy which can overcome the severe side effects observed in immune therapy when genetically engineered T cell receptors are introduced into autologous lymphocytes and re-infused into a human patient. A more specified object of the present invention is to provide novel antigen recognizing constructs which specifically target tumor cells and not healthy cells.

In a first aspect of the present invention, the above objective is solved by a method for the production of a human T cell receptor (TCR) or a T-cell, which is specific for tumorous cells and has reduced adverse effects in adoptive T-cell therapy, comprising the method steps of

a. Providing a host organism expressing un-rearranged human TCR loci,

b. Immunizing said host organism with a peptide comprising an epitope specific for a tumor specific antigen (TSA),

c. Isolating from said host organism or cell a T cell clone having an activity against said human mutated TSA,

d. Optionally, isolating from said T cell clone the TCR,

wherein said TSA is selected out of the class of somatic mutated antigens.

The surprising finding of the present invention is that if, by contrast to the state of the art approaches, mutant cancer-driving oncogenes, specifically TSAs out of the class of somatic mutated antigens, are targeted by adoptive T cell therapy (ATT), many of the problems with TAAs as known in the state of the art are resolved. Except for antigens encoded by cancer viruses, mutated antigens are the only exclusively tumor-specific antigens.

Of course, the TCRs produced in accordance with the herein described method of the invention do not only provide their advantageous effects in adoptive T cell therapy, but also in any other therapeutic approach wherein the specific binding of the TCR to its target is employed.

The TCRs isolated in accordance with the method of the present invention are advantageous over the state of the art antigen recognizing constructs due to their reduced risk for adverse effects which are observed in adoptive T cell therapy. Adverse effects in context of adoptive T cell transfers are mainly due to autoimmune reactions or to off-target reactions. The present invention specifically intends to solve the former problem by providing T cells which are highly specific to tumor cells and do not mediate an immune reaction against a patient's healthy tissue. Adverse events following infusion of human autologous or allogeneic lymphocytes that the present invention seeks to reduce can be various. In a preferred embodiment of the present invention the TCR obtained by the present invention provide a reduced risk when used in adoptive T cell therapy for inducing healthy tissue damage, which might result in edema and necrosis.

In one preferred embodiment of the present invention said host organism further comprises a transgene for the expression of a human major histocompatibility complex (MHC) class I or II allele. Preferably the MHC is a human leucocyte antigen (HLA) type which is known or suspected to be able to present said mutated TSA. Even more preferred is that the HLA type which is expressed in said host organism is known or suspected to be able to present a peptide

derived from said mutated TSA. This peptide should comprise an amino acid sequence including the mutation which is specifically present in the mutated TSA opposed to the corresponding un-mutated (wild type) version of the same protein.

HLAs corresponding to MHC class I comprise the types A, B, and C. HLA class I complexes present peptides which are processed inside the presenting cell (including alien peptides´such as viral peptides if present). In general, such HLA class I peptides are small polymers, about 9 amino acids in length. HLAs corresponding to MHC class II comprise the types DP, DM, DOA, DOB, DQ, and DR. HLA class II complexes present antigens originating from the outside of the cell. They can be of a length between 12 and 18 amino acids. The characterization of the responsible HLA alleles presenting an antigen of choice is a methodology generally known in the art.

In said host organism used in accordance with the present invention – insofar it is not a human – the un-rearranged human TCR loci are preferably present as one or more transgenes in the genome of said host organism. Preferably these loci encode TCR α and β chains, and preferably comprise a plurality, ideally all, of human TCR V, D, J, and/or C genes.

For the method in accordance with the present invention it is preferably a prerequisite that said host organism has an adaptive immune system and/or is able to mount a VDJC rearrangement within said human TCR loci. Furthermore a host organism is preferred which is able to express heterologous TCRs. In certain preferred embodiments of the invention said host organism is a transgenic animal, preferably a mammal, more preferably a non-human mammal, most preferably a mouse, a rat, a donkey, a rabbit, a hare or a monkey, or any animal which is known in the art to be a host for the generation of T-cells.

In the context of such embodiments of the invention which relate to the above method and wherein non-human host organisms are used, such a non-human host organism preferably further comprises inactivated endogenous TCR loci, preferably wherein said endogenous TCR loci encode for the TCR α and β chains of said non-human host organism.

In one very specific embodiment of the present invention said host-organism is an "ABabDII" mouse. The term "ABabDII" mouse refers to the transgenic animal produced as described in Li et al., 2010;16:1029-34 Nature Medicine. Of course it is understood that also any other

transgenic animal produced with the same methodology as described in Li et al. shall be encompassed as a suitable host organism for use in the herein described embodiments of the invention.

An alternative embodiment relates to a method, wherein a human, for example a healthy individual or a human patient suffering from a tumorous disease, is immunized with said peptide as described herein. In this embodiment T cells can be isolated subsequent to the immunization process from the blood of the human subject. This embodiment has the advantage that the improved T cell receptor is expressed on human, ideally autologous, T cells which can then be used for reinfusion in adoptive T cell therapy.

The peptide used for the immunization of the host organism in context of the method of the present invention comprises an amino acid sequence which is in at least one amino acid residue mutated compared to the amino acid sequence of the corresponding wild-type cellular protein. The present invention relates to the use of tumor specific antigens, therefore proteins which were mutated in the development of tumor cells and thus in this specific mutated form exclusively are present in tumor cells. Normal, healthy, cells however might still express the original un-mutated (wild type) protein. Thus, for the herein described invention it is specifically preferred that the peptide used for immunization comprises in its sequence the mutation which differentiates the TSA from the original un-mutated cellular protein. Preferred peptides for use in the method of the invention comprise any of the sequences shown in SEQ ID No. 1 to 27. In preferred embodiments of the invention the peptide for immunization comprises the amino acid sequence shown in SEQ ID No. 1.

Antigens which are specifically expressed in tumor cells and not in healthy tissue can be categorized into four types: (I) mutated antigens develop during tumor-genesis by point mutations or translocations within the tumor cells. Those antigens are strictly tumor-specific. In the context of the invention these antigens are referred to as tumor specific antigens (TSA). (II) cancer/germline antigens are usually expressed solely within the germ cells of an adult organism and not in healthy somatic tissue. In cancer cells, however, due to the loss of epigenetic regulation, germ-cell specific genes can be activated. (III) differentiation antigens are expressed in tumors and their healthy progenitor cells. CTL responses against such antigens often result in auto-immune reactions. (IV) overexpressed TAA show only minor expression in healthy cells

whereas in a tumor those antigens are strongly activated. For the present invention it is preferred that only antigens of the first type are used.

For the invention TSAs formed by any kind of mutation are comprised. For merely illustrative reasons the following types of mutations are described: amino acid substitution, deletion, addition, insertion, or chemical or post-translational modification. Furthermore included are chromosomal translocations and exclusively in tumor cells expressed splice variants, for example which occur by unspecific splicing mutations resulting in new splice sites.

For the immunization process said peptide can have any length. A minimum requirement is however the presence of the epitope containing the above mentioned mutated sequence. Preferred peptides of the invention have a length of 100 amino acids, preferably of 50 amino acids, more preferably of 30 amino acids, even more preferably 8 to 16 amino acids. The exact peptide length might vary depending on whether the TSA is MHC class I or MHC class II presented.

In order to enhance immunization of the host organism, it is preferred that an adjuvant is used together with the peptide. An adjuvant is for example, without being limiting thereto, CpG and/or incomplete Freunds adjuvant. After the initial immunization with the peptide, said host organisms is treated preferably at least one or two, three or four more times with said peptide and/or the adjuvant of choice. Freund's adjuvant is a solution of (mineral) oil wherein the antigen for immunization is emulsified. Incomplete Freund's adjuvant, as preferably used in this invention, does not contain any mycobacterial components.

During and after the immunization process said host-organism should develop T cells expressing rearranged T cell receptors specific against the TSA of the invention. Such T-cell clones are then in a preferred embodiment isolated from said host organism. For example the cells can be isolated from spleen cells, lymph node cells or blood. T cell clones are selected for example via the surface expression of CD4 or CD8, depending on whether the TSA epitope is MHC class I or II. Methods for the isolation of single T cell clones form host organisms are well known for the person of skill in the art. The present invention is not restricted to a specific methodology for isolating T cells. However, in one preferred embodiment of the invention, said T cells or said T cell clone is after isolation further tested for the expression of a TCR binding to the TSA used in the method of the invention. This is preferably done

by tetramer binding (staining) using TSA specific HLA tetramers. Optionally, the isolated T cell or T cell clone is also tested for its specificity to the TSA compared with the un-mutated version of the cellular protein. To this end, T cell reactivity against peptides comprising the mutation and against peptides comprising the wild-type version is compared. In a preferred embodiment such T cells or T cell clones are isolated in accordance with the method of the invention, which are highly selective for the TSA and not the un-mutated version of the cellular protein.

Another embodiment of the invention relates to a method as described herein, where after isolation of the T cell or T cell clone, the TCR sequence is cloned. In this embodiment the method in step d. as described above, comprises the further method steps of (i) preparing cDNA from said T-cell clone, and (ii) amplifying said cDNA, and (iii) cloning the respective TCR α and β genes into a vector. Preferably a retroviral vector for the transduction of human peripheral blood lymphocytes is used as a vehicle for the TCR of the invention. Means and methods for such a cloning procedure are well known to the skilled person.

In another preferred embodiment of the invention the TSA used is expressed in a tumor cell or tumor disease.

As used herein, the term "tumor " or “tumor disease” means both benign and malignant tumors or neoplasms and includes melanomas, lymphomas, leukemias, carcinomas and sarcomas. Illustrative examples of tumor tissues are cutaneous such as malignant melanomas and mycosis fungoides; hematologic tumors such as leukemias, for example, acute lymphoblastic, acute myelocytic, or chronic myelocytic leukemia; lymphomas such as Hodgkin's disease or malignant lymphoma; gynecologic tumors such as ovarian and uterine tumors; urologic tumors such as those of the prostate, bladder, or testis; soft tissue sarcomas, osseus, or non-osseous sarcomas, breast tumors; tumors of the pituitary, thyroid, and adrenal cortex; gastrointestinal tumors such as those of the esophagus, stomach, intestine, and colon; pancreatic and hepatic tumors; laryngeae papillomestasas and lung tumors. Preferred tumors in the context of the present invention are selected from melanoma, lung tumor, endometrial tumors, glioma, lymphoma, leukemia or prostate tumor.

Exemplary TSAs which can be subject to the inventive method described herein – without being limiting for the invention –are described in Krauthammer et al. 2012 (Nature Genetics).

A preferred selection of TSAs which are presented by HLA type A2 are RAC1, RAC2, RHOT1, MAP2K1, MAP2K2, Nos1, EGFR, SMCA4, STK11, ARID1A, RBM10, U2AF1, EP300, CHD4, FBXW7, H3F3A, KLHL6, SPOP, or MED12. Their respective mutated epitope sequences are provided in the examples section herein below.

The object of the present invention is furthermore solved by a nucleic acid molecule encoding for a TCR obtained or obtainable by the method in accordance with the present invention. Furthermore provided in the present invention are nucleic acid molecules which encode for the respective a α or β chains of an TCR of the invention, or for a variable or constant domain of a TCR of the invention, or for a fragment of a TCR of the invention, preferably wherein such a fragment of the TCR still has the activity/ability for binding its TSA. In addition to that, the nucleic acid molecule optionally has further sequences which are necessary for protein expression of the nucleic acid sequence, specifically for an expression in a mammalian/human, most preferably an immune cell. The nucleic acid used can be contained in a vector suitable for allowing expression of the nucleic acid sequence corresponding to the TCR in a cell.

Also provided is a vector or a cell comprising a nucleic acid molecule described herein above, specifically wherein the vector is for use in medicine. Also a cell comprising a vector according to the invention is provided.

In another aspect the invention provides the T-cell receptor (TCR), or a fragment thereof, as obtained or obtainable by the method of the present invention. In this context it is specifically preferred that the TCR of the invention is a TCR which shows reduced adverse effects in immune therapy. The TCR of the invention preferably does not target healthy cells or tissue, which express the un-mutated (wild-type) version of the TSA used for the generation of the TCR. The TCR of the invention in preferred embodiments does not induce necrosis events, and does not mount when given to subject an immune response against healthy cells or tissue. preferred TCR of the invention is a TCR specific for the epitope shown in SEQ ID No. 1.

Preferably a TCR in accordance to the invention may be a TCR as described herein below.

Yet another embodiment of the invention pertains to a single chain TCR (scTCR, preferably an αβ-scTCR, which are derived from a sequence of a TCR of the present invention. Single-

chain TCRs (scTCRs) are artificial constructs consisting of a single amino acid strand. An scTCR can comprise a polypeptide of a variable region of a first TCR chain (e.g., an [alpha] chain) and a polypeptide of 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, as described herein.

Also provided is such a scTCR of the invention or other TCR derived molecule of the invention, which is fused to a human cytokine, such as IL-2, IL-7 or IL-15. TCRs of the present invention can also be provided as a multimeric complex, comprising at least two scTCR or TCR molecules, wherein said scTCR or TCR molecules are interconnected for example by an introduced biotin-streptavidin functionality.

In another aspect of the present invention a host cell is provided, comprising a vector a nucleic acid or a TCR molecule as described herein above. In preferred embodiments of the invention the host cell is a human cell, preferably a human T-lymphocyte, which is positive for the expression of CD4 or CD8. Such a host cell of the invention is preferably obtained by transduction of a nucleic acid or vector in accordance with the present invention. Transduction methods for introducing nucleic acid molecules into T cells are well known in the art and include without being limiting thereto viral transduction vehicles.

In an alternative aspect of the invention a T-cell is provided obtained or obtainable by a method for the production of a human T cell receptor (TCR), which is specific for tumorous cells and has reduced adverse effects in adoptive T-cell therapy as described herein above. Such a T cell is depending on the host organism used in the method of the invention for example a human or non-human T-cell, preferably a non-human T-cell expressing a human TCR.

The provided compounds of the invention are in a further aspect for use in medicine, for example for use in the treatment of a cancerous disease, specifically wherein the cancerous disease is characterized by the specific expression of said mutated TSA. Most preferably the compounds of the invention are used in a cancer treatment that involves an adoptive T-cell transfer.

Yet another aspect of the invention relates to a method of treating a human subject, specifically human subject suffering from a tumor disease. The method of treatment comprises the administration of any of the aforementioned compounds into a patient in need of such a treatment. The administration of the compounds of the invention can for example involve the 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 or TCR of the present invention.

Thus also provided is a pharmaceutical composition, comprising a TCR or TCR fragment according to the invention, or a nucleic acid, a vector, a host cell, or an isolated T cell according to the invention. In a preferred embodiment the pharmaceutical composition is for immune therapy.

Examples of pharmaceutically acceptable carriers or diluents useful in the present invention include stabilizers such as SPGA, carbohydrates (e.g. sorbitol, mannitol, starch, sucrose, glucose, dextran), proteins such as albumin or casein, protein containing agents such as bovine serum or skimmed milk and buffers (e.g. phosphate buffer).

Tumor antigens that are preferably used in the methods of the present invention to obtain a TCR of the invention are listed in tables 1 and 2 below (the mutation is indicated as amino acid exchange within the epitope in brackets):

Table 1:

Gene	Protein	Epitope
Rac1	Ras-related C3 botulinum toxin substrate 1	27-35 (P29S)
TRRAP	transformation/transcription domain-associated protein	715-723 (S722F)
Rac2	Ras-related C3 botulinum toxin substrate 2	28-36 (P29L)
		28-36 (P29Q)
Nos1	Nitric oxide synthase	770-779 (S771L)
ARID1A	AT-rich interactive domain-containing protein 1A	1999-2007 (E2000V)
		1021-1031 (W1022L)
H3F3A	Histone H3.3	28-36 (G34V)
KLHL6	Kelch-like protein 6	48-56 (F49L)
ID3	Inhibitor of DNA binding 3	50-58 (L54V)
FLT3	Fms-related tyrosine kinase 3	835-843 (D835Y)
		835-843 (D835V)
FBXW7	F-box/WD repeat-containing protein 7	456-464 (F462S)
		456-464 (A463T)
Med12	Mediator of RNA polymerase II transcription subunit 12	724-732 (D727E)
CDK12	Cyclin-dependent kinase 12	898-906 (Y901C)
CDC42	Cell division cycle 42	7-14 (G12V)
SMARCA4	SWI/SNF related, matrix associated, actin dependent regulator	1153-1161

	of chromatin, subfamily a, member 4	(G1159W)
SMO	Smoothened, frizzled family receptor	412-420 (L412F)
SF3B1	Splicing factor 3b, subunit 1	693-701 (K700E)
CHD4	Chromodomain-helicase-DNA-binding protein 4	907-916 (L912V)
SPOP	Speckle-type POZ protein	83-91 (Y87N)
		83-91 (Y87C)
MAP2K2	Dual specificity mitogen-activated protein kinase2	154-162 (S154F)
Notch1	Notch1	1568-1576 (L1574P)
		1592-1600 (R1598P)
FOXA1	Forkhead Box A1	221-229 (D226N)
2nd NT5C2	5'-Nucleotidase, Cytosolic II	a) 233-241 b) 236-244 (R238L)
2nd Bcr-Abl	Bcr-Abl	247-255 (E255K)
RHOT1	Mitochondrial Rho GTPase 1	29-37 (P30L)
MAP2K1	Dual specificity mitogen-activated protein kinase1	20-28 (E20K)
EGFR	Epidermal growth factor receptor	717-725 (G719A)
		1125-1133 (H1129Y)
STK11	Serine/threonine-protein kinas	219-228 (P221L)
RBM10	RNA-binding protein 10	316-324 (I316F)
U2AF1	Splicing factor U2AF 26 kDa subunit	28-36 (S34F)
EP300	Histone acetyltransferase p300	1623-1631 (R1627W)
CDK4	Cyclin-dependent kinase 4	23-32 (R24C)
		23-32 (R24L)
PPP6C	Protein phosphatase 6, catalytic subunit	269-277 (S270L)
TACC1	Transforming, acidic coiled-coil containing protein 1	792-801 (C794F)
KRAS	V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	5-14 (G12V)
TRAF7	TNF receptor-associated factor 7, E3 ubiquitin protein ligase	518-527 (N520S)
		531-541 (G536S)
HIST1H3B	Histone cluster 1, H3b	26-35 (K27M)
ALK	Anaplastic lymphoma receptor tyrosine kinase	1272-1280 (R1275Q)
ABL1	C-abl oncogene 1, non-receptor tyrosine kinase	251-260 (E255K)
		247-255 (E255V)
CBL	Cbl proto-oncogene, E3 ubiquitin protein ligase	398-406 (H398Y)
NPM1	Nucleophosmin (nucleolar phosphoprotein B23, numatrin)	283-291 (c.863_864insTCTG Insertion)
		283-291 (c.863_864insCATG Insertion)
		283-291 (c.863_864insCATG Insertion)
EZH2	Enhancer of zeste homolog 2	637-645 (Y641F)
GNAS	GNAS complex locus	201-210 (R201C)
PDGFRA	Platelet-derived growth factor receptor, alpha polypeptide	841-849 (D842V)
TSHR	Thyroid stimulating hormone receptor	451-459 (M453T)
KIT	V-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	636-644 (K642E)
STAT3	Signal transducer and activator of transcription 3	a) 654-662
		b) 659-667 (D661Y)
CTNNB1	Catenin (cadherin-associated protein), beta 1	30-39 (S33C)
		30-39 (S33F)
		30-39 (S33Y)
STK11	Serine/threonine kinase 11	219-228 (P221L)
ERBB2	V-erb-b2 erythroblastic leukemia viral oncogene homolog 2	773-782 (G776V)
SLIT2	Slit homolog 2	8-16 (M8I)
CDKN2A	Cyclin-dependent kinase inhibitor 2A	113-121 (P114L)
XPO1	Exportin 1	568-576 (E571K)

The above described TCR of the invention pertain in preferred embodiments to the following TCR molecules:

The present invention pertains to a TCR alpha chain, comprising a CDR3 region with the sequence shown in any one of SEQ ID NO: 28, 30, 32, 33, 36, 38 or 40. Preferred are TCR alpha chains comprising a variable domain having the sequence shown in any one of SEQ ID NO 42, 44, 46, 47, 50, 52, or 54.

The present invention pertains to a TCR beta chain, comprising a CDR3 region with the sequence shown in any one of SEQ ID NO: 29, 31, 34, 35, 37, 39 or 41. Preferred are TCR beta chains comprising a variable domain having the sequence shown in any one of SEQ ID NO 43, 45, 48, 49, 51, 53, or 55.

Preferred embodiments of the invention pertain to specific TCR isolated, or produced (obtained) according to any one of the methods as described herein. Such TCRs of the invention are preferably TCRs specific for targeting a mutated antigen selected from table 1 or 2. The Rac-1 or TRRAP mutated antigen are preferred. More specifically such TCRs are preferred which have the capacity to specifically bind to the mutated Rac-1 epitope FSGEYIPTV, or the mutated TRAPP epitope KLVFGSVFL.

Preferred TCR of the present invention are furthermore characterized by the presence of a CDR3 region comprising any one of the amino acid sequences shown in SEQ ID NO. 28 to 41. A Rac-1 TCR in accordance with the invention, with an alpha or beta chain, preferably comprises a CDR3 having a sequence shown in any one of SEQ ID NO: 28 to 39. A preferred TRRAP TCR in accordance with the present invention is characterized by the presence of a CDR3 amino acid sequence selected from the sequence shown in SEQ ID NO: 40 or 41.

More preferred is an alpha/beta TCR having an alpha chain comprising the CDR3 sequence shown in SEQ ID NO: 28, and a beta chain comprising the CDR3 sequence shown in SEQ ID NO: 29; an alpha/beta TCR having an alpha chain comprising the CDR3 sequence shown in SEQ ID NO: 30, and a beta chain comprising the CDR3 sequence shown in SEQ ID NO: 31; an alpha/beta TCR having an alpha chain comprising the CDR3 sequence shown in SEQ ID

NO: 32, and a beta chain comprising the CDR3 sequence shown in SEQ ID NO: 34; an alpha/beta TCR having an alpha chain comprising the CDR3 sequence shown in SEQ ID NO: 32, and a beta chain comprising the CDR3 sequence shown in SEQ ID NO: 35; an alpha/beta TCR having an alpha chain comprising the CDR3 sequence shown in SEQ ID NO: 33, and a beta chain comprising the CDR3 sequence shown in SEQ ID NO: 34; an alpha/beta TCR having an alpha chain comprising the CDR3 sequence shown in SEQ ID NO: 33, and a beta chain comprising the CDR3 sequence shown in SEQ ID NO: 35; an alpha/beta TCR having an alpha chain comprising the CDR3 sequence shown in SEQ ID NO: 36, and a beta chain comprising the CDR3 sequence shown in SEQ ID NO: 37; an alpha/beta TCR having an alpha chain comprising the CDR3 sequence shown in SEQ ID NO: 38, and a beta chain comprising the CDR3 sequence shown in SEQ ID NO: 39; an alpha/beta TCR having an alpha chain comprising the CDR3 sequence shown in SEQ ID NO: 40, and a beta chain comprising the CDR3 sequence shown in SEQ ID NO: 41.

The TCR chains comprised in a TCR of the invention may furthermore comprise at least one, preferably two, most preferably all three CDR regions as present in one of the variable regions of any one of TCR 1 to 7. The sequences of said variable regions which contain all three CDR regions are shown in SEQ ID NO 42 to 55.

In another preferred embodiment the TCR of the invention comprises at least one variable region of an alpha and/or beta chain selected from a variable region of an alpha or beta chain of any one of the TCR T1 to T7 of the invention as depicted herein below in table 3.

The TCR as isolated in context of the present invention comprise the following variable regions (CDR3 regions are underlined):

Rac-1 TCR:

TRAV20*02-CAVQTSQGGSEKLVF-TRAJ57*01

MEKMLECAFIV LWLQLGWLSG EDQVTQSPEA LRLQEGESSS LNCSYTVSGL RGLFWYRQDP GKGPEFLFTL YSAGEEKEKE RLKATLTKKE SFLHITAPKP EDSATYLCAV QTSQGGSEKL VFGKGTKLTV NPYIQNPEPA (SEQ ID NO:42)

TRBV4-1*01-CASSQDASGIYYEQYF-TRBD2*02-TRBJ2-7*01

MGCRLLCCAV LCLLGAVPID TEVTQTPKHL VMGMTNKKSL KCEQHMGHRA MYWYKQKAKK PPELMFVYSY EKLSINESVP SRFSPECPNS SLLNLHLHAL QPEDSALYLC ASSQDASGIY YEQYFGPGTR LTVT (SEQ ID NO:43)

TRAV13-1*01-CAASRGGAQKLVF-TRAJ54*01

MTSIRAVFIF LWLQLDLVNG ENVEQHPSTL SVQEGDSAVI KCTYSDSASN YFPWYKQELG KGPQLIIDIR SNVGEKKDQR IAVTLNKTAK HFSLHITETQ PEDSAVYFCA ASRGGAQKLV FGQGTRLTIN PN (SEQ ID NO:44)

TRBV3-1*01-CASSQLAGGPLYNEQFF-TRBD2*02-TRBJ2-1*01

MGCRLLCCVV FCLLQAGPLD TAVSQTPKYL VTQMGNDKSI KCEQNLGHDT MYWYKQDSKK FLKIMFSYNN KELIINETVP NRFSPKSPDK AHLNLHINSL ELGDSAVYFC ASSQLAGGPL YNEQFFGPGT RLTVL (SEQ ID NO:45)

TRAV5*01-CAESKRFSDGQKLLF-TRAJ16*01

MR QVARVIVFLT LSMSRGEDVE QSLFLSVREG

DSSVINCTYT DSSSTYLYWY KQEPGAGLQL LTYIFSNMDM KQDQRLTVLL NKKDKHLSLR IADTQTGDSA IYFCAESKRF SDGQKLLFAR GTMLKVDLN (SEQ ID NO:46)

TRAV12-2*02-CAAQSARQLTF-TRAJ22*01

M MKSLRVLLVI LWLQLSWVWS QQKEVEQNSG PLSVPEGAIA SLNCTYSDRG SQSFFWYRQY SGKSPELIM SIYSNGDKED GRFTAQLNKA SQYVSLLIRD SQPSDSATYL CAAQSARQLT FGSGTQLTVL PD (SEQ ID NO:47)

TRBV20-1*01(/02)-CSARDLITDTQYF-TRBJ2-3*01

MLLLLL LLGPGSGLGA VVSQHPSWVI CKSGTSVKIE CRSLDFQATT MFWYRQFPKQ SLMLMATSNE GSKATYEQGV EKDKFLINHA SLTLSTLTVT SAHPEDSSFY ICSARDLITD TQYFGPGTRL TVL (SEQ ID NO:48)

TRBV3-1*01-CASSPWQETQYF-TRBJ2-5*01

MGCRLL CCVVFCLLQA GPLDTAVSQT PKYLVTQMGN DKSIKCEQNL GHDTMYWYKQ DSKKFLKIMF SYNNKELIIN ETVPNRFSPK SPDKAHLNLH INSLELGDSA VYFCASSPWQ ETQYFGPGTR LLVL (SEQ ID NO:49)

TRAV13-1*01 CAASLGSGNTPLVF TRAJ29*01

M TSIRAVFIFL WLQLDLVNGE NVEQHPSTLS VQEGDSAVIK CTYSDSASNY FPWYKQELGK GPQLIIDIRS NVGEKKDQRI

AVTLNKTAKH FSLHITETQP EDSAVYFCAA SLGSGNTPLV FGKGTRLSVI AN (SEQ ID NO:50)

TRBV28*01 CASSLHSGRDTQYF TRBJ2-3*01 TRBD2*02

MGIRLLCR VAFCFLAVGL VDVKVTQSSR YLVKRTGEKV FLECVQDMDH ENMFWYRQDP GLGLRLIYFS YDVKMKEKGD IPEGYSVSRE KKERFSLILE SASTNQTSMY LCASSLHSGR DTQYFGPGTR LTVL (SEQ ID NO:51)

TRAV13-2*01 CAENRGANSKLTF TRAJ56*01 F

MMAGIRALF MYLWLQLDWV SRGESVGLHL PTLSVQEGDN SIINCAYSNS ASDYFIWYKQ ESGKGPQFII DIRSNMDKRQ GQRVTVLLNK TVKHLSLQIA ATQPGDSAVY FCAENRGANS KLTFGKGITL SVRPD (SEQ ID NO:52)

TRBV12-3*01 CASSFTGGFYGYTF TRBJ1-2*01 TRBD1*01

MDSWTFCCVS LCILVAKHTD AGVIQSPRHE VTEMGQEVTL RCKPISGHNS LFWYRQTMMR GLELLIYFNN NVPIDDSGMP EDRFSAKMPN ASFSTLKIQP SEPRDSAVYF CASSFTGGFY GYTFGSGTRL TVV (SEQ ID NO:53)

TRRAP TCR:

TRAV17*01-CATDWYTGANSKLTF-TRAJ56*01

METLLGVSLV ILWLQLARVN SQQGEEDPQA LSIQEGENAT MNCSYKTSIN NLQWYRQNSG RGLVHLILIR SNEREKHSGR LRVTLDTSKK SSSLLITASR AADTASYFCA TDWYTGANSK

LTFGKGITLS VRPD (SEQ ID NO:54)

TRBV6-2*01-CASSYSGYEQYF-TRBD1*01-TRBJ2-7*01

MSLGLLCCAA FSLLWAGPVN AGVTQTPKFR VLKTGQSMTL

LCAQDMNHEY MYWYRQDPGM GLRLIHYSVG EGTTAKGEVP

DGYNVSRLKK QNFLLGLESA APSQTSVYFC ASSYSGYEQY FGPGTRLTVT (SEQ ID NO:55)

One further preferred embodiment of the invention provides a TCR alpha and/or beta chain, or a fragment thereof, comprising a sequence selected from the group of SEQ ID NO 42 to 55. Preferably the TCR of the invention is heterodimeric TCR comprising an alpha chain comprising a sequence according to SEQ ID NO 42, and a beta chain comprising a sequence according to SEQ ID NO 43, or comprising an alpha chain comprising a sequence according to SEQ ID NO 44, and a beta chain comprising a sequence according to SEQ ID NO 45, or comprising an alpha chain comprising a sequence according to SEQ ID NO 46, and a beta chain comprising a sequence according to SEQ ID NO 48, or comprising an alpha chain comprising a sequence according to SEQ ID NO 46, and a beta chain comprising a sequence according to SEQ ID NO 49, or comprising an alpha chain comprising a sequence according to SEQ ID NO 47, and a beta chain comprising a sequence according to SEQ ID NO 48, or comprising an alpha chain comprising a sequence according to SEQ ID NO 47, and a beta chain comprising a sequence according to SEQ ID NO 49, or comprising an alpha chain comprising a sequence according to SEQ ID NO 50, and a beta chain comprising a sequence according to SEQ ID NO 51, or comprising an alpha chain comprising a sequence according to SEQ ID NO 52, and a beta chain comprising a sequence according to SEQ ID NO 53, or comprising an alpha chain comprising a sequence according to SEQ ID NO 54, and a beta chain comprising a sequence according to SEQ ID NO 55.

In even more preferred aspects of the invention the TCR of the invention is a TCR comprising at least one TCR alpha or beta chain selected from the TCR chains of any one of TCRs T1 to T7 in table 3 below. Most preferred is that the TCR of the invention is an alpha/beta TCR selected from any one of T1 to T7 as depicted in table 3 herein below.

The aforementioned TCRs of the invention may in some embodiments contain altered amino acid sequences. Preferred is that TCR chains are encompassed by the present invention which are at least 70, 80, 90, 95, 96, 97, 98, or 99 % identical to a TCR sequence, or TCR alpha or beta chain sequence, a TCR variable region according to any one of SEQ ID NO: 42 to 55, or a CDR3 sequence as disclosed herein. Most preferably a TCR of the invention comprises an

alpha and/or beta chain which is at least 90%, or 95%, or 99% identical to an alpha/beta chain of any one of TCRs T1 to T7 as depicted in table 3.

The above described TCR are preferably specific for the mutated antigens of Rac-1 or TRRAP as disclosed in table 1 or 2, in particular when presented on a cell, such as a tumor cell or antigen presenting cell. Furthermore comprised by the present invention are functional fragments of the TCR or TCR chains of the invention. The term "functional fragment of the TCR or TCR chain" shall refer to a fragment of the full length receptor molecule, characterized in that the fragment is derived from that molecule and has maintained the same capability to bind the mutated TAA.

In a further aspect, as already disclosed above, the invention also pertains to the nucleic acids encoding for the TCR molecules of the invention as well as cells comprising these nucleic acids, or cells expressing said TCRs of the invention. The invention furthermore pertains to the use of the TCR proteins or nucleic acids, or cells, in the various methods or uses described herein before.

Preferably aspects of the invention relate to the treatment of tumorous diseases with the methods and various materials of the invention. Preferred diseases are cancers which are characterized by the expression of any one of the mutated TAA as disclosed herein. Preferred is a disease that is characterized by the expression of the mutated epitope of Rac-1 or TRRAP. Preferred diseases treated with a TCR of the invention that is specific for the Rac-1 mutated antigen or the TRRAP mutated antigen are selected from cancerous proliferative diseases, e.g. lung cancer, breast cancer, cervical cancer, colon cancer, gastric cancer, kidney cancer, leukemia, liver cancer, lymphoma, ovarian cancer, pancreatic cancer, prostate cancer, rectal cancer, sarcoma, skin cancer, testicular cancer, and uterine cancer. Particular preferred diseases for Rac1 specific TCRs are melanoma and non-small cell lung cancer.

The present invention will now be further described 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 entireties. In the Figures and Sequences:

Figure 1: shows the specific CD8+ T cell response against HLA-A201 restricted mutated RAC1P29S epitope in ABabDII mice.

SEQ ID No 1 to 27: show mutated epitope sequences of HLA type A2 restricted TSAs as depicted in Table 1.

SEQ ID No 28 to 41: show the CDR3 domain sequences of the TCR of the invention.

SEQ ID No 42 to 55: show the variable regions of the TCR 1 to 7 of the invention.

EXAMPLES

Exemplary tumor specific antigen epitopes which are usable and preferred in the context of the present invention are provided in table 2.

Gene	Protein	HLA A2.01 Epitope*
MELANOMA		
RAC1:	Ras-related C3 botulinum toxin substrate 1	FP/SGEYIPTV
RAC2:	Ras-related C3 botulinum toxin substrate 1	FP/LGEYIPTV
RHOT1:	Mitochondrial Rho GTPase 1	FP/LEEVPPRA
MAP2K1:	Dual specificity mitogen-activated protein kinase1	E/KIKLCDFGV
MAP2K2:	Dual specificity mitogen-activated protein kinase2	E/KIKLCDFGV
		S/FLDQVLKEA
Nos1:	Nitric oxide synthase	KS/LQAYAKTL
LUNG TUMOR		
EGFR:	Epidermal growth factor receptor	VLG/ASGAFGT
SMCA4:	Transcription activator BRG1	LLSTRAG/WGL
STK11:	Serine/threonine-protein kinas	FQP/LPEIANGL
ARID1A:	AT-rich interactive domain-containing protein 1A	MW/LVDRYLAFT
		FE/VMSKHPGL
RBM10:	RNA-binding protein 10	I/FLGALAPYA
U2AF1:	Splicing factor U2AF 26 kDa subunit	RHGDRCS/FRL
ENDOMETRIAL TUMORS		
EP300:	Histone acetyltransferase p300	LMDGR/WDAFL
		LMDGR/QDAFL
CHD4	Chromodomain-helicase-DNA-binding protein 4	NLEEL/VFHLL
FBXW7:	F-box/WD repeat-containing protein 7	TLYGHTF/SAV
		TLYGHTFA/TV
GLIOMA		
H3F3A:	Histone H3.3	QLATKAARK/M
		KSAPSTG/VGV
CLL		
KLHL6:	Kelch-like protein 6	KF/LDDAGLSL
PROSTATE TUMOR		
SPOP:	Speckle-type POZ protein	YLSLY/NLLLV
		YLSLY/CLLLV
		FVQGKDWGF/V
		FVQGKDWGF/L
MED12:	Mediator of RNA polymerase II transcription subunit 12	VLYD/EQPRHV

*wildtype/mutated amino acid

Example 1: RAC1 specific TCR against the FSGEYIPTV Epitope

For the generation of T-cells bearing a RAC1 TSA specific TCR, mice deficient in their endogenous TCR loci and expressing the human TCR repertoire were used. The production and setup of the transgenic mice (AB*ab*DII mice) are in detail described elsewhere (Li LP, Lampert JC, Chen X, Leitao C, Popovic J, Muller W, et al. Transgenic mice with a diverse human T cell antigen receptor repertoire. Nat Med. 2010;16:1029-34.).

AB*ab*DII mice were immunized twice with mutated RAC1P29S epitope (see above). Seven days after the last immunization, pooled spleen and lymph node cells were stimulated *in vitro* with RAC1 mutant or wildtype peptides and analyzed for expression of CD3, CD8 and intracellular IFN-γ. Figure 1 shows CD8+ and IFN-γ+ cells within the CD3+ cell population (percentages indicated by numbers). In parentheses, the percentage of CD8+ and IFN-γ+ T cells within the CD8+ T cell population is given.

Example 2: RAC1 and TRRAP specific TCR of the Invention

Table 3: The following TCR could be isolated:

TCR	**Antigen**	**peptide/ *purification***	**TCR sequence**	**CDR3***
T1	Rac-1	FSGEYIPTV	TRAV20*02-CAVQTSQGGSEKLVF-TRAJ57*01	28
		IFNg-CAPTURE	TRBV4-1*01-CASSQDASGIYYEQYF-TRBD2*02-TRBJ2-7*01	29
T2	Rac-1	FSGEYIPTV	TRAV13-1*01-CAASRGGAQKLVF-TRAJ54*01	30
		IFNg-CAPTURE	TRBV3-1*01-CASSQLAGGPLYNEQFF-TRBD2*02-TRBJ2-1*01	31
T3/T4	Rac-1	FSGEYIPTV	TRAV5*01-CAESKRFSDGQKLLF-TRAJ16*01	32
		A2-TETRAMER	TRAV12-2*02-CAAQSARQLTF-TRAJ22*01	33
			TRBV20-1*01(/02)-CSARDLITDTQYF-TRBJ2-3*01	34
			TRBV3-1*01-CASSPWQETQYF-TRBJ2-5*01	35
T5	Rac-1	FSGEYIPTV	TRAV13-1*01 CAASLGSGNTPLVF TRAJ29*01	36
		A2-TETRAMER	TRBV28*01 CASSLHSGRDTQYF TRBJ2-3*01 TRBD2*02	37
T6	Rac-1	FSGEYIPTV	TRAV13-2*01 CAENRGANSKLTF TRAJ56*01 F	38
		A2-TETRAMER	TRBV12-3*01 CASSFTGGFYGYTF	39

			TRBJ1-2*01 TRBD1*01	
T7	TRRAP	KLVFGSVFL	TRAV17*01-CATDWYTGANSKLTF-TRAJ56*01	40
		IFNg-CAPTURE	TRBV6-2*01-CASSYSGYEQYF-TRBD1*01-TRBJ2-7*01	41

*Sequence identifier

Table 3 provides the sequences of the alpha and beta chains of the isolated TCR of the invention (T1 to T7). The sequences are presented by the known TCR allele sequence and the specific CDR3 amino acid sequence of the TCR of the invention. TCR allele nomenclature is derived from the TCR allele Database IMGT

(http://www.imgt.org/vquest/refseqh.html#VQUEST) Lefranc, M.-P. and Lefranc, G. The T cell receptor FactsBook Academic Press, London, UK (398 pages), (2001).

The variable regions of the TCR chains of the TCR 1 to 7 are provided in SEQ ID No 42 to 55.

For T3/T4, the inventors discovered that in particular the chain combination TRAV5/TRBV20-1 (SEQ ID NO: 32 and34) shows good binding to the Rac1-tetramer.

CLAIMS

1. A method for the production of a human T cell receptor (TCR), which is specific for tumorous cells and has reduced adverse effects in adoptive T-cell therapy, comprising
    a. Providing a host organism expressing unrearranged human TCR loci,
    b. Immunizing said host organism with a peptide comprising an epitope specific for a tumor specific antigen (TSA),
    c. Isolating from said host organism a T cell clone having an activity against said human mutated TSA,
    d. Optionally, isolating from said T cell clone the TCR,

    wherein said TSA is selected out of the class of somatic mutated antigens.

2. The method according to claim 1, wherein said host organism further comprises a transgene for the expression of a human major histocompatibility complex (MHC) class I or II, preferably the human leucocyte antigen (HLA) type which is known to present said mutated TSA.

3. The method according to claim 1 or 2, wherein said unrearranged human TCR loci are present as one or more transgenes in the genome of said host organism.

4. The method according to any one of claims 1 to 3, wherein said human TCR loci encode TCR α and β chains, and preferably comprise a plurality of TCR V, D, J, and/or C genes.

5. The method according to any one of claims 1 to 4, wherein said host organism has an adaptive immune system and/or is able to mount a VDJC rearrangement and expression of heterologous TCRs; preferably said host organism is a transgenic animal, preferably a mammal, more preferably a non-human mammal, most preferably a mouse, a rat, a donkey, a rabbit, a hare or a monkey.

6. The method according to any one of claims 1 to 3, wherein said host organism further comprises inactivated endogenous TCR loci, preferably wherein said endogenous TCR loci encode for TCR α and β chains.

7. The method according to any one of claims 1 to 4, wherein said host organism is an AB*ab*DII mouse.

8. The method according to any one of claims 1 to 7, wherein said peptide comprises an amino acid sequence which is in at least one amino acid residue mutated compared to the amino acid sequence of the corresponding wild-type cellular protein.

9. The method according to claim 8, wherein a mutation is selected from a substitution, deletion, addition, insertion, chromosomal translocation or chemical or post-translational modification of said at least one amino acid residue.

10. The method according to any one of claims 1 to 9, wherein said peptide has a length of 100 amino acids, preferably of 50 amino acids, more preferably of 30 amino acids, even more preferably 8 to 16 amino acids.

11. The method according to any one of claims 1 to 10, wherein in step b. CpG and in-complete Freunds adjuvant is additionally administered to said host-organism to en-hance immunization efficiency.

12. The method according to any one of claims 1 to 11, wherein after initial immunization, said host organisms is treated at least one or more times with said peptide plus CpG and incomplete Freunds adjuvant.

13. The method according to any one of claims 1 to 12, wherein said T-cell clone is isolat-ed from spleen cells, lymph node cells or blood of said host organism.

14. The method according to any one of claims 1 to 13, wherein step d., comprises the fur-ther method steps of (i) preparing cDNA from said T-cell clone, and (ii) amplifying said cDNA, and (iii) cloning the respective TCR α and β genes into a vector.

15. The method according to claim 14, wherein said vector is a retroviral vector for the transduction of human peripheral blood lymphocytes.

16. The method according to any one of claims 1 to 15, wherein said mutated TSA is expressed in a tumor selected from melanoma, lung tumor, endometrial tumors, glioma, lymphoma, leukemia or prostate tumor.

17. The method according to any one of claims 1 to 15, wherein said mutated TAA is selected from RAC1, RAC2, RHOT1, MAP2K1, MAP2K2, Nos1, EGFR, SMCA4, STK11, ARID1A, RBM10, U2AF1, EP300, CHD4, FBXW7, H3F3A, KLHL6, SPOP, or MED12.

18. A nucleic acid molecule encoding for a TCR obtained by the method according to any one of claims 1 to 17, or for an α or β chain thereof, or for a variable or constant domain thereof, or for a fragment thereof having the ability to bind to said mutated TSA.

19. A vector comprising a nucleic acid according to claims 18.

20. A TCR obtainable by a method according to any one of claims 1 to 17, or a α or β chain thereof, or a variable or constant domain thereof, or a fragment thereof having the ability to bind to said mutated TAA antigenic fragments thereof.

21. A TCR comprising an alpha and/or beta chain, wherein said alpha and/or beta chain comprises at least one CDR3 domain according to any one of SEQ ID NO: 28 to 41.

22. The TCR according to claim 21, wherein said TCR comprises at least one variable region selected from a variable region of a TCR single chain of any one of TCR T1 to T7 in table 3, preferably wherein said variable region comprises a sequence according to any one of SEQ ID NO 42 to 55.

23. A host cell, comprising a vector according to claim 19, a nucleic acid according to claim 18 or a TCR according to any one of claims 20 to 22.

24. The host cell according to claim 23, which is a human cell, preferably a T-cell.

25. The host cell according to claim 23, which is a CD4 or CD8 positive T-cell.

26. A non-human T-cell, obtainable by a method according to any one of claims 1 to 17.

27. The non-human T-cell according to claim 26, wherein the T-cell is a non-human T-cell expressing a human TCR.

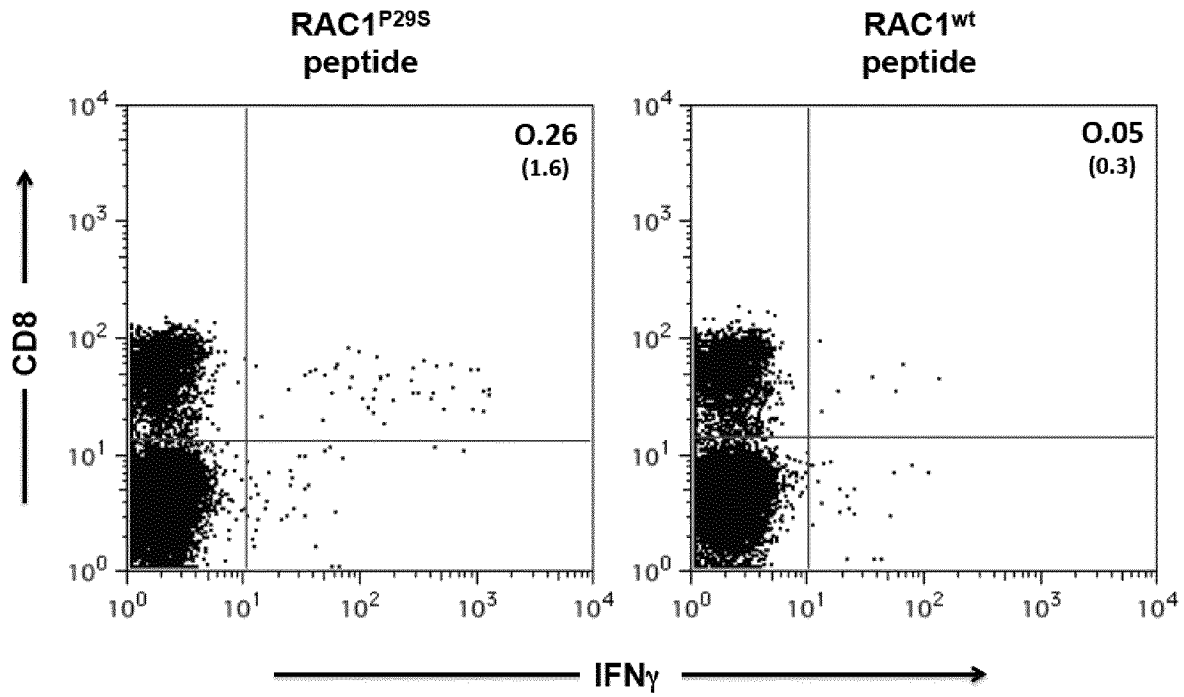
28. A nucleic acid molecule according to claim 18, a vector according to claim 19, a TCR according to any one of claims 20 to 22 or a host cell according to any one of claims 23 to 25, for use in medicine.

29. The nucleic acid molecule, a vector, a TCR or a host cell according to claim 28, for use in the treatment of a cancerous disease, specifically wherein the cancerous disease is characterized by the specific expression of said mutated TSA.

30. The nucleic acid molecule, a vector, a TCR or a host cell according to claim 29, wherein said treatment involves adoptive T-cell therapy.

FIGURES

Figure 1:



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/075141

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/00 C07K14/725 C07K14/47
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, Sequence Search, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NICHOLAS P. RESTIFO ET AL: "Adoptive immunotherapy for cancer: harnessing the T cell response", NATURE REVIEWS IMMUNOLOGY, vol. 12, no. 4, 1 April 2012 (2012-04-01), pages 269-281, XP055034896, ISSN: 1474-1733, DOI: 10.1038/nri3191 the whole document	1-30

Y	MICHAEL KRAUTHAMMER ET AL: "Exome sequencing identifies recurrent somatic RAC1 mutations in melanoma", NATURE GENETICS, vol. 44, no. 9, 29 July 2012 (2012-07-29), pages 1006-1014, XP055098245, ISSN: 1061-4036, DOI: 10.1038/ng.2359 the whole document	1-30

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 January 2014	31/03/2014

Name and mailing address of the ISA/	Authorized officer
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3

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ERAN HODIS ET AL: "A Landscape of Driver Mutations in Melanoma", CELL, vol. 150, no. 2, 1 July 2012 (2012-07-01), pages 251-263, XP055098350, ISSN: 0092-8674, DOI: 10.1016/j.cell.2012.06.024 the whole document	1-30
A	ROBBINS P F ET AL: "A MUTATED BETA-CATENIN GENE ENCODES A MELANOMA-SPECIFIC ANTIGEN RECOGNIZED BY TUMOR INFILTRATING LYMPHOCYTES", THE JOURNAL OF EXPERIMENTAL MEDICINE, vol. 183, no. 3, 1 January 1996 (1996-01-01), pages 1185-1192, XP002914335, ROCKEFELLER UNIVERSITY PRESS, US ISSN: 0022-1007, DOI: 10.1084/JEM.183.3.1185 the whole document	1-30
A	WO 2012/038055 A1 (UNICELL GMBH [DE]; UNIVERSITAETSMEDIZIN DER JOHANNES GUTENBERG UNI MAI) 29 March 2012 (2012-03-29)	1-30
A	-& DATABASE Geneseq [Online] 10 May 2012 (2012-05-10), "Human NY-ESO-1 specific T cell receptor (TCR) beta chain, SEQ ID 31.", XP002719262, retrieved from EBI accession no. GSP:AZU40602 Database accession no. AZU40602 sequence	21,22
A	LIANG-PING LI ET AL: "Transgenic mice with a diverse human T cell antigen receptor repertoire", NATURE MEDICINE, vol. 16, no. 9, 8 August 2010 (2010-08-08) , pages 1029-1034, XP055098052, ISSN: 1078-8956, DOI: 10.1038/nm.2197 the whole document	1-30
A	HOLLY L HANSON ET AL: "Eradication of Established Tumors by CD8+ T Cell Adoptive Immunotherapy", IMMUNITY, vol. 13, no. 2, 1 August 2000 (2000-08-01) , pages 265-276, XP055098234, ISSN: 1074-7613, DOI: 10.1016/S1074-7613(00)00026-1 the whole document	1-30

3 -/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	OFFRINGA ET AL: "Antigen choice in adoptive T-cell therapy of cancer", CURRENT OPINION IN IMMUNOLOGY, vol. 21, no. 2, 1 April 2009 (2009-04-01), pages 190-199, XP026058396, ELSEVIER, OXFORD, GB ISSN: 0952-7915, DOI: 10.1016/J.COI.2009.02.006 [retrieved on 2009-03-16] the whole document	1-30

A	MORRIS ET AL: "Generation of tumor-specific T-cell therapies", BLOOD REVIEWS, vol. 20, no. 2, 1 March 2006 (2006-03-01), pages 61-69, XP005302162, CHURCHILL LIVINGSTONE, AMSTERDAM, NL ISSN: 0268-960X, DOI: 10.1016/J.BLRE.2005.05.001 the whole document	1-30

A	S. P. KERKAR ET AL: "Tumor-Specific CD8+ T Cells Expressing Interleukin-12 Eradicate Established Cancers in Lymphodepleted Hosts", CANCER RESEARCH, vol. 70, no. 17, 20 July 2010 (2010-07-20) , pages 6725-6734, XP055098237, ISSN: 0008-5472, DOI: 10.1158/0008-5472.CAN-10-0735 the whole document	1-30

A	DATABASE EMBL [Online] 20 February 1998 (1998-02-20), "Mus musculus (house mouse) partial T cell receptor beta chain", XP002719327, retrieved from EBI accession no. EMBL:AAC02863 Database accession no. AAC02863 sequence	21

A	DATABASE Geneseq [Online] 13 November 2008 (2008-11-13), "Human T-cell receptor CDR3 peptide, SEQ ID 2592.", XP002719263, retrieved from EBI accession no. GSP:ATF88948 Database accession no. ATF88948 sequence	21
6	& EP 2 116 596 A1 (INT INST CANCER IMMUNOLOGY INC [JP]) 11 November 2009 (2009-11-11)	
3	-----	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2013/075141

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-30(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-30(partially)

method for the production of human T cell receptor (TCR), comprising immunizing a host organism expressing unrearranged human TCR loci with a peptide comprising an epitope specific for a tumor specific antigen, wherein the TAA is selected from RAC1 or RAC2 (SEQ ID NOs: 1, 2, 28-39, 42-53), TCR obtainable from said method, nucleic acid molecule encoding for TCR, vector, host cell, non-human T-cell, for use in medicine

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2-18. claims: 1-20, 23-30(all partially)

method for the production of human T cell receptor (TCR), comprising immunizing a host organism expressing unrearranged human TCR loci with a peptide comprising an epitope specific for a tumor specific antigen, wherein the TAA is selected from RHOT1, MAP2K1,..., or MED12, TCR obtainable from said method, nucleic acid molecule encoding for TCR, vector, host cell, non-human T-cell, for use in medicine

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19. claims: 21-30(partially)

TCR comprising at least one CDR3 domain according to SEQ ID NO: 40 or 41 (TRRAP), host cell, non-human T-cell, for use in medicine

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2013/075141

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012038055 A1	29-03-2012	AU 2011304728 A1	14-03-2013
		CA 2812153 A1	29-03-2012
		CN 103249430 A	14-08-2013
		EP 2618835 A1	31-07-2013
		JP 2013541332 A	14-11-2013
		US 2013273647 A1	17-10-2013
		WO 2012038055 A1	29-03-2012