

21	47	TCR-2 β chain complete
22	48	TCR-3 α chain CDR1
23	49	TCR-3 α chain CDR2
24	50	TCR-3 α chain CDR3
25	51	TCR-3 β chain CDR1
26	52	TCR-3 β chain CDR2
27	53	TCR-3 β chain CDR3
30	54	TCR-3 α chain complete
31	55	TCR-3 β chain complete
2	63	TCR-4 α chain CDR1
3	64	TCR-4 α chain CDR2
4	65	TCR-4 α chain CDR3
5	66	TCR-4 β chain CDR1
6	67	TCR-4 β chain CDR2
7	68	TCR-4 β chain CDR3
87	69	TCR-4 α chain complete
88	70	TCR-4 β chain complete
12	71	TCR-5 α chain CDR1
13	72	TCR-5 α chain CDR2
14	73	TCR-5 α chain CDR3
15	74	TCR-5 β chain CDR1
16	75	TCR-5 β chain CDR2
17	76	TCR-5 β chain CDR3
89	77	TCR-5 α chain complete
90	78	TCR-5 β chain complete
22	79	TCR-6 α chain CDR1
23	80	TCR-6 α chain CDR2
24	81	TCR-6 α chain CDR3
25	82	TCR-6 β chain CDR1
26	83	TCR-6 β chain CDR2
27	84	TCR-6 β chain CDR3
91	85	TCR-6 α chain complete
92	86	TCR-6 β chain complete
94	93	TCR-4 fusion protein
96	95	TCR-5 fusion protein
98	97	TCR-6 fusion protein
102	99	TCR-7 α chain complete
103	100	TCR-7 β chain complete
104	101	TCR-7 fusion protein
108	~~102~~ <u>105</u>	TCR-8 α chain complete
109	~~103~~ <u>106</u>	TCR-8 β chain complete

110	~~104~~ 107	TCR-8 fusion protein
114	~~105~~ 111	TCR-9 α chain complete
115	~~106~~ 112	TCR-9 β chain complete
116	~~107~~ 113	TCR-9 fusion protein

“Nucleic acid molecule” and “nucleotide sequence” generally means a polymer of DNA or RNA, which can be single-stranded or double-stranded, synthesized or obtained (*e.g.*, isolated and/or purified) from natural sources, which can contain natural, non-natural or altered nucleotides, and which can contain a natural, non-natural or altered internucleotide linkage, such as a phosphoroamidate linkage or a phosphorothioate linkage, instead of the phosphodiester found between the nucleotides of an unmodified oligonucleotide. Preferably, the nucleic acids described herein are recombinant. As used herein, the term “recombinant” refers to (i) molecules that are constructed outside living cells by joining natural or synthetic nucleic acid segments to nucleic acid molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above. For purposes herein, the replication can be *in vitro* replication or *in vivo* replication. The nucleic acids can be constructed based on chemical synthesis and/or enzymatic ligation reactions using procedures known in the art or commercially available (*e.g.*, from Genscript, Thermo Fisher and similar companies). For example, a nucleic acid can be chemically synthesized (see Sambrook *et al.*) using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed upon hybridization (*e.g.*, phosphorothioate derivatives and acridine -substituted nucleotides). The nucleic acid can comprise any nucleotide sequence which encodes any of the recombinant TCRs, polypeptides, or proteins, or functional portions or functional variants thereof.

The present disclosure also provides variants of the isolated or purified nucleic acids wherein the variant nucleic acids comprise a nucleotide sequence that has at least 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence encoding the TCR described herein. Such variant nucleotide sequence encodes a functional TCR that specifically recognizes MAGE-A4.

The MAGE-A4-positive HLA-A2 positive tumor cell lines (A375, NCI-H1703, A549-HLA-A2-MAGE-A4) were recognized and lysed by all MAGE-A4-TCR-transgenic T cells. Lysis of the MAGE-A4-negative HLA-A2-positive tumor cell line (A549-HLA-A2) MAGE-A4-TCR-transgenic T cells was not significantly different from the lysis observed using untransduced control T cells (Figure 9).

EXAMPLE 11

T CELLS EXPRESSING MAGE-A4 FULLY HUMAN TCRs CONTROL MAGE-A4-POSITIVE TUMORS IN VIVO

5×10^6 MAGE-A4 positive A375 tumor cells were injected in each flank of 10 NSG mice. Ten days after tumor engraftment, 3.5×10^7 MAGE-A4-TCR-transgenic T cells, 3.5×10^7 control untransduced T cells or vehicle PBS were administered to the mice. After treatment, all mice had their tumor volumes measured twice a week by a caliper.

Results:

Untransduced T cell and vehicle-PBS treated mice failed to control tumor growth and were sacrificed once tumors reached maximum size permitted per protocol. MAGE-A4-TCR transgenic T cell treated mice controlled tumor growth for up to 35 days post T cell infusions (Figure 10).

EXAMPLE 12

ENHANCED HUMAN MAGE-A4 TCRs

The TCR polynucleotide sequences identified in Example 1 were modified to enhance expression and functional avidity. The TCR α and β chain constant regions were minimally murinized and hydrophobic amino acid substitutions were introduced into the transmembrane domain of TCR α chain constant region. Exemplary polynucleotide sequences for the enhanced MAGE-A4 TCRs are set forth in SEQ ID NOs: ~~97-99~~99-101, ~~103-105~~105-107, and ~~109-111~~111-113. Exemplary polypeptide sequences for the enhanced MAGE-A4 TCRs are set forth in SEQ ID NOs: ~~100-102~~102-104, ~~106-108~~108-110, and ~~112-114~~114-116.

Lentiviral vectors encoding polycistronic TCR constructs were used to express the enhanced MAGE-A4 TCRs (TCR-7, TCR-8, and TCR-9). The polycistronic TCR constructs contain a TCR α or β chain, an optional furin cleavage site, a P2A ribosomal skipping sequence, and the corresponding TCR α or β chain. Lentiviral vectors were produced according to known methods. See e.g., Kutner et al., BMC Biotechnol. 2009;9:10. doi: 10.1186/1472-6750-9-10; Kutner et al. Nat. Protoc. 2009;4(4):495–505. doi: 10.1038/nprot.2009.22.

The polycistronic polynucleotide (SEQ ID NO: ~~99~~101) encoding the MAGE-A4 TCR-7 polypeptide (SEQ ID NO: ~~102~~104) contains a β chain encoded by SEQ ID NO: ~~98~~100, a polynucleotide encoding a ribosomal skip sequence, and an α chain encoded by SEQ ID NO: ~~97~~99.

The polycistronic polynucleotide (SEQ ID NO: ~~105~~107) encoding the MAGE-A4 TCR-8 polypeptide (SEQ ID NO: ~~108~~110) contains a β chain encoded by SEQ ID NO: ~~104~~106, a polynucleotide encoding a furin cleavage site, a polynucleotide encoding a ribosomal skip sequence, and an α chain encoded by SEQ ID NO: ~~103~~105.

The polycistronic polynucleotide (SEQ ID NO: ~~111~~113) encoding the MAGE-A4 TCR-9 polypeptide (SEQ ID NO: ~~114~~116) contains a β chain encoded by SEQ ID NO: ~~110~~112, a polynucleotide encoding a furin cleavage site, a polynucleotide encoding a ribosomal skip sequence, and an α chain encoded by SEQ ID NO: ~~109~~111.

EXAMPLE 13

T CELLS EXPRESSING MAGE-A4 FULLY HUMAN TCRs OR ENHANCED MAGE-A4 TCRs CAN BE EFFICIENTLY EXPRESSED ON HUMAN T CELLS.

Peripheral blood mononuclear cells (PBMCs) were isolated from three independent healthy donors, activated, and transduced with lentiviral vectors encoding a fully human MAGE-A4 TCR (TCR-5) or an enhanced MAGE-A4 TCR (TCR-8) or not transduced as a negative control. The cells were cultured for expansion in vitro and analyzed for vector integration by measuring vector copy number (VCN) and for expression by using flow cytometry against cells stained with a MAGE-A4_{GVY}-MHC-multimer (MAGE-A4²³⁰⁻²³⁹, GVDGREHTV; immuneAware) and antibodies against CD3.